SCRIBE

Science Chronicles in Research and Investigation Based Education

Annual Science Journal

(supported by DBT Star Status)

Sophia College (Autonomous), Mumbai

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CONTENTS

NOBEL PRIZE IN SCIENCE 2021

Editorial

Bhavna Daswani, Rajbinder Kaur Dehiya, Hema Subramaniam

It is with utmost pleasure that we bring to you the third issue of SCRIBE (Science Chronicles in Research and Investigation Based Education), our Annual In-house Peer-Reviewed Inter-Disciplinary Science Journal, supported by DBT Star Status.

We are truly indebted to our former Principal, Dr. (Sr.) Ananda Amritmahal and also to our in-charge Principal, Dr. Anagha Tendulkar Patil, for their unflagging support. Thanks are due to our expert Reviewers who have critically evaluated articles and helped raise the bar for this journal.

Much has changed in our world since the first issue of SCRIBE was published on 28th February 2020. The second issue was released online in 2021 in the midst of rampant SARS CoV-2 infections. The development of vaccines has helped abate the pandemic to an extent, so with a small sigh of relief we bring out the third issue of SCRIBE in 2022. However, the world is reeling under new problems, both as a direct outcome of the consequence of disease as well as due to geo-political conflicts and resultant economic crises. All is not well yet. In this context, this issue's cover has a white background as a mark of solidarity for peace and harmonious co-existence on this planet.

On a happier note, we embarked on this journey of publishing SCRIBE with the mandate of encouraging undergraduate and postgraduate science students of our college to inculcate scientific writing skills and provide a platform to publish their work. Our editorial committee comprises of students (along with faculty) who get trained into the editorial process. We are thrilled to share that our former student editors Ms Shreyasi Chatterjee and Ms. Michelle Pereira have landed themselves good careers due to their experience with this Journal - Ms Shreyasi Chatterjee is now employed as Associate Academic Editor at Crimson Interactive and Ms Michelle Periera as Clinical Research Associate with a major role in scientific literature preparation at Vitasoft Technologies Pvt. Ltd.

In the current issue of SCRIBE, we are pleased to include the following (3 research articles and 12 review articles):

This issue commences with an 'Invited Article' on cervical cancer by an alumna of Sophia College, Dr. Dhananjaya Saranath, a distinguished scientist and a stalwart in the field of Oncology research.

A short research article on solid state physics sheds light on a low-cost semiconductor composite with exciting potential applications. An interesting research article on the use of Zebrafish as a model organism to study Foetal Alcohol Spectrum Disorder (FASD) provides insightful information on assays that can be performed using Zebrafish embryos and also the threshold percentage dose of alcohol that shows significant effects on these embryos. This is followed by a research article on the psychology of colour preferences with age, mood, and other parameters.

'Peto's Paradox' and 'Cardiovascular Disease - a Comorbidity in COVID-19' are mini reviews. The former is an interesting quick read on how some larger animals (despite their size), have mechanisms that protect them from cancer. The latter describes the connection between the two in a crisp manner focusing on acute cardiac injury and acute myocardial infarction.

A review on the toxic effects of DEHP (Di-2-ethylhexyl phthalate) offers an in-depth understanding of the effects of this extensively used plasticizer on the body and also touches upon some regulatory

aspects on the use of DEHP in our country. Further, the world is now more aware of the impact that zoonotic diseases can have on human health and the underlying issues such as climate change. A thought-provoking review article describes this relationship in a nutshell and also touches upon socioeconomic factors involved with climate change.

The next two review articles are on neurobiology. 'The science behind sleep paralysis' provides a bird's eye view on a medical condition called sleep paralysis (an in between state sleep and wakefulness wherein a person is unable to move and that usually lasts a few seconds) and the neurotransmitters involved. 'The Plight of Bright Nights: Assessing the Effects of Exposure to Light at Night (LAN)' delves deep into how circadian rhythms have been disrupted in this modern world of technology and how bright light at night can have adverse effects on our psychological and physiological health.

Ensuing this is a review on general facets of 'autophagy', a much-celebrated term in biology which involves many intricate components in the cell but when simply put it means intracellular recycling. Finally, a review article on the use of nanotechnology for agriculture provides an interesting angle as the use of nanotechnology is usually associated with drug delivery in medicine; however, its application in agriculture is under-rated.

This issue would be incomplete without including articles on the recent Nobel Prizes in the Sciences as has been our practice every year. We thank Dr. Tressa Jacob, Department of Life Sciences, for encouraging students to write and for co-ordinating these Nobel write-ups. The articles on the Nobel Prize for Physiology or Medicine 2021, Nobel Prize for Physics 2021, and Nobel Prize for Chemistry 2021 not only showcase the fascinating scientists and their discoveries but also allow us to pay a tribute to their work.

We wish you a happy reading!

Invited article

Cervical Cancer in India: Prevention and Control

Dhananjaya Saranath

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Introduction

Cervical cancer is a cancer in women that occurs in the cells of the cervix, the lower part of the uterus that connects to the vagina **(Fig. 1).** Cervical cancer ranks as the second most common cancer in females in India with 123,992 new cases annually, 77,348 deaths (25% of global burden) due to cervical cancer and annual age-standardized incidence rate (AAR) 14.7 per 100,000 women (The Union for International Cancer Control's (UICC), 2020; World Health Organization (WHO)). Every 7-8 minute a woman in India dies due to the cancer [IARC Information Centre on HPV and Cancer (HPV Information Centre), 2021]. The death rate due to cervical cancer is 8 times higher in low-income and low to middle-income countries (LMICs) as compared to high income countries (HICs) (The Union for International Cancer Control's (UICC), 2020; World Health Organization (WHO)).

About 85% of the women approach a medical doctor in advanced stages of the disease, hence the high death rate and associated poor prognosis; whereas, the cure rate is about 80% if diagnosed in the early stages (Dutta et al., 2013).

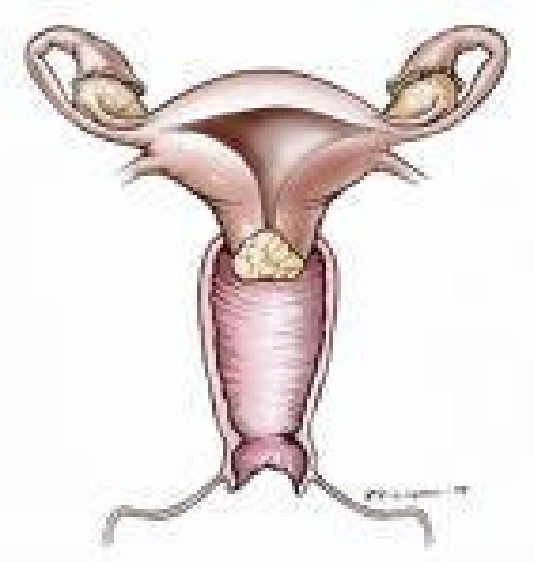

Fig 1: Cervical cancer

Cervical Cancer is caused by High-Risk Human Papilloma Virus (HR-HPV) **(Fig. 2)** and infection with the virus is the first necessary step on the path of cervical cancer (World Health Organisation-International Agency for Research on Cancer, 2005). Consequently 99.7% of the cancers are positive for HR-HPV, with the negative predictive value being 100%. Fifteen HPV types are classified as HR-HPV

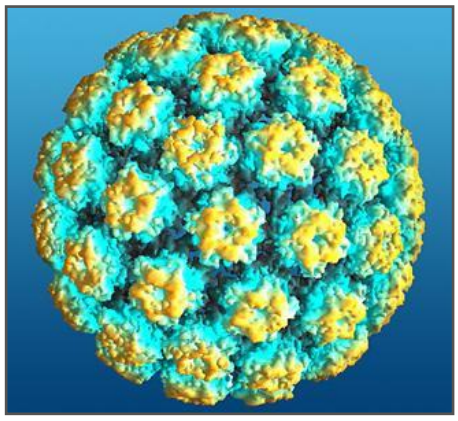

Fig 2: Human Papillomavirus (left) and Prof. Zur Hausen (right)

types i.e.16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 72, and 82 (Dutta et al., 2013; IARC Information Centre on HPV and Cancer (HPV Information Centre), 2021; World Health Organisation-International Agency for Research on Cancer, 2005).

HPV Infection is generally transient, as our immune system neutralizes and inactivates 90% of the virus within 12 to 24 months post infection. Persistent presence of the virus in the cervical cells is critical for conversion of the infected cells to a malignant phenotype.

Cervical Cancer is a Preventable Cancer: The good news is that cervical cancer is preventable by simple means such as HPV vaccination and systematic, organized HPV screening, leading to prevention and protection against cervical cancer. Besides, HPV positivity can result in early detection, downgrading of the cancer, better prognosis and high rate of cure. Three HPV vaccines are available globally, with two of the vaccines, a bivalent vaccine - Cervarix and a quadrivalent vaccine Gardasil, available and accessible in India. The vaccine is administered in the deltoid muscle, preferably in the arm (Joura et al., 2007; Skinner et al., 2016; World Health Organisation-International Agency for Research on Cancer, 2005). The vaccines are safe and efficacious, and approved by several regulatory authorities and professional bodies, as also the Drug Control of India since 2007 (Department of Cytology & Gynaecological Pathology Postgraduate Institute of Medical Education and Research, Chandigarh, India, 2006; Simms et al., 2019). Globally 350 million vaccines, and about four hundred thousand vaccines have been administered in India, without any severe adverse reactions related to the vaccines.

Prevention and elimination of cervical cancer is feasible, using the strategy of cervical cancer awareness, HPV vaccination and systematic and organized screening through detection of HR-HPVs and Papanicolau (Pap) test described by Professor George Papanicolau **(Fig. 3)** (Department of Cytology & Gynaecological Pathology Postgraduate Institute of Medical Education and Research, Chandigarh, India, 2006; Simms et al., 2019).

Challenges in Cervical Cancer in India: Contemporary Issues

The causative agent of cervical cancer is the High-Risk Human Papilloma Virus (HR-HPV), as unequivocally determined by Nobel laureate Professor Harald Zur Hausen.

The virus is a sexually transmitted virus, detected in 99.7% of cervical cancers (IARC Information Centre on HPV and Cancer (HPV Information Centre), 2021; World Health Organisation-International Agency for Research on Cancer, 2005). Thirteen high risk HR-HPV types are recognized as responsible for cervical cancer, with 70-77% of cervical cancers caused by two types - HPV16 and HPV18. Since 2007, two prophylactic vaccines protecting against infection with HPV16/18, the two most ommon HR-HPV types, are available in India, manufactured and marketed by multinational Pharmaceutical Companies – Glaxo Smith Kline (GSK) and Merck Sharpe (MSD). The vaccines are administered to girls between ages nine upto eighteen years in two doses, with the second dose administered at a six to twelve-month interval after the first dose. The vaccine is also administered as three doses in women 18 to 45 years. The first dose is administered at Day 1, second dose between 1-2 months after the first dose, and third dose after a twelve-month interval (Sankaranarayanan et al., 2016). Regulatory authorities including World Health Organization, European Medicines Agency, Drug Controller of India, have approved the vaccine. WHO proposed that 'Elimination of Cervical Cancer' should be placed on a fast track, and the Resolution was discussed and passed by the World Health Assembly, Geneva, in November 2020. One hundred and ninety-four countries are signatories to the resolution including India. The safety and efficacy of the vaccines have been proven and 350 million doses of the vaccines have been delivered to women globally, and about 400,000 doses of vaccines given to women in Indian women. No serious adverse effects have been reported in the past fifteen years. The antibody level in the vaccinated women indicates that booster dose is not required at least fifteen years and the protectionmay last a lifetime.

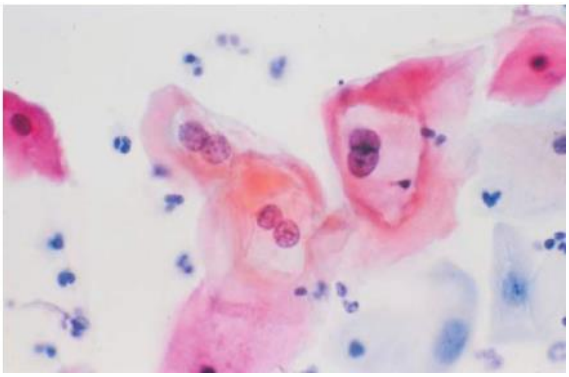

Fig 3: Koilocytes visible in Pap test (left) and Prof. George Papanicolau (right)

The vaccines are safe and efficacious and recommended by the World Health Organization, Geneva, and used globally towards eliminating cervical cancer. Thus, cervical cancer is preventable with the possibility of eliminating cervical cancer and resulting in 'Zero Cervical Cancer' in Indian women. Currently about 469 million women are in the age group susceptible to the high risk of cervical cancer.

Strategies for elimination of cervical cancer:

1. Cervical Cancer awareness:
A major drawback in our country is lack of awareness of various aspects of cancer

including women's cancer of Breast, Cervix, Ovary, Mouth and Stomach, the most common cancers recorded in women in India. Our Organization, Cancer Patients Aid Association (CPAA), is a Non-Government Organization registered with the Charity Commissioner since 1970. The vision of CPAA is 'Total Management of Cancer' with the goal to help and support socio-economically underprivileged cancer patients (Sharma & Saranath, 2021). The emphasis of CPAA is on cancer awareness through interactive sessions highlighting the facts and fictions, misconceptions and myths, and association of lifestyle with cancer risk factors including tobacco habits, alcohol, obesity; early detection through screening camps for cancers such as cervical/breast/oral/lung/prostate/etc with awareness campaigns for various sectors including schools, colleges, slums, corporates; financial and medical aid to cancer patients; advocacy for availability and accessibility of cancer drugs to all patients; rehabilitation of cancer survivors through vocational training and employment in our Cancer Rehabilitation Centre; cancer research with analysis of psychosocial aspects of delay in diagnosis and non-compliance of treatment by patients, as well as identification of biomarkers for cancer diagnosis/prognosis/response to treatment in our high incidence oral/cervical/breast cancers.

A concern in promoting control of cervical cancers is the lack of awareness of the pros and cons of HPV positivity, contributing to adverse psychological responses in HPV-positive women. Further, the individual and the society is often shocked by the link between a sexually transmitted infection and cancer. Stigma, shame and anxiety of HPV positive women is immense and can be alleviated by accompanying extensive health education to inform women and destigmatize infection. Information on intervention is likely to be the key to minimize negative psychological consequences of HPV positive results (McCaffery et al., 2006; Ngu et al., 2018). A recent study in Hong Kong observed that information provided either face-to-face or by a leaflet, successfully increased HPV knowledge and psychological well-being in HPV-positive women (Ngu et al., 2018). Thus, cervical cancer awareness is a critical factor in control and prevention of the cancer.

2. Early Detection of premalignant lesions through HR-HPV Screening:
An international state-of-the-art modality system - Hybrid Capture 2, is used to detect presence of HR-HPVs in cervical smears (Cuzick et al., 2006; Ronco et al., 2014; Sankaranarayanan et al., 2009; Saranath D., 2000). The test is recommended by WHO and several regulatory authorities, and used routinely in most countries for early detection of cancer. The test detects clinically relevant HR-HPVs, for indication of the risk of cervical cancer in women. Initiatives to provide free HPV tests for socio-economically underprivileged women is advised, for estimation of the risk of cervical cancer, and forward path advised for the high-risk women. CPAA has screened about 10,000 women in the past years (Sharma & Saranath, 2021). At any given time, about 6.8% of women in the general population in India, show cervical infection with HPV. The HPV 16 and 18 accounts for 70% – 80% of cervical cancers in India. The HR-HPV positive women are alerted and monitored closely for development of premalignant and malignant lesions of the cervix, and also early detection of cervical cancer (International Agency for Research on Cancer Working Group, 1986; Sharma & Saranath, 2021)

3. HPV Vaccination: The emphasis of CPAA for HPV Vaccination is through safe and efficacious vaccines protecting against cervical cancer (Bhatla et al., 2010). HPV vaccination has been adopted by 85% of high developed index (HDI) countries in the world, whereas only a small number of low-to-middle income countries have introduced HPV vaccination, due to limited financial resources and infrastructure facilities. Thus unaffordability, inaccessibility and unavailability of the vaccine is the bane in several low to middle income countries including India.
Two HPV vaccines are available in India (Bhatla et al., 2010). These are Gardasil – a quadrivalent vaccine against HPV16/18/6/3, and Cervarix – a bivalent vaccine against HPV16/18. These vaccines are prophylactic to be administered prior to

HPV infection. HPV vaccination has been adopted by 85% of high developed index (HDI) countries in the world although increased incidence of cervical cancers is primarily the low-to-middle income countries (LMIC), only a small number LMICs have introduced HPV vaccination (Brisson et al., 2020). The limitations are due to limited financial resources, infrastructure facilities and unawareness of cervical cancer and prevention and protection by the HPV vaccines. Thus unaffordability, inaccessibility and unavailability of the vaccine is the bane in several countries including India (Gallagher et al., 2017; Kane et al., 2012). In India we can move to Zero cervical cancer (Cx Ca) as indicated in **Fig. 4.**

- Approved by the Drug Controller of India

CPAA initiated free HPV Vaccination Camps for girl students from marginalized socio-economic background in villages of Maharashtra and urban slums, with about 12,500 vaccines administered to school girls and women in the age group 9 – 45 years (Sharma & Saranath, 2021). The screening and vaccination will result in reducing the incidence of cervical cancers, downgrade cancer, and result in better prognosis and low mortality in cervical cancer in Indian women. Minor adverse reactions such as swelling and redness at the site of injection, mild pain, headache and giddiness lasting a short duration.

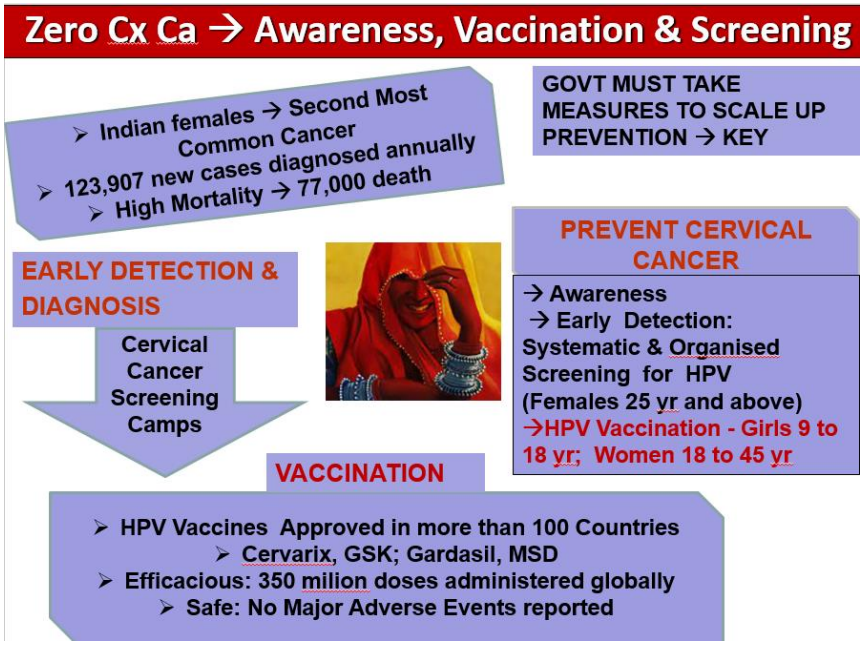

Fig 4: Strategies to move to Zero cervical cancer (Cx Ca)

Characteristics of HPV Vaccines:

- Protection against Cervical Cancer
- Proven efficacy and safety
- 99-100% efficacy against relevant HPV types
- Vaccine injections are given intramuscularly in the deltoid muscle in the upper arm or the thigh region
- No severe adverse side-effects are reported
- Contraindicated in pregnant women

Certain additional risk factors in cervical carcinogenesis include early age at marriage, multiple pregnancies, multiple sexual partners, poor genital hygiene, obesity, malnutrition, long term oral contraceptive usage and basic lack of cervical cancer awareness **(Fig. 5).** (Sharma & Saranath, 2021; Denny & Sankaranarayanan 2006).

Game Changer in Prevention of Cervical Cancer: Update: The very recent evaluation of the World Health

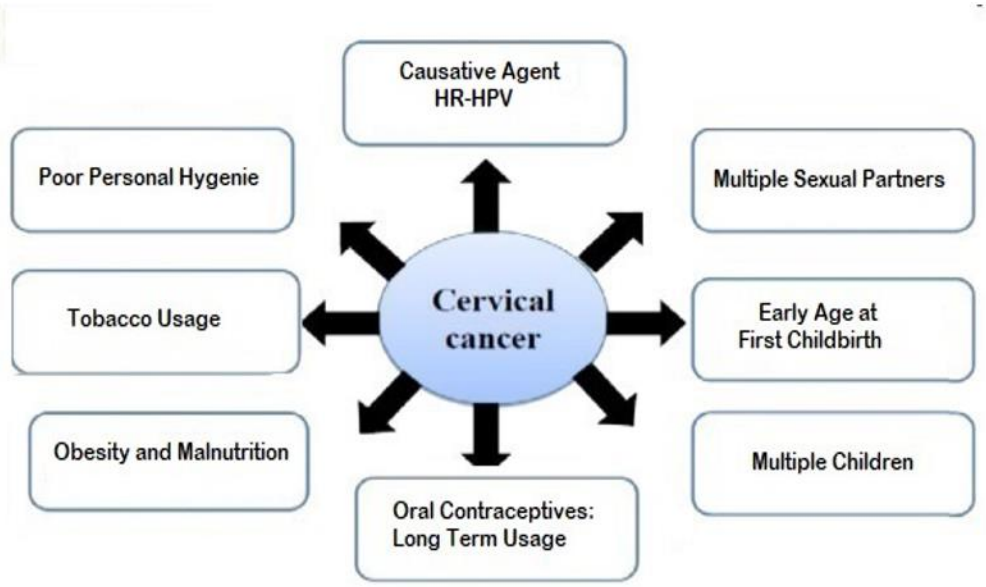

Fig 5: Additional risk factors in cervical cancer

Organization Strategic Advisory Group of Experts on Immunization (SAGE), held on April 11th 2022, categorically stated that 'One-dose Human Papillomavirus (HPV) vaccine offers solid protection against cervical cancer', with comparable efficacy to 2-3 dose regimens (WHO, 2022).

This will be a game-changer for the prevention of the disease, enabling HPV vaccine doses to prevent and protect against cervical cancers in a larger number of women with the life-saving jab given to more girls. SAGE recommends updating dose schedules for HPV as follows:

- one or two-dose schedule for the primary target of girls aged 9-14
- one or two-dose schedule for young women aged 15-20
- Two doses with a 6-month interval for women older than 21

The unanticipated potency of a single dose of the HPV vaccine has been linked to the structural features of the virus-like particles in the vaccines and specificities of the virus life cycle, resulting in the induction of high and durable antibody titers and exceptional susceptibility of the virus to these antibodies Secondary prevention of cervical cancer:

Systematic and organized screening of cervical smears in women 25 to 65 years (International Agency for Research on Cancer Working Group, 1986) with primarily HPV DNA PCR testing, followed by a histopathological and cytopathological examination of cervical smears, Papanicolaou test (Pap test), is the basis of secondary prevention of cervical cancer (Saranath et al., 2002). The Pap test has high specificity (96%), but low sensitivity (28-53%). In 2007, Professor Harold Zur Hausen received the Nobel Prize in Physiology and Medicine, for unequivocally proving that HR-HPVs are the causative agents of cervical cancer, with application in primary and secondary prevention (International Agency for Research on Cancer Working Group, 1986; Tewari et al.,2018). HPV testing is critical and shows high specificity (90.7%), as well as high sensitivity (>89%) for identification of precancerous lesions and cancer (Brisson et al., 2020; Department of Cytology & Gynaecological Pathology Postgraduate Institute of Medical Education and Research, Chandigarh, India,

2006; Simms et al., 2019). Further, the negative predictive value of HR-HPV test is 100% (Saranath et al., 2002; Sharma & Saranath, 2021). In developed HICs, Pap test and HPV test have been the two screening strategies recognized and routinely used to detect precancer and diagnose early cervical cancers. The cervical cytology-based Pap test is either conventional cytology or liquid-based cytology, and HPV tests are PCR based including the globally used, WHO recommended, Hybrid Capture 2 test (Diaz, 2008; International Agency for Research on Cancer Working Group, 1986). In several LMICs including India, recently visual inspection with 3-5% acetic acid (VIA) application or a comparable visual inspection with Lugol's iodine (VILI) has been recommended as the primary screening test due to monetary constraints and additional limitations (Bhatla et al., 2007; Sankaranarayanan et al., 2007).

Screening for cervical cancer in the susceptible 469 million Indian women is a very expensive proposition and a monumental task, perhaps beyond our government health budgetary allocation. However, the overall lifetime cost in 100,000 women with no screening facilities is estimated to be INR 194 million and 90% (INR 175 million) of the cost is the treatment expenditure for ICC (Campos et al., 2017; Kawai et al., 2012). The quality of life for women with cervical cancer is poor and negatively affects the family members. Introduction of systematic and organized screening has led to reduction in 58% cervical cancer cases and 70% deaths as compared to no screening in a lifetime cohort of 100,000 women. The reduction in cancer cases and associated mortality translates into a gain of up to 6848 life years and 8198 Quality Adjusted Life Years (QALYs).

Absence of systematic and organized prevention programs, lack of sufficient numbers of medical doctors, trained paramedical staff, healthcare infrastructure and socio-religious beliefs present mega obstacles to the implementation of cervical cancer screening programs.

Limitations of HPV Vaccination: HPV vaccination in pregnant women is contraindicated, as the effect of the vaccines on the fetus is not extensively studied. Pregnancy outcomes from pregnancy registries has indicated no increased birth defects in HPV vaccinated women as compared to non-vaccinated women during pregnancy, confirming that HPV vaccination is not associated with adverse pregnancy outcomes, including major malformations (Dana et al., 2009). Per chance the vaccine is administered inadvertently during pregnancy, it is not necessary to become unduly concerned and consider termination of pregnancy. The remaining doses should be delayed for a period of two months post-delivery of the child. If a woman has received HPV vaccine and gets pregnant, the additional doses of vaccination should be given post birth of the child.

The state Health Ministries in India have generally not been proactive in introducing HPV vaccination. A proactive stance, directives and policies for elimination of cervical cancers in Indian women by our Central and State governments will result in elimination of cervical cancer in Indian women. Elimination of smallpox and polio through vaccination, has been extremely successful with the support and commitment of the Health Ministries.

Limitations of Screening Tests:

Besides poor sensitivity, disadvantages of cytology-based Pap test include the turnaround time of around two weeks for test reports, resulting in a high loss to follow up. Further, the test requirement of qualified and specialized cytopathologists or histopathologists for interpretation of the test results, trained paramedics and Auxiliary Nurses and Midwives (ANM) for sample collection, makes it difficult to implement the test universally. Besides, in India the location of cytopathology laboratories in public health sectors are based at tertiary care centers in urban areas, making the test inaccessible to women in rural areas. Diversification of district hospital laboratories is required to implement cytology-based screening programs at district hospitals. In general, logistic limitations of cytology-based cervical cancer screening programs are factors limiting the use of Pap test in LMICs. Organized and systematic cytology-based cervical cancer screening program is

currently not operational in India. The interpretation of Pap smear is subjective and hence the increasing trend towards more objective and automated HPV DNA testing of cervical screening is justified (Almonte et al., 2007; Denny & Sankaranarayanan, 2006; Salmerón et al., 2003).

Major limitations of HPV based tests are that these are generally provided in private commercial laboratories and hence expensive. Generally, in several LMICs including India, the underprivileged and socio-economically backward women in urban slums and rural regions do not have health and medical insurance to cover HPV testing cost, and the family has to bear the test expenses, which they can ill afford. Hence, often visual inspection with acetic acid (VIA) test is recommended despite the relatively poor specificity of VIA test. The rank-ordering of screening strategies from most effective to least effective, is a two-visit HPV DNA testing, single visit VIA, and three-visit cytology, for screening adult women two to three times in their lifetime in addition to pre-adolescent vaccination.

With our experience on cervical cancer screening, we recommend differential pricing for the test with free HPV testing for underprivileged women, and pricing at two tiers with discount for the medium income group women, and normal pricing for privately referred women. Thus, it is critical to enable availability, accessibility and affordability of screening tests for women in LMICs. It should be emphasized that women with pre-cancer are detectable only through screening. It is critical that relatively high screening coverage rates of 70% women, counseling, diagnostic testing, treatment for precancer and early and advanced stages of cervical cancer, should be mandatory.

Future Perspectives:

In India women susceptible to and at a high risk of developing cervical cancer, is large, around 469.1 million, including about 50 million school girls (Bhatla et al., 2007). These numbers require both large financial and infrastructure resources, often lacking in our country and included in our annual budget. Hence a feasible and practical solution is a collaborative effort between public private partnerships (PPPs) and NGOs. The NGO group need expertise in HPV vaccination and HPV screening with professional medical/paramedical staff, education institution faculty and administrators, social activists and local coordinators, and a commitment and dedication to the social cause. The Corporate organizations should take the initiative of providing financial resources, resulting in no cost to the participating school girls or women to be administered the vaccines or women to be screened for cervical cancer. The task should be taken up state wise with the State Health Ministry and Education Ministry directing the schools and various organizations to promote HPV vaccination. There is a dire need for the preventive measures of cervical cancer by HPV vaccination in the Indian National Immunization Program (NIP), and means to implement the same. In the absence of further intervention, it is estimated that 44·4 million cervical cancer cases will be diagnosed globally in the next 50 years (2020-2069), with almost two-thirds of cases occurring in LMICs. The consequences and implications of cervical cancer are dire not only for the women, but will profoundly be reflected in the families, communities and nations, with the cancer affecting women at a very productive age, vital to social and economic stability. HPV vaccination in school girls, pre-adolescents and older women upto 45 years, with 70% vaccination coverage, followed by screening two to three times per lifetime between ages of 35 and 45 years, is estimated to prevent more than 1.25 million cervical cancer deaths over the lifetimes of 10 consecutive birth cohorts (Brisson et al., 2020). Fewer than five percent of the eligible women in India have ever been screened and there are no government-sponsored population-based cervix screening programs in the country, although it is critical to screen and even repeat screening of cervix with high coverage of targeted women.

Summary and Conclusion:

Current data suggests that 85% of all cervical cancer cases occur in LMICs, primarily due to poor access to HPV vaccination, cervical cancer screening, early detection and treatment of both pre-cancers and cancers. Cervical cancer is currently the leading cause of cancer deaths in women in 42 countries,

although it is one of the most preventable and successfully treatable forms of cancer. Increase in cervical cancer awareness, appropriate implementation of HPV vaccination, screening in women in the 25-65 age group, and treatment for precancerous and early-stage cancer will make it feasible to 'Eliminate cervical cancer in women in India'. The available prophylactic HPV vaccines protects against the most common cancer-causing HPV types i.e HPV 16/18, offers cross immunity against additional HR-HPVs, provides herd immunity, is safe and efficacious, and can significantly reduce the risk of cervical cancer.

The WHO cervical cancer elimination initiative suggests 90% coverage of HPV vaccination in girls by 15 years of age, 70% coverage for screening with a high-performance HPV test between the ages of 35 and 45 years, and 90% treatment of precancerous lesions and ICC cases. WHO estimates predict that achieving and sustaining the 90:70:90 targets will avert 74 million new cases of cervical cancer and 62 million deaths in 78 LMICs in the coming decade. Increasing coverage of HPV vaccination and cervical cancer screening will be a challenge, but is an excellent opportunity for prevention of cervical cancer translating into saved lives in women and leading to healthy women, happy families and a healthy nation and advanced stages.

In India and several other developing countries, an alternative test VIA is considered an affordable and effective method for cervical cancer screening, although the associated reduction in cervical cancers is relatively less. An alternative out-of-the-box approach to this health problem in our women population needs to be worked out, and dedicated and committed Public Private Partnerships must be seriously considered. The financial contribution may be planned from the corporate organizations for HPV DNA screening, and state-of-the-art technology-based screening to be provided by technically qualified expert groups. Adoption of specific rural regions and urban slums facilitated by individual Corporations in conjunction with Health based NGOs, may be a viable solution to elimination of cervical cancers in Indian women. Primary prevention by HPV vaccination in school girls and a systematic and organized cervical cancer screening is anticipated to result in eliminating cervical cancer by reducing cervical cancer incidence to 4 per 100,000 women. In India it is estimated that the earliest possible period for elimination of cervical cancer would be about 50 years around 2079, provided HR-HPV vaccination, screening and treatment of precancers and early-stage cancers are implemented. Australia has planned elimination of cervical cancers in the country by 2040, and the progress and indications point to their success.

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Research article

Thermally stimulated discharge conductivity of Polystyrene: Poly vinyl chloride & Meta nitro aniline doped Polyblend thin films

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Abstract

D.C. electrical conductivity of Meta nitro aniline (MNA) doped polystyrene (PS) and Poly vinyl chloride (PVC) thin films (~46.14 μm) were studied. The electrets of undoped (50% PS + 50% PVC) and MNA doped thin films were prepared at different polarizing electric fields like (6, 12, 15, 18 & 24KV/cm). Thermally Stimulated Discharge Conductivity (TSDC) has been carried out in the temperature range 303°K to 403° K at five different polarizing fields (6, 12, 15, 18 & 24KV/cm). Results are discussed on the basis of mobility of charge carriers in the sample. The present study reveals that both temperature and addition of dopant influence the D.C. electrical conductivity of undoped and doped polyblends samples.

Introduction

In today's world of sophisticated mechanical, electro – mechanical and electronic applications, polymers are playing an increasingly important role. These materials are of great interest because of their enhanced electrical, mechanical and magnetic properties. One of the most valuable properties of polymers is their capability to store space charge near the surface or in the bulk for long period of time. The dielectric materials which exhibit a 'quasi – permanent' charge storage and /or dipole orientation is called electrets (Sessler, 1982). Electrets formed by thermal method are referred as thermo – electrets. Thermally stimulated (Sessler, 1982) discharge conductivity (TSDC) from thermo – electrets are induced by thermal release of dipoles, ions and trapped electrons (Sangawar et al., 2007). n polymeric electrets, charge storage and dipole orientation depend on many characteristics such as chemical impurity, chemical constitution, macro molecular arrangement, degree of crystallinity, metal – insulator or interfaces at amorphous – crystalline regions etc. In this work, the study was performed on undoped and Meta nitro aniline (MNA) doped Polystyrene (PS) and Poly vinyl chloride (PVC) blends. PS and PVC has been extensively used in numerous applications and therefore intensively studied. A blend of Polystyrene and Poly methyl methacrylate was studied by Keller (Keller et al., 1991). Polyblends of Poly methyl methacrylate and Poly vinyl chloride was studied by Khare (Khare et al., 1993). Thermally stimulated depolarization current characteristic of ethylene-co-vinyl acetate (EVA) with conductive polypyrrole (PPY) composites was studied by Thabet (Thabet et al., 2020). The thermally stimulated discharge current (TSDC) is an important tool to identify and evaluate the dipole reorientation process, trapping and recombination in polymeric matrix.

Methodology

Sample preparation: Polystyrene (PS) and Poly vinyl chloride (PVC) was supplied by Reliance Industries, Mumbai. Meta Nitro Aniline (MNA) was supplied by New Modern Chemical Corporation, Mumbai. The thin films of undoped (50% PS + 50% PVC) and MNA doped (49.5 % PS + 49.5 % PVC + 1.0 % MNA) were prepared in the laboratory by weight percentage method using an electronic mono pan balance (Adiardutt - 180) having an accuracy of 0.0001g. The percentage ratio of undoped polyblend sample is (PS + PVC) is 50: 50 and MNA doped polyblend sample is 1.0 percentage of MNA is dissolved in common solvent i.e., cyclohexanone (A R Grade). All films were prepared by isothermal evaporation technique (Sangawar and Johari, 2009). These films were subjected to 12 hours heat at constant temperature of 400C and for another 12 hrs. at room temperature to remove traces of solvent. The resultant specimens were used for electrical studies.

The thickness of polyblend samples were measured by micro meter screw guage with a least count of 0.001 cm. But for greater accuracy a compound microscope in conjunction with an occulometer having least counts 15.38 μm at the magnification of 1:100 was used (Besare and Deogaonkar, 1998). The thickness of all samples was kept constant and it is of the order of ~46.14 μm. For good ohmic contact, both sides of thin films were coated by quick drying conductive silver paint supplied by Eltecks Corporation, Bangalore. Metal – polymer – Metal sandwich structure was made by placing the coated film in between circular brass electrode. The sample holder having Metal – Polymer – Metal sandwich structure was placed in a furnace and heated up to the poling temperature. The sample was allowed to remain at that temperature for about 30 min. then electric field of particular strength was applied for 1 hour at poling temperature. The sample was allowed to cool, down at room temperature in the presence of electric field. Total time of polarization was adjusted to be 2 hours in each sample. At room temperature the sample was short circuited for 20 min. to remove stray charges. The electrets were prepared at different D.C. polarizing fields (6, 12, 15, 18 & 24KV/cm). The D.C. electric conductivity was measured by determine the resistance of a sample with in a range of 303°K – 403°K at a rate of 2°/min. The measurement of voltage drops across high resistance was taken on digital multimeter (Systronics, 435) having accuracy of ±1mV[2]

Results and Discussion

The results of the present work are in the form of thermo – grams (**Fig. 1** & **Fig. 2**)

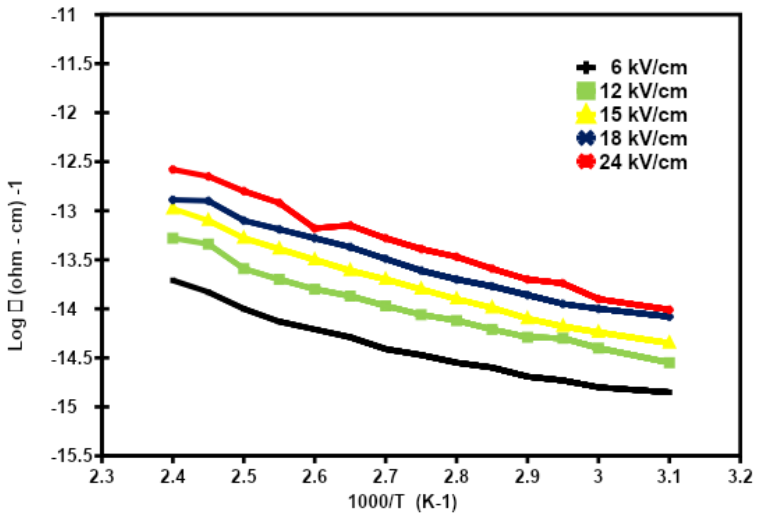

Fig. 1: PS+PVC (50%+50%)

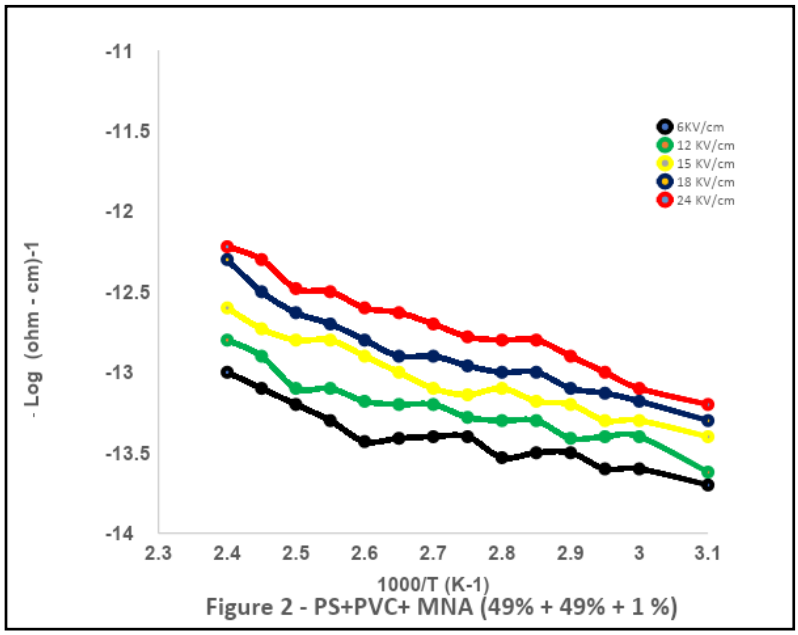

Fig. 2: PS+PVC+MNA (49%+49%+1%)

which are plotted between log of thermally stimulated discharge conductivity σ (ohm - cm)-1 and temperature (103/T) at different polarizing fields (6, 12, 15, 18 & 24KV/cm).

Fig. 1 is the thermo – gram of undoped PS and PVC sample. For all polarizing fields the D.C. electrical conductivity remains in the insulating region.

Fig. 2 shows slight change in conductivity due to addition of 1.0 weight percentage of MNA in PS and PVC poly blend. The slight decrease is observed at low temperature region and then continuous increase in conductivity is observed up to 4030K. Our study reveals that D.C. electrical conductivity increases due to increase in temperature approximately follows the equation shown in **Fig. 3**.

Table 1 – 2 shows the activation energy values within low (LTR) and high temperature region (HTR).

Under the influence of applied electric field the localized (short range) motions of the trapped charges in the sample serve as effective electric dipoles (Bengoechea et al., 2002). As the strength of the electric field increases the degree of distortion in the sample increases resulting increase in conductivity. The main constituents of the Polyblends are PS and PVC, which are amorphous in nature. This insulating polymer material requires extra thermal energy for excitation of charge carriers (Sangawar, 1996; Sangawar et al., 2007). The slight decrease in electrical conductivity at low temperature may be due to local motion of molecular groups present in the sample.

$$\sigma = \sigma_0 \frac{E_0}{KT}$$

Fig. 3: Equation wherein σ is conductivity, σ_0 the pre-exponential factor, E_0 activation energy of conduction and K is Boltzmann's constant.

Table – 1: Activation Energy (Ea) values from TSDC thermograms of 50 wt. % PVC in 50% PS polyblend thermo – electret.

Electric field (kV/cm)	Activation Energy (Ea) eV	
	LTR	HTR
6	0.110	0.3961
12	0.330	0.198
15	0.242	0.396
18	0.176	0.033
24	0.242	0.59

Table – 2: Activation Energy (Ea) values from TSDC thermograms of 1.0 wt. % MNA doped in 49.5 % PS: 49.5 % PVC polyblend thermo – electret.

Electric field (kV/cm)	Activation Energy (Ea) eV	
	LTR	HTR
6	0.176	0.396
12	0.374	0.363
15	0.242	0.330
18	0.198	0.561
24	0.264	0.429

Addition of dopant increases the conductivity of polymeric sample caused by increase in mobility of ions and polymeric bond rotations. Initially increase in conductivity at low temperature may be due to the injection of charge carriers directly from electrode. The dopant in the sample formed hertrocharges and discharge by dipolar disorientation is thermally activated and so can be speeded up by increasing temperature. At high temperature due to softening of the sample the injected charge carrier can move easily into the volume of the sample resulting large current.

At high temperature enhancement in D.C. conductivity is mainly due to increase in ionic mobility of activated charge particles (Ateria et al., 1998). Our results are in good agreement with other studies (Gwaily et al., 1993; Ramadin et al., 1994; Ateria et al., 1998).

Conclusion The present study reveals D.C electrical conductivity of undoped Polystyrene (PS) and Poly vinyl chloride (PVC) and Meta nitro aniline (MNA) doped polyblends thermo electrets in the temperature range 303^{o}K to 403^{o} K increased with increasing temperature as well as polarizing field. The slight decrease in the activation energy at high temperature region of doped polyblend sample enhances the conductivity and it is mainly attributed to the increase in ionic mobility of dopant. These low-cost semiconductor composite materials are utilized in many applications ranging from photocatalysis, photovoltaic cells, photo

electrochromic displays, and light-emitting devices to sensors etc. (Rajeshwar et al., 2010).

Conflict of interest

The author declares no conflict of interest.

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Research article

Response of developing zebrafish (*Danio rerio*) embryos under hypoxic stress to ethanol toxicity

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Abstract

Foetal Alcohol Spectrum Disorder (FASD) is a known cause of congenital craniofacial abnormalities, poor functioning of the nervous system including cognitive and motor functioning in children exposed to alcohol during gestation. Several other factors are known to affect the outcome of ethanol toxicity. We have used zebrafish as a model organism to study the effect of hypoxia on alcohol toxicity during early development. Zebrafish embryos were treated to chemical hypoxia at 4 hours post fertilization (hpf) followed by ethanol treatment an hour later. The embryos were exposed to their respective treatments for 24 hours. Criteria such as viability, hatching rate, heart rate and motility were monitored and recorded at appropriate time points. Morphometric analyses were performed on images that were taken at 72 hpf. Neurocranial cartilage was studied at 5 days post fertilization (dpf). Ethanol at a dose of 2.5% showed severe abnormalities in all the parameters monitored while 1.5% ethanol had no significant effect. Hypoxia pretreatment did not have any further effect on these embryos. The study shows that zebrafish is a good model to study FASD and that chemical hypoxia had no adverse effects beyond that observed for alcohol under these conditions.

Introduction

Maternal alcohol consumption during pregnancy often leads to varying degrees of abnormalities in the foetus that are termed as Fetal Alcohol Syndrome Disorder (FASD). The incidence of FAS (which is the most severe form of FASD) globally ranges between 0.09–0.23% of all annual births (Popova et al., 2017) while the incidence of FASD in some regions may be as high as 2% to 5% (May et al., 2009; May et al., 2014). The symptoms vary from child to child and manifest as physical deformities and intellectual and cognitive deficits (Popova et al., 2017). Physical deformities include small head due to craniofacial developmental abnormalities and reduced brain size. Poor development of the brain and central nervous system leads to poor motor coordination and intellectual disability and learning disorder (Riley at al., 2011).

Most studies carried out on developmental FASD have used in vitro tissue culture cells or the rodent model. The ease of tissue culture makes it a convenient system to study molecular effects; however, it is often difficult to extend these observations to an in vivo situation. The rodent model has yielded very useful information especially in studying response to nervous system development and behaviour but is not suitable for large scale screening. The zebrafish overcomes both the limitations. It is a vertebrate model system that is very simple to handle, yet complex enough to manifest behavioural changes and yields a large number of progeny for bulk scale

investigations. Addition of ethanol to the medium directly ensures that all embryos are exposed to the same amount of ethanol, overcoming animal to animal variation. It has been determined that tissue levels of ethanol post exposure are between 25% and 35% of the ethanol in medium (Fernandes et al., 2018). Further, human and zebrafish genomes share approximately 75% similarity. They are transparent during the initial few days as a result of which the developmental stages can be easily monitored in real time. Embryogenesis is rapid, due to which the vital organs like the brain, heart, liver, and kidney can be observed by 96 hours post fertilization (hpf). Their nervous system, cardiovascular system and digestive systems are similar to that of mammals. Any deformities or changes in the phenotypic structure can easily be observed and further analyzed (Spitsbergen & Kent, 2003; Hill et al., 2005; Chakraborty et al., 2009).

It has been shown that the response to ethanol toxicity is dependent on other variables such as genetic background, nutritional status, smoking and drug use. Many of these variables lead to the generation of hypoxia and free radicals which are prime causes of cell damage (Abel and Hannigan, 1995; Burd et al., 2007). Oxygen levels to the foetus are dependent on the umbilical vasculature. Increased blood alcohol levels are known to constrict the vessels leading to decreased oxygen levels (Abel & Hannigan, 1995). Further the foetus may be exposed to hypoxia during parturition. Hence it would be interesting to determine if hypoxia will influence the effect of alcohol exposure. Several studies have shown conflicting results of the effect of alcohol and hypoxia. Simultaneous exposure of ethanol and hypoxia in an in vitro model of hepatoma cell line showed enhanced effect of lower levels of alcohol (Wang & Wu, 2009). Similar results of enhanced death were obtained for cortical neurons in culture due to the synergistic effect of low levels of alcohol and hypoxia (Genetta et al., 2007). A meta-analysis done on the effect of maternal drinking on foetal development suggests that the effect of alcohol on poor foetal development may be due to hypoxia and oxidative stress (Bosco & Diaz, 2012). On the other hand, additional studies have shown that intermittent hypoxia training can protect against alcohol withdrawal excitotoxicity (Wang et al., 2013; Jung & Mallet, 2018).

Our study was designed to understand the effect of hypoxia on alcohol toxicity using zebrafish as the model system. Chemical hypoxia was given to 4 hpf embryos followed by ethanol treatment of various doses at 5 hpf. The treatments were continued for a further 24 hours and then the embryos were monitored till 5 dpf for effects of toxicity using various parameters. Our results indicate that prior exposure to hypoxia did not have a significant effect on the toxicity observed for ethanol alone for most of the parameters studied.

Materials and Methods

Animal Husbandry and collection of embryos: Wild type (Tübingen) zebrafish were maintained as per standard procedure (Westerfield, 2000). Embryos were obtained by natural crossing and were reared in E3 embryo media. Embryos were kept at 28˚C throughout the course of the experiment. Developmental stages were monitored (Kimmel et al., 1995). 4 sets were performed using 8 embryos for every treatment.

All procedures were conducted according to the policies of Institutional Animal Ethics Committee.

Chemical hypoxia and ethanol treatment: Most protocols give physical hypoxia in a chamber. which is usually given by bubbling nitrogen gas in the media (Kamei and Duan, 2018). We however, found that chemical induced hypoxia is also effective on model systems. Sodium sulphite is a potential hypoxia inducer that mimics hypoxic stress in *Caenorhabditis elegans* (Jiang et al., 2011) Hence sodium sulphite at a concentration of 0.2g/lit (Sorathia et al., 2019) dissolved in embryo medium was used as a scavenger of the dissolved oxygen in the media. Winkler's method was used to measure dissolved oxygen in control and hypoxic media (Delzer & McKenzie, 2003).

Earlier studies have shown that concentrations from 1% ethanol are toxic to zebrafish (Bilotta et al., 2004; Sylvain et al.,

2010; Ramlan et al., 2017). According to a meta-analysis review, a consensus of 25%-35% of the alcohol in the media has been reported to be taken up by the tissue (Fernandes et al., 2018). In this study we have used 1.5% and 2.5% ethanol along with hypoxia to ascertain its toxic effect.

Chemical hypoxia was given at 4 hpf and the respective ethanol treatments were given at 5 hpf.

All treatments were terminated after 24 hours (when embryos are about 30 hpf) by washing them with the embryo media and were then reared in E3 till the termination of the experiment.

Criteria to determine toxicity: The following parameters were studied to ascertain the effect of both ethanol and hypoxia, alone and together. Viability was noted upto 72 hours of the respective treatments and the percentage of surviving embryos was calculated. Effect on hatching rate was determined at 48-50 hpf. Heart rate was monitored at 48 hpf for 15 seconds per embryo under a stereo microscope. Controls and ethanol treated embryos were compared to their respective hypoxic counterparts.

Effect on morphology was checked and images were taken using Carl Zeiss (Stemi 2000C). Morphometric analysis was done on 72 hpf embryos using the ImageJ software. Morphological characters such as the body length, head length, head to body ratio, eye area, and area of the pericardial region were measured and analysed. Locomotor behaviour was studied by gently touching the embryo at the tail with an eyelash brush. Zebrafish embryos with normal motor development show an immediate darting behaviour when touched. This behaviour was observed at 72 hpf and the number of embryos that spontaneously darted were recorded.

Embryos were fixed at 5 dpf in buffered paraformaldehyde and stained with Alcian blue for cartilage to ascertain the effect on craniofacial development (Javidan & Schilling, 2004).

Statistical analysis: Students t-test was performed to determine the level of significant toxicity between treated and control groups.

Results

80.6 mg/L of dissolved oxygen was estimated in E3 media using Winkler's method. 0.2g/L of sodium sulphite for 15 minutes scavenged all the dissolved oxygen from the media.

The effect of toxicity of ethanol or hypoxia and of simultaneous treatment with both stressors was determined using several standard toxicity parameters.

Viability: Viability of embryos was determined at 24, 48 and 72 hpf. The percent viability after only ethanol or ethanol and hypoxia was ascertained (Table 1). Neither ethanol nor hypoxia had any significant effect on the mortality of zebrafish embryos except at 72 hpf where all embryos were dead with 2.5% ethanol alone and with 2.5% ethanol and hypoxia ($p < 0.05$).

Hatching rate:

Hatching of embryos starting from 48 hpf is an active process and an indication of the normal development of the embryo. The number of embryos hatched at approximately 53 hpf was noted and the percentage of hatched embryos was calculated (Table 2). Hypoxia reduced the hatching rate but not significantly. Treatment with 1.5% ethanol reduced the hatching rate significantly ($p<0.05$). The hatching rate of embryos exposed to combined treatment of 1.5% ethanol and hypoxia was better than to treatment with ethanol alone, however, it was also significantly reduced compared to control ($p<0.05$). None of the embryos hatched with 2.5% ethanol treatment alone or with hypoxia.

Table 1: Percent viability of zebrafish embryos at 24 hpf, 48 hpf and 72 hpf following exposure to ethanol

	Control	Hypoxia	1.5% EtOH	1.5% EtOH + Hypoxia	2.5% EtOH	2.5% EtOH + Hypoxia
Percent viability at 24 hpf	93.75 ± 8.8	87.5 ± 17.6	100	100	68.75 ± 8.8	62.5 ± 17.6
Percent viability at 48 hpf	87.5 ± 17.6	87.5 ± 17.6	75	81.25 ± 8.8	37.5 ± 17.6	50
Percent viability at 72 hpf	87.5 ± 17.6	100	68.75 ± 8.8	68.75 ± 8.8	0	0

Table 2. Percentage of embryos hatched at 54 hpf following hypoxia and ethanol treatment

Control	Hypoxia	1.5% EtOH	1.5% EtOH + Hypoxia	2.5% EtOH	2.5% EtOH + Hypoxia
72.9 ±15.7	50 ±12.5	21 ±14	35.4 ± 13	0	0

Heart Rate and Motility:

Heart rate was measured as an indicator of developing cardiac function. The heart of zebrafish embryos is located anterior to the yolk sac and can be easily distinguished as embryos during the early stages are transparent.

Heart rate significantly decreased at the concentration of 2.5% ($p<0.01$) ethanol and 2.5% ethanol and hypoxia ($p<0.01$) **(Fig. 1).**

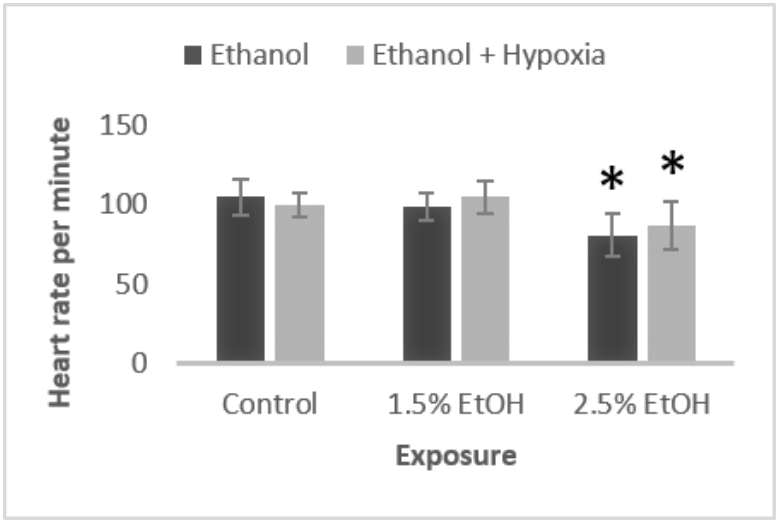

Fig 1: Mean heart rate (beats/minute) in embryos treated with hypoxia and varying doses of ethanol (*- p< 0.01).

Motility:

To determine the effect of these stressors on zebrafish motor development, a motility assay was performed. The embryos were gently touched at the tail at around 72 hpf. Normal embryos show some swimming behavior by this time and dart on being touched. The percentage of embryos that responded to touch was calculated.

Ethanol reduced the motility of zebrafish embryos in a concentration dependent manner. 87.61% and 46.87% in 1.5%, and 2.5% ethanol treated embryos respectively showed swimming or an instant darting behavior when touched at the tail. Hypoxia for 24 hours during early development did not affect motility of the embryos. However, only 30.20% of the embryos showed motility in 2.5% ethanol plus hypoxia treatments ($p<0.01$) suggesting severe impairment of either the motor circuitry or muscle development **(Fig. 2).**

Morphometric analysis:

Any abnormality during the early development causes an obvious change in the morphology of the embryo. In zebrafish

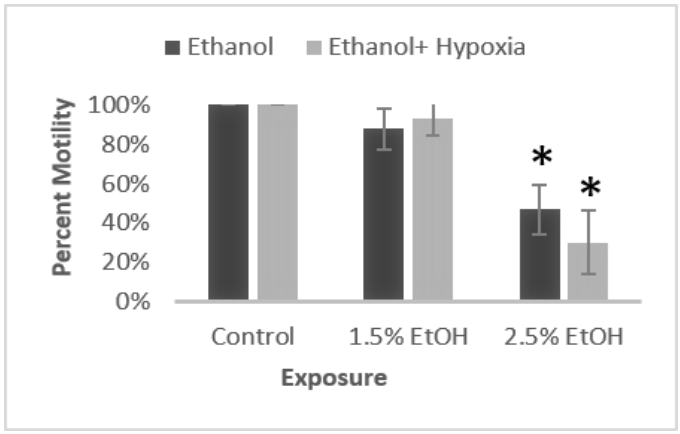

Fig 2: Percentage of the embryos that respond to touch in hypoxia and varying doses of ethanol (*-p<0.01).

very obvious morphological deformities are observed if the embryo is under stress. Here, we have looked for the body length, the head length, area of the eye and the area of the pericardial region indicating severity of oedema near the heart.

Fig. 3 shows images of the embryos following various treatments. Embryos exposed to hypoxia for 24 hours looked very similar to the control ones. 1.5% ethanol **(Fig. 3C)**, and 1.5% ethanol under hypoxia **(Fig. 3D)** showed mild oedema in the heart region. Embryos that were treated with 2.5% ethanol **(Fig. 3E)** and 2.5% ethanol under hypoxia **(Fig. 3F)** showed severe oedema in the heart region, stunted growth of the body, very obvious cyclopia and curling of the tail.

Body length, head length and head to body ratio:

The length of body **(Fig. 4a)** and head **(Fig. 4b)** both significantly decreased in the embryos exposed to 2.5% ethanol ($p<0.01$) and 2.5% ethanol with hypoxia ($p<0.01$). However, just hypoxia, 1.5% ethanol and 1.5% ethanol with hypoxia did not have an effect on this parameter.

The ratio of the head to body length was determined to ascertain if there was a differential effect on either of these parameters or that the head size was smaller due to decreased size of the entire embryo. This ratio was significantly higher in embryos treated with 2.5% ethanol with or without hypoxia suggesting that the body length is more affected than head length **(Fig. 4c).** Hypoxia treatment did not appear to have any further effect.

Eye area and area of the pericardial region:

Eye development is affected in FASD (Fernandes et al., 2018). In the zebrafish model too, the area of the eye significantly decreased in 1.5% ($p<0.01$) and even more in 2.5% ($p< 0.01$) ethanol treated embryos **(Fig. 5)**. Hypoxia also reduced the eye area of the embryos. Hypoxia, given along with both concentrations of ethanol, more or less mimicked results of that particular concentration of ethanol alone.

As pericardial oedema was observed, we measured the area of the pericardial region **(Fig. 6).** 1.5% ethanol did not cause a significant pericardial oedema. However, 1.5% ethanol with hypoxia showed a significant pericardial oedema both when compared to control ($p<0.01$) and when compared to 1.5% ethanol alone ($p<0.05$). 2.5% ethanol and 2.5% ethanol with hypoxia

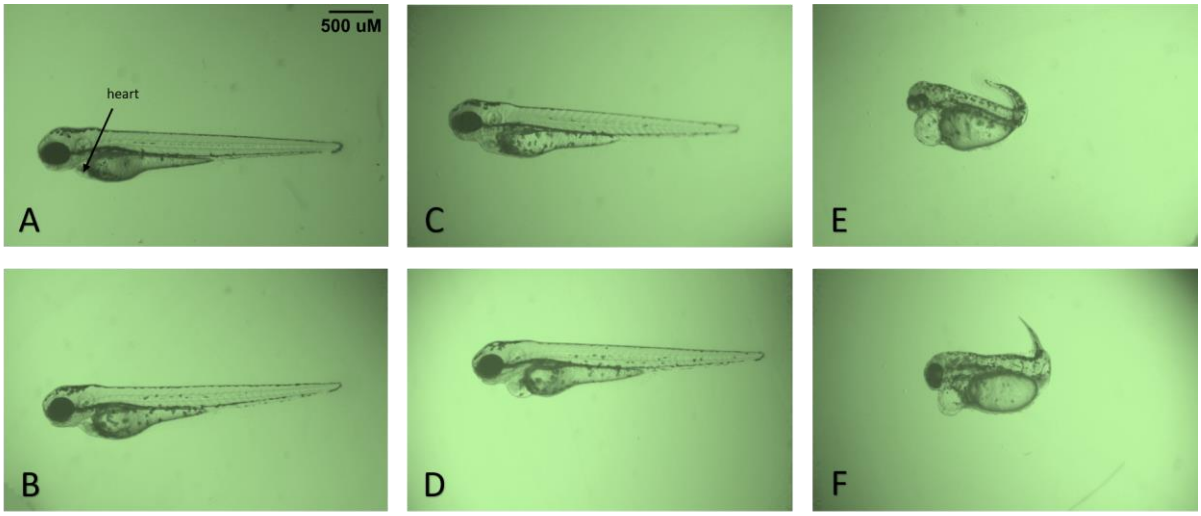

Fig 3: Images taken at 72 hpf under a stereo microscope. A- Control; B- Hypoxia; C- 1.5% Ethanol; D- 1.5% Ethanol+ Hypoxia (mild pericardial oedema); E- 2.5% Ethanol (severe pericardial edema and curling of the tail); F- 2.5% Ethanol + Hypoxia (severe pericardial oedema and curling of the tail)

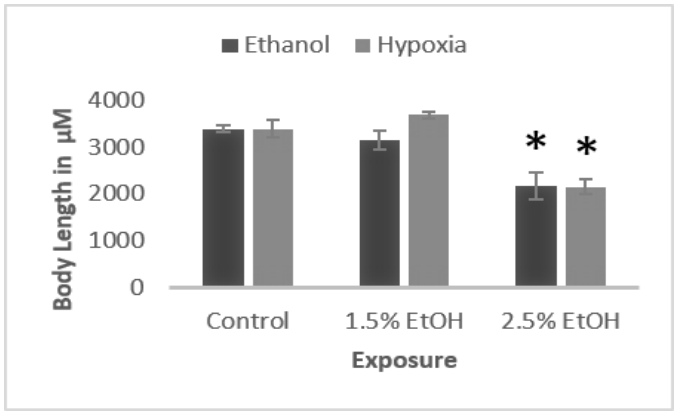

Fig 4a: Mean body length at 72 hpf after treatment with hypoxia and varying doses of ethanol (*- p< 0.01).

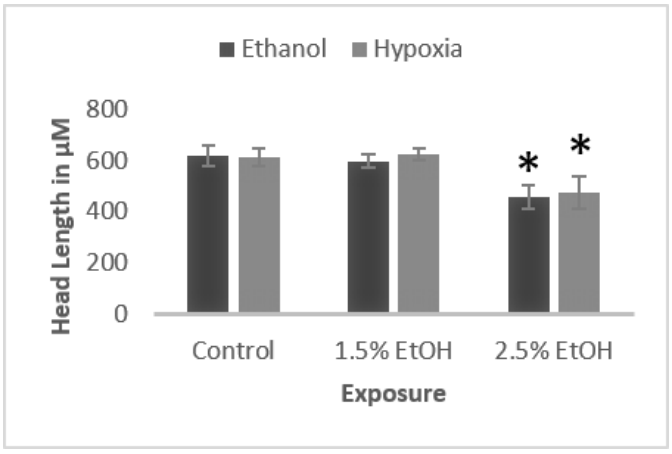

Fig 4b: Mean head length at 72 hpf after treatment with hypoxia and varying doses of ethanol (*- p< 0.01).

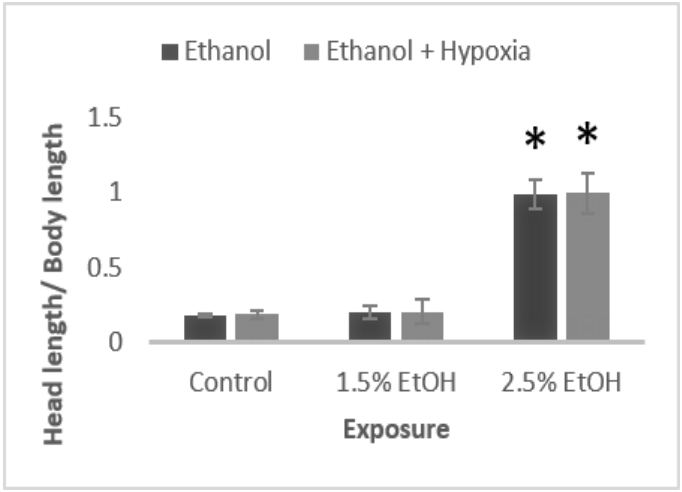

Fig 4c: Head to body ratio of embryos at 72 hpf after treatment with hypoxia and varying doses of ethanol (*- p< 0.01).

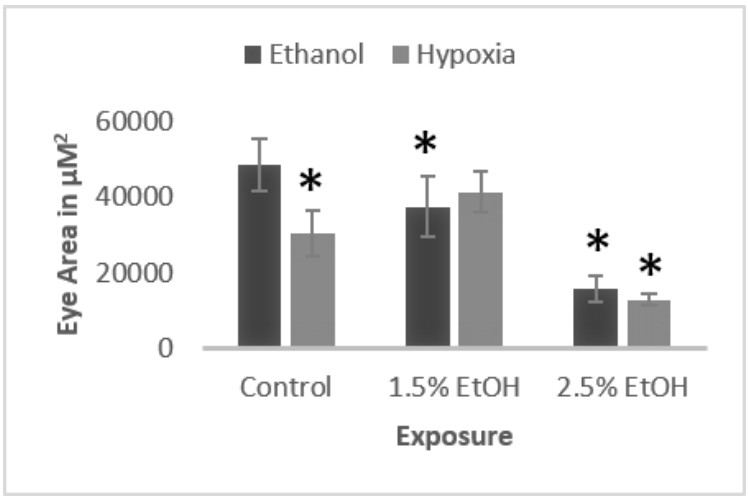

Fig 5: Mean area of the eye at 72 hpf after treatment with hypoxia and varying doses of ethanol (*- p< 0.01).

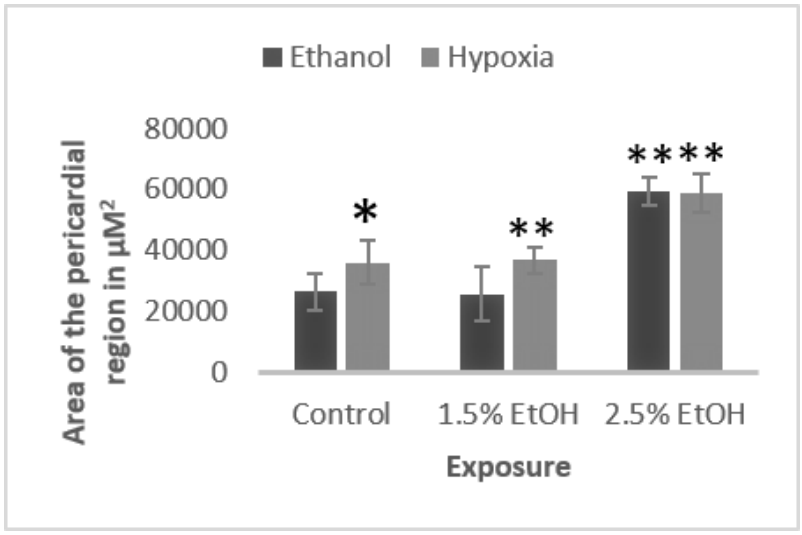

Fig 6: Mean area of the pericardial region at 72 hpf after treatment with hypoxia and varying doses of ethanol (- p< 0.01, *-p<0.05).**

treated embryos showed more severe oedema of the pericardium. However, there was no significant difference between the two.

Craniofacial development:

To study the developing craniofacial cartilage, embryos were stained with Alcian blue to reveal the cartilage pattern. The developing craniofacial cartilage consists of the ventral pharyngeal cartilage and dorsal neurocranial cartilage.

Ethanol (1.5%) exposure for 24 hours from 5 hpf did not have a significant effect on the development of craniofacium (data not shown). However, 2.5% ethanol exposure during the same time point drastically affected the anterior region of the craniofacial cartilage, reducing the width of the ethmoid plate in the neurocranium, and reducing the distance between the Meckel's and the ceratohyal cartilage in the branchial arch preparation **(Fig. 7c)**. Hypoxia in general did not affect the morphological pattern of the craniofacial cartilage **(Fig. 7b)**. However, 2.5% of ethanol followed by hypoxia further reduced the width of the ethmoid plate drastically, hence affecting

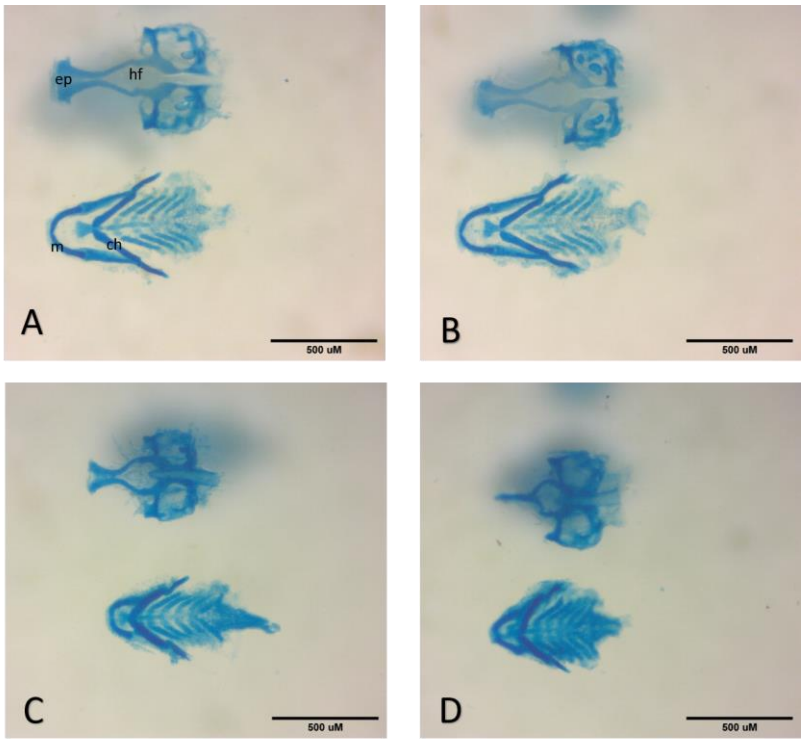

Fig 7: Neurocranial preparation (upper) and seven arch preparation (lower) of Alcian blue stained embryos at day 5. A- Control; B- 24 Hypoxia for 24 hours; C- 2.5 % Ethanol; D- 2.5% Ethanol + Hypoxia; Abbreviations: ep, ethmoid plate; hp, hypophyseal fenestra; m, Meckel's; ch, ceratohyal

the morphology of the hypophyseal fenestra **(Fig. 7d)**. The overall length of the neurocranium was significantly reduced in the 2.5% ethanol treatments **(Fig. 7c & 7d)** which was evident even when the external head length was measured.

Discussion

FASD, today is considered the most preventable developmental disorder, however, its occurrence remains high and can reach 2 to 5% in some regions (May et al., 2009). Understanding the pathology of ethanol toxicity and determining other contributing factors hence, is of paramount importance. The zebrafish embryo model used in this study has proved of immense value for understanding this disorder. Zebrafish model of FASD not only shows all the morphological manifestations observed in children born with FASD, but also permits study of more subtle defects such as in behaviour and cognition (Lovely et al., 2016). Further zebrafish shares 70% homology to the human genome (Araujo-Silva et al., 2018). Our current study confirms the occurrence of all the morphological defects observed in FAS in the zebrafish embryos treated with ethanol.

Several studies have reported that the manifestation of FASD is also dependent on other factors, one of which is hypoxia. Hence, we have used the zebrafish model to ascertain if a pretreatment with mild hypoxia alters the effect of ethanol on development. Further we have standardized a technique of giving hypoxia using a chemical method in zebrafish. Sodium sulphite gets converted to sodium sulphate in oxygen rich conditions (Jiang et al., 2011) thereby scavenging the dissolved oxygen in the media.

Of the two doses of ethanol used (ie. 1.5% and 2.5%) the lower dose of 1.5%, showed minimal effect on most of the parameters monitored and pre-exposure to hypoxia did

not show any further effect. However, 2.5% ethanol treatment caused severe abnormalities in all the parameters monitored. Though the ethanol did not directly affect viability upto 48 hpf, all embryos were dead by 72 hpf, with or without hypoxia. There was significant delay in hatching rate, at the higher concentration, indicative of abnormal overall development, or an abnormality in the motor functions. Morphological analysis, including measurement of body and head length, eye diameter and pericardial region all were indicative of gross defects in the overall development of the embryos exposed to 2.5% ethanol alone and with hypoxia. In all the parameters hypoxia had no further effect beyond that observed with ethanol.

Prenatal hypoxia is known to affect the development and behaviour of many organisms, which may later cause cognitive abnormalities and mental retardation (Nalivaeva et al., 2018). Also other studies using in vitro cultures have shown that hypoxia enhances the toxic effects of ethanol (Wang et al., 2013). However, in our model pre-exposure to hypoxia showed no adverse effect on the embryos, except for a mild reduction in hatching rate. There was also no synergistic effect with alcohol except for malformation of the ethmoid plate of the craniofacium when given along with 2.5% ethanol. On the other hand pre-exposure to hypoxia did not have any protective effect either, as reported for cortical granule neurons (Genetta et al., 2007) or during withdrawal from ethanol (Jung & Mallet, 2018). Our study has used a chemical method of inducing hypoxia by scavenging oxygen from the water, while most other studies report data from physical depletion of oxygen. It needs to be ascertained whether the two methods of depletion of oxygen result in the same level of hypoxia.

Motor development begins at an early stage in zebrafish. The nervous system in zebrafish begins to develop by 12 hpf (Schilling and Kimmel, 1994; Kimmel et al., 1995) and they exhibit swimming behavior in response to touch by 27 hpf (Saint-Amant & Drapeau, 1998). A normal swimming behavior is an indication of normal motor development in the brain and spinal cord (McKeown et al., 2009). The motor response, as determined by the darting behaviour in response to a gentle touch to the tail, was severely affected in 2.5% ethanol and further worsened in embryos exposed to hypoxia, suggesting abnormal development of the motor system as well.

Growth retardation and abnormalities of the craniofacial cartilage are hallmarks of FASD, both of which were observed in zebrafish exposed to 2.5% ethanol. Hypoxia did not have any further effect on the morphology. Interestingly 1.5% ethanol treatment showed no adverse effect on the morphology. Two signaling pathways important in early development are retinoic acid (RA) and sonic hedgehog (Shh). Other studies have shown that ethanol reduces RA production by inhibiting the dehydrogenases that form the active form of RA (Duester, 1998; Kot-Leibovich & Fainsod, 2009), and it has also been demonstrated that RA can partially rescue ethanol induced facial defects (Marrs et al., 2010). One of the targets that cross talks with RA is Shh; a reduction in Shh is known to cause apoptosis of cranial neural crest cells (Zhang et al., 2013), and would lead to abnormal craniofacial development. Cyclopia, another abnormality often seen following alcohol exposure, can also be explained by abnormal Shh signaling (Fernandes et al., 2018).

The craniofacial cartilage develops from cranial neural crest cells that begin to migrate around 12 hpf and they complete their differentiation and migration at 72 hpf (Schilling and Kimmel, 1994). Meckel's and ceratohyal cartilages are the first to chondrify at around 54 hpf. The five ceratobranchial cartilages chondrify between 60- 72 hpf (Javidan and Schilling, 2004). Ethanol-treated embryos showed an overall reduction in the size of the neurocranium and major malformations in the 1st and the 2nd branchial arches (Meckel's and the ceratohyal). In both the neurocranium and the pharyngeal arches the anterior region was more malformed than the posterior. Studies have shown that ethanol exposure during gastrulation affects not only the expression of RA but also has an impact on some hox gene expression (Kot-Leibovich and Fainsod, 2009). Hox genes

are important for craniofacial development (Yan et al., 1998).

Kuchler and his group have studied the effect of hypoxia on craniofacial development (Küchler et al., 2018). They reported that 30% to 50% hypoxia for 24 hours after 8 hpf resulted in clefts in the ethmoid plate which later results in cleft palate in children. In our experiments, however, we did not observe any clefts in the hypoxic embryos. Hypoxia did worsen the development of the ethmoid plate in the embryos treated with ethanol.

Our study clearly establishes zebrafish as a good model to study FASD related pathology and investigate the underlying molecular mechanisms. We did not observe a direct dose dependent response as 1.5% ethanol showed no obvious effects while 2.5% showed gross abnormality of development with all the hallmarks of FASD related morphology. Similar lack of concentration dependence had been reported earlier (Carvan et al., 2004). In addition, several studies have shown that the effect may vary depending on the strain of fish used (Fernandes et al., 2018). Further studies will be conducted to determine if ethanol and /or hypoxia toxicity could be due to cellular death or is due to disruption of signalling pathways.

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Conflict of interest

The authors declare no conflict of interest.

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Research article

Does colour preference change with age? – A preliminary report

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Abstract

Colours are a significant part of our life. Interestingly, colours influence our mood in ways we do not realise, even through the smallest of things. The fact that a single colour can have a meaning or interpretation, differing from person to person, makes it fascinating to study. Thus, colour psychology is the study of how colours influence our ideas and feelings. Colours, consciously or unconsciously, can evoke emotions, inspire reactions, and alter our thought process. This study was survey-based, carried out for individuals over 15 years of age. The survey questions revealed how the warm and cool colours were differently perceived by individuals. The survey had 127 respondents, of which, 71 individuals were between 15-20 years and 56 individuals were above 20 years. It was observed that individuals had varied choices and moods for warm and cool colours.

Introduction

Colours have a powerful influence on people, without them even realising it. Colours are everywhere, changing our perception of every single thing. The colours that surround us in our daily lives, according to various studies, have a significant effect on our mood and behaviour (Kurt & Osueke, 2014). Perhaps, it is no longer surprising that the colour of food influences how it tastes, the colour of your room influences how you feel, and the colour of your clothes influences how appealing you are to others. It can be used to "balance" emotions or to create diverse moods (Rizomyliotis et al., 2018).

Moreover, each colour is denoted by a particular wavelength. The spectral sensitivities of the light receptors interact with the spectrum of light in the eye, resulting in colours (Elliot & Maier, 2014). When light strikes a coloured item, it absorbs only those wavelengths that fit its atomic structure perfectly and reflects the rest to the viewer. The human eye can perceive a narrow band of radiation ranging from 380 nm (red) to 760 nm (violet) in wavelength. Within this range, wavelengths of light striking our eyes are transmitted to our brain, particularly the hypothalamus, in the form of electrical impulses (Kurt & Osueke, 2014). Colour is one of the most powerful variables for influencing how one expresses one's emotions (Valdez &Mehrabian, 2020).

Each colour may denote a different mood. Often, the setting in which people express

their feelings is strongly impacted by the colour given to that setting (O'Connor, 2011). Additionally, colours can even influence our way of advertising, stage design, and costuming (Kumar et al., 2013). Colours are known to offer a wide range of emotional states, including warmth, strength and weakness, hardness and softness, and activity and calmness (Birren, 2006). Many colours in nature may be exciting; however, a favourite colour does not guarantee that the colour has the potential to calm them or stimulate positive emotions (Cherry, 2020). Colours create a different impact depending on whether they are deemed warm or cool (Kwallek et al., 1988). Too much of a warm colour can be unpleasant or aggravating. Contrastingly, cool colours are more commonly associated with creating a quiet and relaxing environment in daily life. Blue and green are thought to be the most pleasant, tranquil, and relaxing colours (Cherry, 2020).

The objective of this survey was to check whether there is a significant association between the choice of colours (warm/cool) and teen age (below/above 20 years).

Methodology

This survey was conducted on individuals categorised into 2 age groups: 15- 20 years and above 20 years. A total of 127 respondents,71 teenagers (15-20 years) and 56 adults (above 20 years), answered the survey. The aim was to find out how individuals select their colour preferences – whether warm or cool colours - and associate it to an emotional state of mind. This survey was conducted online using google forms which were circulated on various social media platforms. Chi-square test was used to analyse the date, with a p value less than 0.05 indicating statistically significant.

Results

Colours can influence the feelings of happiness or unhappiness, which is based on the impact the colour creates on an individual's emotion (Elliot, 2015). Perhaps, respondents were asked about their preferred colours and how those colours affect their mood. It was observed that 36 respondents chose warm colours and 91 chose cool colours. The observations have been tabulated and represented using a segmented percentage column graph below.

As mentioned above, colours can be interpreted differently from individual to individual. Thus, the table below gives an account of the interpretation by the respondents to some of the common colours we come across in our daily lives

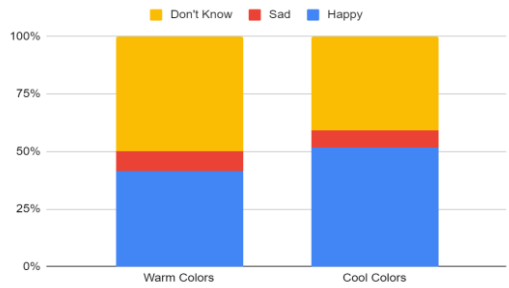

Fig 1: Graph for the favoured colour versus respondents' mood

Table 1. The colours participants favour and their mood

Type of colours	Happy	Sad	Don't Know
Warm colours	15(41.7%)	3(8.3%)	18(50%)
Cool colours	47(51.6%)	7(7.7%)	3(3.3%)

Table 2. Participants opinion on a given colours

Colours	Meaning of the colours(According to participants' responses)
Red	Love (69), Energy(26)
Blue	Calm (51), Loyal (30)
Green	Freshness (54), Calm (27)
Yellow	Happy (31), Warm (23)
Black	Confident (48), Luxury(41)
White	Pure (46), Calm (46)
Brown	Rugged (41), Warm(19)
Orange	Warm (34), Energy (27)
Purple	Royal (36), Luxury (30)

Table 3. Chi-square test

Age of the respondents	Colours they favour	
	Warm Colours	**Cool Colours**
Below 20	21	50
Above 20	15	41

p value = 0.7286

Discussion

It is crucial to remember that colour preferences are subjective; what makes one person happy may make another person sad, based on their previous experiences. Colours and emotions are inextricably linked; therefore, we must consider their consequences (Elliot, 2015). Based on Table 1, it can be said that individuals do have a preference for warm or cool colours and this also tends to link to a particular state of mind. It is very evident that individuals prefer cooler colours than warm ones. Moreover, not all individuals feel alike seeing a particular warm or cool colour and this distinct interpretation can be observed through Fig. 1, where in the graph shows the variation.

On the other hand, Table 2, gives an account of the different interpretations individuals had for the different colours. Some of the common colours were selected at random and respondents selected what that particular colour meant to them. These results can be compared to several previously done studies where it has been proposed that each colour can have more than one symbolic meaning (Elliot, 2015; Cherry, 2020)

To understand whether age impacted the colour preference, a chi-square test was carried out. There was no significant difference observed between the colour preference and age groups. This is because a wide age group was considered for two broad categories of warm and cool colours. Interestingly, other studies have proven a significant difference between age and colour preference for certain specific colours like red and green while no significant difference for the colour blue (Dittmar, 2001). Hence, further work on this aspect needs to be done, with more precise colour selection and age groups with a larger sample size.

Conclusion

Colours do have a vital part in expressing information, establishing emotions, and even influencing people's decisions. Colours have been shown to have psychological impacts in two ways: warm and cool. Nevertheless, warm and cool colours tend to create a different sense of feeling for different individuals. With respect to age, colour preference does play a role, but for specific colours only. Further work is required to explore how these colours influence the emotions/feelings and how these preferences change with age. Colour choices also impact the items individuals buy, the clothes they wear, and the way they organize their surroundings. Thus, future work can be done by studying colour choices with different parameters like gender and occupation, along with the age.

Conflict of interest

The authors declare no conflict of interest.

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Review article

Peto's paradox and cancer resistance: A mini review

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Abstract

The mechanisms that reduce the accumulation of genetic damage and subsequently the uncontrolled proliferation of somatic cells in multicellular organisms remains unknown. Theoretically, larger animals should have more chance of getting cancer because of their larger size (number of cells) and greater life expectancy (number of cell divisions) as there are more chances for accumulation of mutagenic cells resulting in malignant transformation. Cancer risk should increase with species lifespan and body size if all mammalian cells are equally susceptible to oncogenic mutations but it is not observed and Peto's paradox explains this phenomenon. Hence it is important to identify different mechanisms for cancer resistance in larger animals which can be helpful in human cancer prevention.

Introduction

The occurrence of cancer is postulated to be related to the number of cells an organism possesses. If each cell of the body has the probability of being cancerous, then larger animals should have more risk of cancer than the smaller ones as more cell division means more chance of mutations (Brown et al., 2015). Peto's paradox states that the occurrence of cancer is not related to the number of cells and lifespan of the organism (Caulin & Maley, 2011). Larger mammals like elephants and whales show more cancer resistance despite their bigger body size and longer life span (Caulin et al., 2015). The natural mechanism of cancer resistance in such organisms can help us find a solution to reduce the occurrence of cancer in humans.

Mechanism of cancer suppression in larger animals:

The metabolic rates are said to be proportional to the frequency of cancer. The metabolites and the by-products of the metabolic pathway can cause mutation and can alter the genes. Studies have shown that metabolic rates are higher in small animals and hence the chances of mutations are higher (Dang, 2015).

Leukemia inhibitory factor (LIF6) is a gene in mammals that induces apoptosis of cancerous cells. Recent research on elephants has shown that LIF6 has reactivated in them. Previously, the LIF6 gene was not functional but over the years of evolution, elephants have evolved to make it functionalized. This helps them to either

arrest or eliminate tumor cells to attain their adult sizes (Vazquez et al., 2018). LIF6 acts in response to genetic errors and DNA damage and destroys the cell that can be cancerous through apoptosis. TP53 is a tumor suppressor gene that prevents oncogenic mutations and destroys cancerous cells. One copy, that is two alleles of the TP53 gene, is present in humans, whereas elephants have multiple alleles of the same (Abegglen et al., 2015). Inactivation of this gene can lead to uncontrolled cell division leading to malignancy. The absence of the two alleles can lead to cancer development. Peto made observations that the elephants would have naturally evolved to suppress cancer. Moreover, the TP53 has been amplified in them with greater apoptotic response. From birth to being an adult, younger elephants grow in less than 10 years which is an approximately 30-fold increase in their cellular mass and a gain of 1kg weight per day. Such a high rate of cellular division and expansion in the growing elephant requires an especially efficient system of cancer prevention. But discovering these mechanisms has been challenging because it requires an ideal study system in which a large, long-lived species is deeply nested within a monophyletic group of smaller, short-lived species, all of which have sequenced genomes. Since larger bodies might have evolved many times hence not many lineages have the same pattern and might have evolved a different mechanism to prevent cancer (Vazquez et al., 2018). The evolutionary mechanism for this phenomenon is studied only on mice. Mice have a lifespan of a year hence they do not need a cancer-suppressing mechanism that might be required in long-lived species. Whereas elephants, which have a decades-long life expectancy and more cells, need to avoid cancer to remain reproductively fit.

Conclusion

Elephants appear to have lower cancer rates compared to other mammalian species due to the presence of multiple copies of TP53. Compared with human cells, elephant cells show an increased apoptotic response to DNA damage. Also, larger animals might have improved immune surveillance which can eliminate cancer cells even before turning malignant. It is important to identify an evolutionary-based approach for understanding mechanisms related to cancer suppression in humans. The cancer suppression mechanism, if studied from such large organisms, could be used to prevent cancer in humans even before it begins rather than treatment after it has been detected and could help to eradicate cancer. Evolution has been modifying cancer suppression mechanisms for billions of years while we humans have started looking at it (Caulin & Maley, 2011). Further in-depth studies of the mechanism of cancer suppression in large and long-lived animals might help reduce the incidences of cancers in humans.

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Review article

Cardiovascular Disease - a Comorbidity in COVID-19: A Mini Review

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Abstract

SARS-CoV-2 is a risk factor for the development of myocarditis, pericarditis, myocardial infarction [MI], arrhythmias, and an aberrant coagulation mechanism. The binding location for the viral spike (S) protein of SARS-CoV-2 is the angiotensin-converting enzyme 2-(ACE2) surface receptors of endothelial cells of multiple organs, that are implicated in the replication process of SARS-CoV-2. There is a higher fatality rate among individuals with cardiovascular diseases due to SARS-CoV-2, maybe because of the virus' affinity for ACE2 and the vulnerability of such individuals. In this mini review, we have discussed the risks of how individuals with cardiovascular diseases are more susceptible to Covid-19 sequelae and conversely how SarsCoV2 can lead to cardiac damage.

Introduction

Heart diseases, often known as cardiovascular disease (CVDs), involves the following conditions: coronary artery disease (CAD), also known as coronary heart disease (CHD), peripheral artery disease (PAD), and aortic atherosclerosis.

Viral infections are dependent on viral infiltration into the host cell, where the virus exploits the host's cellular machinery to generate numerous viral copies, which are then shed by the host cell.

It is now known that coronaviruses such as SARS-CoV-2 and SARS-CoV-1 employ the host protein angiotensin-converting enzyme-2 (ACE2, EC 3.4.17.23) as a coreceptor to enter cells. (Chappell, 2016; Chappell et al., 2014). SARS-CoV-2 is a positive-sense, single-stranded RNA virus. Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. SARS-CoV2 attaches to host cells via its spike protein and the angiotensin-converting enzyme 2 (ACE2) as a membrane fusion receptor. Two functional subunits make up the spike protein. The receptor-binding region of the S1 subunit binds to ACE2, while the S2 subunit is responsible for fusion and entrance into the host cell. (Huang et al., 2020). Only after the viral invasion of the lungs the virus is able to penetrate the heart; the indirect damage mechanism also commences due to the induction of immune responses during viral invasion of the lungs. (Wang et al., 2020). Based on single-cell RNA sequencing, more than 7.5% of cardiac cells express ACE2, which might facilitate SARS-CoV-2 entrance into cardiomyocytes and induce direct cardiotoxicity.

SARS-CoV2 and its connection to cardiovascular diseases:

The presence of ACE-2 receptors on cardiac muscle cells explains the high risk of COVID-19 infection in pre-existing CVD patients. This implies that the cardiovascular system may be involved in SARS-CoV-2 infection. Patients with cardiovascular disease are more likely to develop acute coronary syndrome during an infection. This syndrome causes myocardial rate to increase, resulting in myocardial damage or infarction. Furthermore, in COVID-19 cases, an elevated rate of inflammatory cytokines mediates atherosclerosis, procoagulant activation, and hemodynamic instability, resulting in ischemia and thrombosis. COVID-19 patients with cardiovascular comorbidities require urgent treatment to decrease morbidity and mortality (Ejaz et al., 2020).

A recent study found that individuals with CVD, diabetes, and hypertension were more likely to require ICU admissions among 1527 COVID-19-infected patients (Li et al., 2020). A five-fold increase in the death rate in individuals with previous CVD problems compared to patients without CVD was observed using large COVID-19 patient data from China (10.5% vs. 2.3 %) (Zhou et al., 2020). Multiple studies have found that patients with previous CVD have a higher risk of mortality.

According to a meta-analysis encompassing over 6000 COVID-19 patients, the risk of cardiac injury varies from 15% to 42% depending on age and disease severity (Schultheiss et al., 2021). The rate of positive SARS-CoV-2 RNA detection in the heart was 61.5 percent in 39 patients who died as a result of coronavirus infection, according to post-mortem analysis of cardiac tissue from 39 patients who died as a result of coronavirus infection (Lindner et al., 2020). Furthermore, a study involving 40 SARS-CoV-2 positive individuals identified a connection between COVID-19 infection and immediate cardiac injury. Another post-mortem examination of nine COVID-19 patients who died of cardiogenic shock revealed that all compartments of the heart were involved, including intramural arteries, conduction tissue, and the subepicardial ganglia. There is substantial evidence that the SARS-CoV-2 S protein interacts directly with TLR4 in myocardium, activating the TLR4 signaling cascade (which includes pro-inflammatory cytokines and type I interferons) and even up-regulating ACE2 surface expression (Aboudounya & Heads, 2021).

Acute cardiac injury

Acute cardiac injury is characterised by a rise in cardiac troponin levels, with or without ejection fraction loss or electrocardiographic abnormalities. It was detected in 10–23% of COVID-19 patients, with a higher prevalence among intensive care unit (ICU) patients (22.2%) vs non-ICU patients (2.0%) and non-survivors (59% vs 1%) (Zhou et al., 2020). Patients with acute cardiac injury showed higher levels of C-reactive protein (CRP), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and creatinine, as well as more multiple mottling and ground-glass opacity, and were more likely to need noninvasive or invasive ventilation. Acute cardiac injury was also connected to cardiac dysfunction and malignant arrhythmias. Prior to and after the onset of symptoms, individuals with acute cardiac damage had a considerably higher mortality risk. Both the magnitude and frequency of cardiac troponin increases were associated with an increased mortality risk. ACE2 may be involved in the mechanism of acute myocardial damage caused by SARS-CoV-2 infection. Other suggested mechanisms of cardiac injury include a cytokine storm caused by an unbalanced response between type 1 and type 2 T helper cells, as well as respiratory failure and hypoxemia triggered by COVID-19 that harm myocardial cells (Huang et al., 2020; Wong et al., 2004). The mechanism of acute myocardial injury triggered by SARS-CoV-2 infection could involve ACE2. Other hypothesized pathways of myocardial damage include a cytokine storm created by an imbalanced response between type 1 and type 2 T helper cells, as well as respiratory dysfunction and hypoxemia induced by COVID-19, which injure myocardial cells. (Huang et al., 2020; Wong et al., 2004). More than 7.5% of myocardial cells have positive ACE2 expression, based on single-cell RNA sequencing,14 which could mediate SARS-CoV-2 entry into cardiomyocytes and cause direct cardiotoxicity.

Acute myocardial infarction

Type 1 of myocardial infarction happens when an atherosclerotic plaque ruptures, causing thrombosis, while type 2 occurs when an acute illness causes tachyarrhythmia, hypoxia, or hypotension without atherosclerotic plaque rupture. It is possible that a pro-inflammatory condition in individuals with COVID-19 may lead to the instability of a coronary atherosclerotic plaque, a process previously documented during influenza outbreaks (Bonow et al., 2020). In the event of acute respiratory insufficiency, Type 2 AMI may occure due to the mismatch between decreased oxygen supply and increased myocardial oxygen demand as a result of sympathetic activation, tachycardia, hypotension, and hypoxemia (Schiavone et al., 2020). In a COVID-19 electrocardiographic study, newly diagnosed AMI was reported in 5.3% of cases, and in 2.9 % in another echocardiography research (Li et al., 2020). ST-elevation myocardial infarction (STEMI) might be the first symptom of COVID-19, and 33.3–39.3 % of COVID-19 patients who had STEMI were found to have non-obstructive coronary artery disease (Stefanini et al., 2020). This finding suggested that COVID-19 may be linked to both endothelial dysfunction and the hypercoagulable condition.

Conclusion and Discussion:

As a result of SARS-CoV2 infections, ACE2 levels are affected, which may lead to cardiovascular disease. More than 7.5% of myocardial cells have positive ACE2 expression, based on single-cell RNA sequencing, which could mediate SARS-CoV-2 entry into cardiomyocytes and cause direct cardiotoxicity. It is not yet clear if SARS-CoV-2 directly affects the cardiovascular system by targeting the ACE2-expressing cells. Individuals having a history of cardiovascular disease already have an increased chance of developing COVID-19 severity, which might lead to worse clinical outcomes. As a result, those with cardiovascular disease (CVD) account for the majority of fatalities from COVID-19.

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Review article

Toxic effects of DEHP used as Plasticizer

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Abstract

Due to increase in the production and use of plastic everywhere, significant increase in plasticizer concentration has found its way into the ecosystem. The most commonly used plasticizers are esters of phthalates, adipates and citrates. Among phthalates, Di-2-ethylhexyl phthalate (DEHP) is one of the extensively used plasticizer in furniture, cosmetics, medical devices, etc. DEHP is bound non-covalently to plastics and hence leaches out from different products into the environment after repeated over use. Industrial settings and plastic waste disposal sites majorly act as sources through which it enters the soil and aquatic ecosystems. It can enter the body through inhalation, ingestion, and dermal contact on a daily basis, which has raised concerns about its safety and its potential effects on human health as well as the ecosystem. The primary objective of this review is to highlight the toxic effects of DEHP on vital organs and also on endocrine and reproductive system.

Introduction

Advances in polymer science and technology have led to the development of a variety of manmade materials such as plastics. 'Plastic' is the term commonly used to describe a wide range of synthetic or semi-synthetic materials that are utilized in huge and ever-expanding range of applications. Worldwide it is seen that the production and application of plastic is burgeoning over the last few years. Further, theflexibility and utility of plastics are usually ameliorated with the addition of several chemicals to it (Cadogan, 1991). These chemicals are collectively known as additives. Of these, 'Plasticizers' are one of the most important additives. These are low molecular weight polymers which lead to an increase in the spacing between chains of various crystalline polymers. The most commonly used plasticizers are esters of phthalates, adipates, citrates, etc., majority of which are used in polyvinyl chloride (PVC) products.

Plasticizers are also added to the products in cosmetic and personal care industry to enhance the staying capacity of scented products like lotions, body washes and shampoos, to increase clinging properties of hair styling products, nail polish and to promote skin penetration (Walsh, 2019).

Plasticizers tend to egress from several products, thus escaping into the environment and in turn finding their way into the food chain (Erythropel et al., 2014). The Environmental Working Group (EWG) in their 2002 report found phthalates in three fourths of products ranging from deodorants, nail polish, hairgels, lotions, perfumes, etc.

(Houlihanet al., 2002). The widespread and pervasive use of plasticizers has made them an ideal candidate for extensive research on the toxic nature of these products.

Phthalates as Plasticizers: Phthalates are esters of phthalic acid commonly used as plasticizers, solvents and additives in many consumer products (Frederiksen et al., 2007). They are produced in high volumes and used in many products that we consume in our day-to-day life. Phthalates can get accumulated in the fat tissues after easy absorption in the blood (Walsh, 2019). Public concerns on phthalates distribution in the environment have been increasing because of their ability to cause liver cancer, structural and developmental abnormalities and reduced sperm count in males (Bauer & Herrmann, 1997). Male and female reproductive systems are more susceptible to phthalates. It has been seen that compared to urine, human milk contains relatively higher percentage of hydrophobic phthalate (Frederiksen et al., 2007). Hence exposure of the human foetus and infants to phthalates via maternal exposure is a rising matter of concern.

Because of major concern around paediatric exposure recent research has received considerable attention to two phthalates namely, Di-(2-ethylhexyl) phthalate (DEHP) and Di-isononyl phthalate (DINP). Like all the phthalates these two phthalates are ubiquitous contaminants in air, food, water bodies, soil and sediments.

Di-(2-ethylhexyl) phthalate (DEHP) used as a Plasticizer: DEHP which is also known as bis(2-ethylhexyl) phthalate or dioctyl phthalate (DOP) is the most common member of the family of phthalates having molecular formula $C_{24}H_{38}O_4$ and structural formula as shown in fig. 1. This colorless to pale yellow oily liquid is produced at an annual rate touching more than 2 million tons across the globe (Rowdhwal & Chen, 2018) and is outrageously used for its plasticizing properties and low cost.

DEHP is found everywhere in the environment and is a key pollutant in many countries. It is universally considered to be an omnipresent environmental contaminant because it is the most comprehensively used plasticizer belonging to the group of phthalates. It is mainly used in PVC formulation for its wide range of applications including medical devices, cosmetics, personal care products, clothing, furniture and other home appliances, car products, etc. (Koo & Lee, 2004).

Release of DEHP causing Environmental Hazard

DEHP fails to tightly bind to the polymers of plastic and hence can easily enter into the environment at the time of production, transport, storage, use and disposal. Sewage sludge and solid waste disposal sites are the primary sources of release of this phthalate into the air and water bodies (Li et al., 2018). When DEHP is released into soil, it generally attaches strongly to soil particles and does not move very distant from where it has been released. When DEHP finds its way into water, it dissolves at a slow pace into underground or surface water which comes in contact with it. DEHP in air binds to dust particles and can be carried backtrack to earth through gravity and rain or snow. Indoor releases of DEHP to the air from plastic materials, coatings, and flooring in home and work environments, although small, can cause higher indoor levels than are found in the outdoor air (Rowdhwal & Chen, 2018).

According to the results of standard biodegradation test, it is not readily biodegradable and hence tends to be more harmful to the environment (Li et al., 2018). DEHP is probably the only substance whose ubiquitous occurrence in the environment makes it one of the most nefarious substances on our planet. As per estimates it has been concluded that essentially all of the DEHP manufactured in a given year will become a part of the environment within succeeding 20 years (Meeker et al., 2009).

Since DEHP can easily find its way into the environment, it can readily enter the body through inhalation, ingestion and dermal contact. According to epidemiological studies carried out in different organisms it is found that DEHP can be found in meat and lipid-rich products such as fats and dairy products at higher concentrations (Serrano et al., 2014; Rowdhwal & Chen, 2018). One can be exposed to DEHP through drinking water. When people consume water

from a well located near a landfill or waste site, they may get exposed to its higher-than average levels. In little amounts, it can be transferred by skin contact with plastic clothing or other articles. Since plastic articles of clothing do not have direct contact with skin, exposure via this route is likely to be low and transfer is probably minimal even if they do touch to the body. However, it can enter the body during certain medical procedures. Blood products that are stored in plastic bags and used for transfusions, flexible tubing used to administer fluids or medication can transfer DEHP to the patient. The plastic tubing used for kidney dialysis and plastic tubing of respirators often contain this phthalate (Roslev et al., 1998; Rowdhwal & Chen, 2018). This has, therefore, raised some concerns about its safety and potential effects on human health.

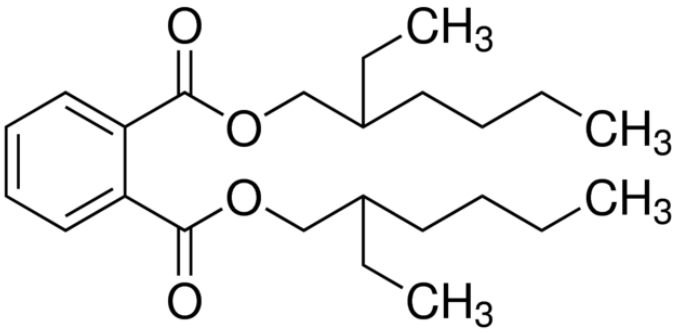

Fig 1: Di-(2-ethylhexyl)-phthalate (DEHP)
(Source – PubChem)

Elimination of DEHP from the Body

Half-life of DEHP in humans is about 2 hours. About 50% of the initial dose is eliminated in urine after 44 hours in the form of MEHP (Koch et al., 2004). Much of ingested DEHP leaves the body in the faeces. Humans and non-human primates excrete DEHP metabolites principally in the form of glucuronidated conjugates, while mice, guinea pigs, and hamsters excrete smaller amount of these conjugates, and none of the metabolites in rat urine have been found to be conjugated (Rusyn et al., 2006).

Hepatic toxicity caused due to DEHP

Liver serves as a vital organ for detoxification and hence is the main target organ for DEHP toxicity. Liver contains the highest amount of DEHP and its metabolites under the condition of repeated exposure (Rhodes et al., 1986; Rusyn et al., 2006). Both Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are liver specific enzymes. A significant increase in liver weight, and aminotransferase levels have been reported after DEHP and other phthalates exposure in laboratory animals (Shi et al., 2020; Mo et al., 2019). Hepatomegaly and hepatocellular tumors have also been observed in different laboratory animals (Talsness et al., 2009). The modes of action of DEHP in hepatocytes include - proliferation of peroxisomes and induction of peroxisomal proteins, induction of non-peroxisomal metabolism proteins, induction of cell proliferation, suppression of apoptosis, production of reactive oxygen species, oxidative DNA damage, etc. (Rusyn et al., 2006).

Hepatotoxicity results in oxidative stress thereby causing the disturbance in the balance between the production of reactive oxygen species (ROS) (free radicals) and antioxidant defences. It has been found that DEHP upsets oxidant–antioxidant balance in many laboratory animals (Hamid et al., 2020; Ustundag et al., 2017). Build-up of ROS, inhibition of superoxide dismutase (SOD) activation in cells and increase in catalase activity in liver has been observed in experimental animals (Lu et al., 2021; Reddy et al., 1976; Reubsaet et al., 1991).

Neurotoxic effects of DEHP

Exposure to DEHP can affect the brain due to its toxic effects on neurodevelopment, especially due to teratogenic anomalies in

foetal brain development. It is mainly because DEHP can cross the placenta and enter the foetal circulation. Further, its prenatal exposure impairs neurobehavior (Barakat et al., 2018). Harmful effects on laboratory animals' brain development and function have been found due to gestational and postnatal DEHP exposure (Lin et al., 2015; Xu et al., 2007). The neurotoxicity of DEHP has also been observed in a nematode, *Caenorhabditis elegans*. Its exposure can also result in an accumulation of ROS intracellularly, which causes neurotoxic effect due to oxidative stress (Wojtowicz et al., 2018).

Toxicity of DEHP in Other Organs

The toxic effects have also been seen on kidney, heart, gills, and immune system. Nephrotoxicity has been reported in lab animals when exposed to DEHP, with aggravated pathological changes leading to chronic progressive nephropathy (David et al., 2000). It has been seen that patients receiving long-term dialysis may acquire Polycystic Kidney Disease (PKD) secondarily from their exposure to chemicals leached from artificial kidneys and plastic materials used in dialysis equipment (Crocker et al., 1988). Decrease in heart rate and prolongation of PR and QT intervals have been observed in the experimental animals on exposure. It is found to induce micronuclei frequency in gill cells and decreased sodium, potassium, chloride levels in gills of different fishes. Leydig cell tumors and leukemia have been detected in rodents (Talsness et al.,2009).

DEHP as an Endocrine Disruptor

DEHP is an exogenous substance that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny, or (sub)populations and hence is best known as an endocrine disruptor (Grindler et al., 2018). Declined T4 levels and histopathological thyroid changes have been seen in the lab animals exposed to DEHP (Hinton et al., 1986). Studies on exposed zebrafish larvae have shown that DEHP alters the levels of thyroid hormones and affects its synthesis, regulation, and action (Zhai, et al., 2014; Jia et al., 2016).

Kim et al., (2013) conducted a research study on elderly people to check the effects of DEHP on insulin activity. The researchers recruited 560 elderly participants, and obtained blood and urine samples during repeated medical examinations. For the determination of phthalate exposure, urinary levels of mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) as metabolites of DEHP and Malondialdehyde (MDA), an oxidative stress biomarker, were measured in urine samples. They also measured serum levels of fasting glucose and insulin, and derived the homeostatic model assessment (HOMA) index to assess Insulin Resistance (IR). A mixed-effect model and penalized regression spline were used to estimate the associations among phthalate metabolites, MDA, and IR. From the results it was seen that oxidative stress leads to an increase in insulin resistance after DEHP exposure in elderly people.

Reproductive toxicity due to DEHP

According to several reports, DEHP has an adverse impact on reproduction followed by developmental abnormalities in many animal models (Oehlmann et al., 2009). It is found to bio-accumulate in aquatic organisms and is highly toxic, with an LC_{50} of 0.50 ppm, and consequently leads to embryo mortality and typical toxicity symptoms, such as necrosis, hepatomegaly, cardiac oedema, tail curvature, in zebrafish (Kwan et al., 2021).

DEHP which is a man-made or artificial estrogen mimicking chemical, also known as xenoestrogen, has been reported to create infertility issues in both males and females as it confuses body's natural estrogen receptors (Carnevali et al., 2010). Research in mammalian model animals indicates that it can interfere with male endocrine functions and sexual development, by causing testicular toxicity which can lead to testicular atrophy, reduced epididymal sperm density and motility, and increased numbers of abnormal sperm (Agarwal et al., 1986; Rowdhwal & Chen, 2018). Embryonic exposure to DEHP in lab animals has been found to disrupt testicular germ cell disorganization and impaired spermatogonial stem cells of the progeny of multiple generations (Doyle et al., 2013).

Ovaries can become easy target through the monoethylhexyl phthalate (MEHP) which is the primary metabolite of DEHP. The abnormalities in ovaries can cause irregular estrogen secretions, absence of ovulation, and sterility. DEHP can enhance estrogenic activity (Rowdhwal & Chen 2018). Further, it has been found that it induces oxidative stress which can lead to follicular atresia (Agarwal et al., 2005; Wang et al., 2012). Low doses of DEHP can alter estrous cycle and interfere with normal reproductive functions in mice (Hannon et al., 2014). An increase in ROS generation and a resultant decreased expression of glutathione peroxidase in human endometrial stromal cells has been detected due to its exposure (Cho et al., 2015). Oral administration of DEHP and its metabolite MEHP has been shown to have adverse impacts on oocyte meiotic maturation and development *in vivo* (Absalan et al., 2016). A study to evaluate the possible association between phthalate esters including DEHP and occurrence of endometriosis has shown that there is a positive correlation between the exposure concentration and severity of endometriosis is strong (Reddy et al., 2006). *In vitro* treatment of human endometrial cells with DEHP has been shown to increased ROS, thereby leading to increases oxidative stress. It has been found that DEHP-associated endometrial stromal cell alterations may be associated with the progression of the pathogenesis of endometriosis (Cho et al., 2015; Kim et al., 2010).

Regulatory aspects of DEHP use in Plastic in products

From all the above literature cited it was revealed that DEHP enters and pollutes the environment through the effluents coming from industries and waste disposal sites whereby aquatic environment and the life present there end up becoming victims of the toxic effects of this plasticizer. Phthalates are an ever-increasing and noxious source of water and soil pollution and their toxicity has traumatized the entire food chain. Hence, steps towards the elimination of these harmful chemicals warrant immediate and serious attention through strict regulatory majors.

The US Department of Health and Human Sciences has prohibited the use of DEHP in children's toys at concentrations higher than 0.1% (Consumer Product Safety Improvement Act 2008). The European Commission identified DEHP as reproductive toxicant (Directive 2005/84/EC), and the European Union prohibits their use as ingredients in cosmetics (Directive 2005/90/EC).

India has also set the standards for five common phthalates (DEHP, DBP, BBP, DIDP, DNOP & DINP) in various children's products. However, there is no such regulation in place for baby diapers (The Hindu, 2020). Food Safety and Standards Authority of India (FSSAI) has framed the draft of Food Safety and Standards (Packaging) Amendment Regulations, 2020 related to specific migration limits of DEHP. Under this regulation, the use of food grade packaging material of plastic intended to be in contact with articles of food must have maximum migration limit of DEHP up to 1.5mg/kg (FSSAI, 2020). This draft is open in public domain to submit comments online to *regulation@fssai.gov.in*. Meanwhile as recommended by the Hon'ble National Green Tribunal (NGT), in order to reduce plastic usage and managing the plastic waste, it has been decided to permit the use of food grade packaging materials for packaged water other than those mentioned in the regulations and to operationalize the specific migration limit as mentioned.

Toxicological research pertaining to DEHP, thus has been significant in enforcing such regulations against its extensive use. This would help ensure mitigation of its environmental and health hazard.

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Review article

Climate Change and Zoonotic Diseases

A *look into zoonotic diseases and how they have taken over the world*

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Abstract

With the current COVID-19 pandemic just beginning to release its 2-year long grip on the global community, the world of 2022 is a far cry from what we expected it to be in 2019. The pandemic reshaped society and its attitude towards disease and epidemiology in a very fundamental way. With society still trying to find a way out of this current pandemic, the lingering question is "are there more such pandemics on the way?"

Cornonaviruses are, as far as we know, zoonotic in nature- in which that these viruses are transmitted between animals and humans. This sort of transmission is known as "zoonotic spillover" which is the transmission of a pathogen from a non-human animal to humans. This is not an uncommon phenomenon- in fact several diseases that exist today are zoonotic in origin (rabies, anthrax, West Nile virus etc). The wet markets of Wuhan offered a variety of processed meat and bushmeat- including bats and pangolin, which are today considered the possible zoonotic origins for the SARS-CoV-2 virus in humans.

This review attempts to summarise the impact of climate change and how it can cause further debilitating pandemics on three different levels: a) the history of zoonosis in conjunction with climate change, b) the direct impact and migration of animals and/or vectors due to climate change, causing zoonosis, c) the socioeconomic genesis of zoonotic diseases.

Introduction

The evolution of humans started a long 6 million years ago, with the prototypic ancestors of the *Homo sapiens* starting around 200-300,000 years ago (Callaway, 2017). The culture of civilisations started, earliest about 6000 years ago, with the Mesopotamian, Egyptian, Indus Valley and Chinese societies forming root (Gangal, 2014). These mechanisms paved their way into the late 17th to 18th century, where the genesis of the Industrial Revolution took place. It has been a long 6 million years, venturing outside Africa, populating the continents, laying down the foundation for civilisation and communities. What has always however stayed with us, and guided us through evolution, is disease.

Diseases are fundamentally abnormalities to the general human condition; physiologically or mentally. These can range from tooth decay, the oldest known "disease" to humanity, seen all the way back in the Taforalt human's jaws from approximately

10-15 thousand years ago (Oxilia, 2015). Today we recognise that most infectious diseases arose from the Old World, back when we were stumbling around as hunter/gatherer populations, pre agriculture, approximately 11,000 years ago (Diamond, 1999, Wolfe et al., 2007). The germ theory today recognises that "certain diseases are caused by the invasion of the body by microorganisms", but what we have to understand is that most of these diseases existed on this planet, longer than we did. They just had different hosts.

Zoonosis the portmanteau of two Greek words, arising from zoon meaning "animal" and *nosos* meaning "disease." Zoonosis is the transfer of an infectious disease from a "non-human animal" to humans. These diseases can "jump" from direct contact through food, water, sexual intercourse, milk and environment (WHO, 2020).

Today, wildlife is estimated to be responsible for approximately 72% of emerging infectious diseases in humans (Congressional Research Service, 2021). Zoonotic diseases have been affecting humans since the prehistoric hunter-gatherer era. This is predominantly due to the bushmeat consumption that was common among tribes back then (Nibert, 2013). Bushmeat comprises of meat that hunted down and consumed. While bushmeat is a popular source of animal protein in Africa, Latin America and Asia, particularly among the poor and rural communities, where it can even act as a cash commodity (Secretariat of the Convention on Biological Diversity, 2008), it is threat to both to biodiversity and human life. Bushmeat activities and their subsequent pathogen spillover into human communities have been linked to emerging infectious diseases (EID's) such as Ebola (Leroy et al. 2004), HIV (Van Heuverswyn & Peeters 2007), and monkeypox (Rimoin et al., 2010). Bushmeat transfection isn't localised simply to the communities that merely consume the meat but also pathogen spillover can occur through other mechanisms of exchange of bodily fluids, for example injured hunters and injured animals can have an exchange of blood through open wounds.

For a disease to go from affecting a particular non-human animal to becoming a zoonotic or even an anthroponotic problem, the health bodies like the WHO and the CDC have laid out two pathways: a direct pathway that goes as (animal ⇌ animal) with the transmission to humans directly through the animal, or (animal ⇌ vector ⇌ animal) with the vector infecting the human (CDC, 2021).

Climate change on the other hand can be described as a gradual, long-term transition in weather patterns and temperature (United Nations, 2011). Climate change is not new to the planet, the earth goes through periods of warming and cooling based on several inorganic factors. However, the post-industrial revolution earth has had an active part in excessive carbon emissions that has accelerated the rate of climate change, much faster than the ecosystem or even humanity was expecting (Snyder, 2016).

The mechanism of zoonotic diseases

We must first understand that zoonotic diseases are typically kept in check to prevent rampant zoonotic spillover. These processes can be described as a "series of barriers" that a pathogen must persist through to make the transition from a non-human species-specific disease to a zoonotic disease (Plowright et al., 2017). These mechanisms include climate, aridity, salinity in water, predator-prey relations that can keep the host in check etc. Pathogens must overcome these barriers in order to "host jump" or horizontally transfer between species (Ellwanger & Chies, 2021).

Pike, 2010 has described the 5 stages of zoonotic disease emergence in the form of an illustration that starts from Stage 1 while is exclusive to animals and Stage 5 which is exclusive to humans (refer to Fig. 1 and 2). There are several barriers that exist between each stage, in fact it is well established that most pathogens do not even make it to from Stage 1 to Stage 2. This divide is said to decrease with the increase in phylogenetic distance, rate of interactions with humans and also the pathogens' ability to proliferate among the new host's cells and its ability to overcome this new set of immune challenges (May et al, 2001; Antia et al, 2003; Moya et al., 2004; Morens et al, 2004). If a pathogen survives the transition from Stage 1 to Stage 2, it often finds itself at a dead end. While this doesn't always mean the virus is passive: pathogens like anthrax and rabies are lethal;

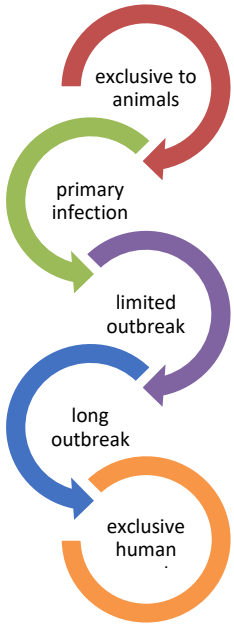

Fig 1. Pike 2010's hierarchy of the 5 stages of zoonotic disease emergence

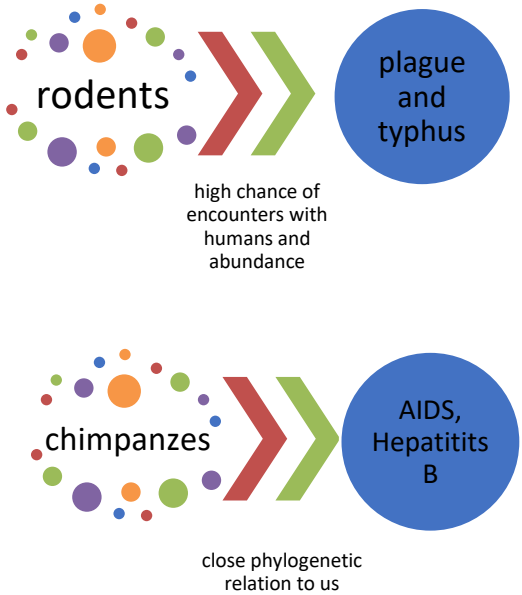

Fig 2. High chance encounters and close phylogenetic relationships between the non-human animals and humans can cause the barriers between the stages mentioned by Pike, 2010

but their spread is often limited. This stunting in progress for the pathogen is typically barricaded by the ability of the pathogen to adapt to the immune system of the new host or even the possibility of a new vector to infect people. The barriers between Stage 3 and 4 are more statistically stunted due to human population sizes and the low efficiency of transmission between humans (Morse, 1995; Wilson, 1995; Weiss, 2004, Morens, 2004).

Climate change and animals

While it is well established that the earth is used to both seasonal warming and cooling as well as periodic heating and cooling phases, it is the haste at which human induced climate change is progressing that is causing worry. In 2021, the IPCC reported the global temperature had already risen as high as 1.1°C since 1850-1990 (pre-Industrial revolution) and warned that a 1.5°C increment was to be expected by 2040. While nominal on paper, it has been predicted that a 2°C change in the global climate would result in a severely irreversible catastrophe. (Climate Change Widespread, Rapid, and Intensifying, 2021).

According to the IPCC, several species will lose their habitat due to an increase of 1.5°C. An estimated 13% of land areas are set to entirely change their biomes which is approximately 50% more than the estimated prediction for the 1.5° climate change. Marine ecosystems are set up for low oxygen and high acidity which will be amplified due to the high amounts of carbon dioxide at 1.5-2°C increments (IPCC, 2021).

Animals today have only three options, to move, adapt or die and not enough time to pick the "right" one. Distributional range changes have already been observed in animals and this has led to invasive or deficient species in different ecosystems.

Historical perspective of climatic imbalance and zoonosis

Epidemics are not a new concept in history. Several historic and folklore mentions of various plagues have existed throughout history- the Bible even names *pestilence* as a

sign of an apocalypse. The earliest known plague in history was the Plague of Justinian which was caused due to rats that boarded a ship from Egypt to Europe. The rats thrived on the grains available in the granaries and affected the communities en masse. The displacement of the vector to a region with more favourable conditions caused it multiply out of control, causing an epidemic (Harbeck et al., 2013).

Evidences from ancient China also showed epidemics that were spread across 3-4 provinces and corroborated with the annual heating and cooling seen in the region (McNeill, 1976). The smallpox outbreak in the Western Roman Empire between 312-313CE was linked to the lack of winter precipitation. The dysentery outbreak in the Sydney cove settlement during the drought was accredited to famine/food rationing crisis of 1790-1792 (Klein, 2014).

The Great Plague also known as the Black Death was the plague that wiped out 1/3rd of Europe's population after its introduction to Europe from Asia in 1347. The pathogen responsible for the same was *Yersinia pestis*, an Asian rodent plague that travelled down the Silk Road system. The pathogen primarily established itself in the European urban rodents and wildlife as its primary reservoir. The black rat *Rattus rattus* was the vector that kept the plague outbreaks on ships but it's more likely that it infected other wildlife as opposed to infect the population itself. A study reported a link where climatic fluctuations facilitated "synchronised plague outbreaks" in across central Asia and climatic unfavourabilities would cause the collapse of the rodent populations infected by the plague, causing the fleas to find new hosts. A series of progressive plagues like this are accelerated the arrival of the plague to Europe (Schmid et al., 2015).

In the 16th century, during the Spanish acquisition of the Aztec Empire, the Spaniards introduced the stowaway measles and smallpox virus to the Aztec population, which previously wasn't exposed to these diseases. However, droughts that were interrupted by intense rainfall caused zoonotic spillover of indigenous rodent borne diseases, which are today called the Cocoliztli epidemic (Acuna-Soto, 2002).

Modern zoonosis and climate change

Today we recognise the acceleration in several infectious diseases such as malaria due to climate change. Zoonotic vector borne diseases include most tick-borne diseases such as Lyme disease, babesiosis, ehrlichiosis, anaplasmosis, tularaemia etc. This is predominantly because vectors such as mosquitos, flies, ticks etc breed well in humid, warm climates. The accelerated warming is highly ideal for most of these vectors which are cold blooded in origin. A warmer global temperature simply gives a lot of these vectors, which were previously climatically constrained by the colder temperatures towards the poles to migrate towards these areas. This is particularly devastating because this is the preliminary exposure most of these populations will have to these diseases and a global warming will release these vectors at a much faster rate than incidentally expected (Gibb, 2020).

Take for instance, Lyme disease. Extensive deforestation in the United States caused most of the deer to vacate the forest lands and thus the Lyme disease pathogen *Borrelia bugdorferi* was isolated in tick populations on the east side of the Long Island Sound River. This is because deer were reintroduced for sport in the northeast. Post suburbanisation meant that in the late 70s, hunting was limited and the deer had no particular predators to be worried about and it caused an overpopulation of deer (Fish, 2020). Today Lyme disease is an epidemic that affects most of Northern United States. In the beginning, Lyme disease was only caused by deer but today dogs, horses and even cattle and small rodents can carry Lyme disease. The blacklegged tick is now posed to spread all the way upto Montana and Ontario, states in Canada that were previously immune to this disease due to their colder climatic condition (Climate Atlas of Canada, 2016).

Climate change can also push for direct transmission from animals to humans, sans vectors. A study conducted in New Zealand modelled "climate change-based scenarios" and proposed that climate change could push feral cats towards areas that were previously uninhabited by them. Feral cats are carriers of *Toxoplasma gondii*. Climate change can also trigger massive overpopulations of cats which can cause

feline breeding seasons to accelerate from 2 times a year to upto 4 times a year due to warmer climates (Aguilar et al., 2015).

Mycobacterium bovis is an aerobic bacterium that killed more than half the population of cattle in the early 20th century. It's typically transmitted to humans and even other mammals through unpasteurised milk or aerosol drops through faeces, air, urine, pus, etc. Tuberculosis is still a major global challenge for public health which has been impacted severely by population migration and climate change (Kuddus et al., 2019).

Climate change is also severely impacting the Arctic. In 2020, the land surface temperature of North Eastern Siberia was 45°C. The Arctic is warming twice as fast as it should and it is causing several ecological problems, including the rising sea levels (Arctic Report Card, 2020). A 2018 study shows that the rates of infectious diseases are affected by the changing climate. This is because the warming climate causes the habitat of the zoonotic animal or the vector to change, typically in a more favourable direction (Waits et al., 2018).

A red tide is a phenomenon where the marine coastal sea surfaces are discoloured to a red colour due to harmful algal blooms. This process, while sometimes natural, can be accelerated by human activities like industrial runoff and livestock waste, which can make the water nutrient dense and ideal for algal blooms (Guy, 2014). Warmer temperatures also allow for faster growth and perpetuation of algal blooms which grow thicker and faster due to the stagnancy of water and the ability of the blooms to absorb and trap sunlight, which makes the water warmer and promotes additional growth (Sarkar et al., 2019). *Kareina brevis* is a dinoflagellate that has been found in the waters of the Gulf of Mexico and has also been accredited for the Florida Red Tides. *K. brevis* produces a "suite" of neurotoxins that are called *brevotoxins* that can cause neurological and digestive problems and have caused large die offs in marine birds and organisms. The *K. brevis* can cause harm to humans, through a condition called Neurotoxic Shellfish Poisoning (NSP). While not lethal (yet), poisoning does result in severe nausea and vomiting (Hwang & Chen, 2016).

The socioeconomics of zoonotic diseases

Advancements in medical science, industrial revolution and improved agricultural and animal productivity has led to a population boom that started accelerating from the end of the Great Plague (Greenwood, 2014). The population has exponentially grown, from 1 billion in the 1800s to 7.9 billion in 2020. Along with the population growth, the life expectancy has also doubled. Pre industrial revolution, the life expectancy of humans at birth was 35 years whereas the life expectancy today is an average 65 years from birth (Hodges, 2015).

However, the life expectancy is not equivocal across tax brackets. The increased life expectancy coupled with the rapid population boom means that the supply of resources isn't enough to cover both the young and the old (*Humanium*, 2017). Climate change also impacts the people who live in rural or agricultural lands and the First Peoples drastically. The current estimate is that over 2 billion people are poor and face threats to their livelihood. 78% of the world's poor are in agro-based fields (World Bank Group, 2019). Climate change can cause acid rains which leads to high soil acidity which can lead to failed crop cycles, droughts, flooding, natural disasters etc (Kumar & Das, 2014; The Royal Society, 2020).

While capitalism and excessive production cycles impacts natural resources through draining, poverty cyclically impacts the environment due to excessive pressure on natural resources. Poor people are more likely to harvest wood from forest lands and consume bushmeat. Today, the population growth and the subsequent poverty are considered a reason for the degradation of the world's resources (Borgen Project, 2013).

While not exactly a Malthusian reality, there is something to be said about the rate of carbon emissions tied to industrial agriculture and meat. Today, world governments are willing to make the trade-off of the inappropriate pressure the poor people put on the strained resources. Countries like China have even promoted the use of farming wildlife as a means of alleviating rural populations out of poverty

in an effort to close the rural-urban divide. However, these sorts of wet-markets or in China's case, wildlife markets, can be prime spots for zoonotic spillover due to the limited, cramped conditions of the animals and poor sanitation (Beech, 2020).

Poverty also limits personal sanitation and hygiene, both of which are essential for sustained good health (Leibler et al., 2017). Public resources like water fountains are frequently unhygienic in that handles of water fountains are some of the most infected surfaces in public school settings (Walters & Geoff Cram, 2002). Rationed grain on the other hand has been found to contain rat faeces or hair in certain cases (Hussain, 2002). Poverty also means the exposure to stray animals and the dependence on bushmeat and unpasteurised milk is much more likely. (Davies et al., 2007)

Heightened population also causes cramping and limited space for both humans and animals. The encroachment of humans into forest lands beyond measure has pushed a lot of animals into the urban spotlight. Communities that are dependent on animal husbandry can see an uptick in the number of TBD (tick borne diseases) that affect cattle. Lack of housing can mean that the distance between humans and strays will be even lesser, causing them to be more likely to be infected by diseases that strays carry, such as rabies and toxoplasmosis (Leibler et al., 2017).

Conclusion

Zoonosis and further epidemics or even pandemics can be controlled through litigation and environmental protection which can in turn lower carbon emissions to a much more manageable rate (Nijman, 2021). There should be preventative barriers put in place at each stage of zoonotic transference from animals to humans to prevent the continued aggravation of zoonotic spillover. Natural and chemical preventatives for both the vector and human exposure should be instilled along with litigation that does more for the vulnerable populations who can unwittingly turn into a reservoir for further pandemics. Alongside this, vector control must also be taken seriously by using vector-related predators at each stage (Sokolov, 2019). Sanitation and hygiene should take up a more central stage with respect to preventative measures. Alongside stern litigation, education and vaccination should also be available for a broader population. The pandemic of 2020 was not the first one and it certainly will not be the last one in the domino effect of climate change but we must do our best to prevent it while we are ahead (Rodó et al., 2021).

Zoonosis and further epidemics or even pandemics can be controlled through litigation and environmental protection which can in turn lower carbon emissions to a much more manageable rate (Nijman, 2021). There should be preventative barriers put in place at each stage of zoonotic transference from animals to humans to prevent the continued aggravation of zoonotic spillover. Natural and chemical preventatives for both the vector and human exposure should be instilled along with litigation that does more for the vulnerable populations who can unwittingly turn into a reservoir for further pandemics. Alongside this, vector control must also be taken seriously by using vector-related predators at each stage (Sokolov, 2019). Sanitation and hygiene should take up a more central stage with respect to preventative measures. Alongside stern litigation, education and vaccination should also be available for a broader population. The pandemic of 2020 was not the first one and it certainly will not be the last one in the domino effect of climate change but we must do our best to prevent it while we are ahead (Rodó et al., 2021).

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Review article

The Science Behind Sleep Paralysis

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Abstract

Sleep paralysis is a medical condition that is correlated with the inability of a person to move when one is on the verge of sleeping or awakening. It can be categorized as isolated sleep paralysis when it has no association with other sleep related disorders such as narcolepsy. This medical condition has been described vividly amongst multiple cultures, each having diverse interpretations. Sleep paralysis has been identified as a medical condition in 2.4 - 40% of the population. Governed by the release of neurotransmitters; GABA and Glycine, this condition can sow the feelings of fear and anxiety in a person's mind. It is a condition that has got major attention by the non-scientific community presenting multiple myths. This increases the need to spread awareness about sleep paralysis, its symptoms and its mechanism and treatment.

Introduction

"I imagined that somebody was lying in bed with me, but I could not see them because I was struggling to turn over but could not move." is a modern-day interpretation of a medical condition that has been experienced over the years (Dahlitz & Parkes, 1993). Dating all the way back to the 1st century BC, where Themison of Laodicea termed it as a nightmare related to a supernatural entity called the Incubus (Khodadoust, 2012), sleep paralysis has been an unsolved puzzle. In the earlier times it was described as a visit from an evil being that tends to press the life out of their victim. Suffering people often complained of vivid hallucinations and a heavy chest or a suffocating feeling when one is asleep. Due to these hallucinations primarily in correlation with the evil entity, there has been some stigma attached to this condition.

Sleep paralysis is a condition that is associated with the inability to move when a person is on the verge of sleeping or awakening. This phase of sleep is known as Rapid Eye Movement sleep in which one can have the most emotional and vivid dreams. These dreams are so vivid that if one is to act them out, he or she would be at the risk of hurting themselves. To prevent the body from hurting itself the brain induces a state of temporary paralysis in the body (John Fakoya et al., 2018). Sleep Paralysis is the most common form of parasomnia associated with Rapid Eye Movement sleep. Rapid Eye Movement sleep is a state of the body that is very similar to the state of the body when fully awake. This is because of the similarity seen in neuronal activity. In fact, a person in Rapid Eye Movement sleep can sometimes show more neuronal activity. This activity is usually centered in the pons, lateral geniculate nucleus, and occipital cortex (John Fakoya et al., 2018).

The state of paralysis is initiated by the pons and the ventromedial medulla. This trigger acts as a signal for the cells to secrete glycine and GABA which are inhibitory neurotransmitters. These affect

the motor neurons such that the person starts to wake up and starts to gain awareness while in Rapid Eye Movement sleep.

The cause of sleep paralysis remains unknown but there are some risk factors such as anxiety disorders, poor sleep quality, consumption of alcohol, exposure to traumatic events, and a family history of sleep paralysis (Denis et al., 2015). Sleep paralysis can be familial, suggesting the predisposition of genes such as PER2 and ABCC9 possibly playing a role in it, genes that play a prime role in governing the circadian rhythms.

Sleep paralysis has been estimated to affect 2.4-40% people, out of which the major population comprises students (Cheyne, 2005). Patients suffering from the same usually report some stressful events in life, changes in their work schedule, or emotional experiences preceding the episodes. Physically normal signs of Rapid Eye Movement sleep can be noticed; these include muscle atonia or the absence of normal muscle strength, rapid eye movements, decreased respiration and increased heart rate (Peever & Fuller, 2016). Some evidence also suggests a relation with bipolar disorder, schizophrenia and narcolepsy. In fact, it is a part of the "tetrad of narcolepsy" This tetrad includes four classic symptoms, Sleep attacks, cataplexy, hypnagogic hallucinations and sleep paralysis being the fourth contender. Neurologically the reduction of respiratory muscle movement caused due to the inhibition of motor neurons can explain the feeling of heavy chestedness, or the 'incubus'. The hallucinations can be explained by the activity of serotonergic receptor 5-HT_{2A}R that are seen in abundance in the visual cortex (Jalal, 2018).

The Role of Neurotransmitters and Their Receptors in Mediating Sleep Paralysis

Sleep paralysis is characterized as a period in which a person is unable to perform movements voluntarily while the onset of sleep (hypnagogic form) or upon awakening (hypnopompic form) (Khodadoust, 2012). It is a state where one wakes up mentally while in Rapid Eye Movement Sleep, where the body protects itself from possible physical injury during the state of semi wakefulness.

Rapid eye movement sleep is a state where the neuronal activity of an individual is equivalent to that mixed frequency activity seen during wakefulness. It is marked by a substantial rise in the heart rate and blood pressure along with an irregular breathing pattern. This phase of semi wakefulness acts as a trigger (*Brain Basics: Understanding Sleep | National Institute of Neurological Disorders and Stroke*, z.d.) since Rapid Eye Movement Sleep Paralysis is initiated upon the action of the glutamatergic SubC cells (sleep promoting cells) that in turn activate the neurons that are present in the ventromedial medulla, this causes the secretion of GABA and glycine which give inhibitory signals to skeletal motor neurons.

When studying the role of GABA and glycine receptor mediated inhibition it was found that the metabotropic $GABA_B$ and the ionotropic $GABA_A$/glycine affect the motor neurons of the skeletal muscles (Brooks & Peever, 2012). Somatic neurons too show the presence of $GABA_B$,$GABA_A$ and glycine receptors indicating some relation in their roles in controlling Rapid Eye Movement sleep (Lalley, 1986; Araki et al., 1988; Persohn et al., 1992; Okabe et al., 1994; Margeta-Mitrovic et al., 1999; O'Brien and Berger, 1999; Charles et al., 2003; O'Brien et al., 2004). It is also seen that the motor neurons are hyperpolarized during inhibitory postsynaptic potentials (I.P.S.Ps) (Nakamura et al., 1978).

As mentioned earlier, sleep paralysis is a result of suppressing the muscle tone by pons and ventromedial medulla under the inhibitory action of gamma-aminobutyric acid (GABA) and glycine (Brooks & Peever, 2012)**.** It is said that in most of the sleep related disorders there is a role of flawed brainstem structures, in this case the amygdala. Another active role player is the limbic system. During Sleep Paralysis it is seen that a person's brain functions such that there is continuous signalling from the cortex to the inactive limbs (Jalal & Ramachandran, 2014).

Role of Serotonin Receptors in Causing Hallucinations Seen in Sleep Paralysis

The hallucinations seen in patients that suffer from sleep paralysis are similar to serotonergic hallucinations. It is suggested that there is an active role of the 5-HT_{2A} Receptors that are present in the visual cortex. During the process of waking from sleep the orexin producing neurons excite the serotonin producing neurons that promote cortical arousal (Bayer et al., 2004; Pace-Schott, 2008). In the process of sleep paralysis there is a similar transition that occurs which works under the same mechanism but the person is said to remain in a paralyzed state. This can lead to the possible serotonin overactivity causing the overactivation of 5-HT2A receptors which could lead to an increase in excitation in the cortex, causing release of glutamate in the neocortex that could further enhance the activation of the amygdala (Jalal, 2018). It has also been seen that in sleep paralysis the increased sense of awareness and threat can be a function of the activation of the amygdala (Leonard, 2008). The amygdala is responsible for processing memory, decision-making, and emotional reactions. It is pointed out that these impulses are carried forward by the lateral amygdala to the basolateral complexes giving the illusion of an intruder in the room (Phelps et al., 2005).

Association Of Sleep Paralysis with Other Disorders

Sleep paralysis has been associated with multiple other medical and psychiatric conditions ranging from narcolepsy, hypertension, seizure to insomnia, jet lag or even the commonest sleep disturbances such as stress and occupation. Sleep paralysis was also consequently noted in varied percentages with African Americans having different disorders. Like those who had panic disorders showed higher incidences of isolated sleep paralysis as compared to anxiety disorders. Patients of anxiety disorder and college students are assumed to suffer from sleep paralysis likely due to a disrupted sleeping pattern, while some studies suggest the use of antidepressants as a cause. Sleep paralysis has been reported in children that have faced childhood sexual abuse. They experience scary episodes of tactile, auditory and visual hallucinations. The sleep paralysis in such individuals has been accompanied along with depression and PTSD (Abrams et al., 2008). Sleep paralysis is a major contributor in the tetrad of narcolepsy. The tetrad consists of four classic symptoms, *Sleep attacks*- which are characterised by a sudden urge to sleep; *cataplexy*- which is a partial or generalised form of paralysis that is caused by laughter, anger, surprise, anticipatory excitement, *hypnagogic hallucinations*- which are scary, unsettling hallucinations that occur at the onset of sleep and the final contender being *sleep paralysis* (Mitler et al., 1990). There are some questionnaires that are devised to identify the risk factors too, these include the Beck Depression Inventory, Eysenck Personality Questionnaire, Clinician-Administered PTSD scale, Hamington Anxiety Rating Scale and Liebowitz Social Anxiety Scale (Paradis et al., 2009; Wróbel-Knybel et al., 2020).

Treatment Options for Sleep Paralysis

Sleep paralysis can be diagnosed with the help of many questionnaires that have been developed to assist in the process of evaluation of sleep paralysis and identifying its risk factors. They include the 'Unusual Sleep Experiences Questionnaire' (USEQ) and the 'Sleep Paralysis Experiences and Phenomenology Questionnaire' (SP-EPQ) (Paradis et al., 2009; Wróbel-Knybel et al., 2020). However, there is no direct treatment for sleep paralysis.

There are multiple attempts that point towards the physical and physiological factors that have the ability to trigger an episode, but there is nothing that focuses on eradicating the episodes of sleep paralysis. There are studies that have focused on muscle relaxation therapy along with focused attention meditation that have shown clinical benefits. They could be treated as methods of direct treatment (Jalal, 2016).

A strong correlation is seen between multiple sleep disorders and sleep

paralysis. Thus, suggesting that improving one's sleep hygiene may help in tackling this condition. This refers to a person's daily habits and routines that influence sleep quality. Some of these include fixing a sleep timing i.e. having a scheduled time to go to bed and wake up. The bed should be comfortable and should have a comfortable pillow. The room should not have too much light coming in. Another practice that can help with patients of sleep paralysis is having a pre-bedtime routine that would include avoiding the use of televisions or mobile phones, in the bedroom or, 1 hour before sleeping. Consumption of coffee and alcohol before sleeping can also hamper one's sleep cycle. Hence, keeping all these things in mind and following a rigid routine before sleep can help a person suffering with sleep paralysis.

There are no medicinal approaches that can be put to use per se, but the 5-HT_{2A} Receptors function in the development of hallucinations is under study. It proposes that a mechanism of down regulating the expression of these receptors could be an effective approach towards managing the fearful hallucinations one can have during an episode of sleep paralysis.

Discussion

Although very little is known about this subject in the current world, building research on the condition will not only pave a path for a better future but will also play an important role in understanding different sleep related disorders and medical conditions such as narcolepsy, idiopathic hypersomnia, obstructive sleep apnea, and insomnia disorder. Sleep Paralysis can be very draining and distressing for a patient and can put them in a loop of anxiety and fear accompanied with increased frequencies of episodes. Hence it is better to recognize the condition and intervene on the early onset to help the patient (Denis, 2018).

An initiative to ease the patients and help in identification is questionnaires created by reliable sources. These questionnaires help determine the triggering factor for these episodes and may aid in the management of this condition. Although there are no therapeutic modalities available Pimavanserin has been proposed as a therapeutic intervention for ameliorating the hallucinations. Further research and development can help find a direct therapy or treatment strategy for sleep paralysis.

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Review article

The Plight of Bright Nights: Assessing the Effects of Exposure to Light at Night

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Abstract

Circadian rhythms are mental, physical, and behavioral patterns that follow a 24-hour cycle in most living organisms. In humans, it is regulated by light and dark cycles, which were driven in the past by the solar day. However, since humans have started using light at night (LAN) for various reasons, their circadian rhythm has lost its natural regulator, i.e., sunlight. Naturally, this has disrupted our circadian rhythm, leading to a cascade of different psychological and physiological issues. This review summarizes the possible impacts of this unnatural use of LAN on human life.

Introduction

Among all the planets in our solar system, the Earth is the only planet that definitively hosts life. Naturally, most life forms on Earth have evolved to make the most of Earth's 24-hour rotation pattern. The alternating light and dark periods that organisms are exposed to have not only dictated the behavioral patterns of life forms but also affected them down to the molecular level. Since the advent of the industrial revolution, the boundary between days and nights have started blurring. The invention of artificial light sources and the rise of shift work ensured that optimum productivity levels were maintained even at night. While the productive output has undoubtedly increased in terms of material work, the psychological and physiological impacts of aberrant exposure to light have long been overlooked. But the effects of this unnatural exposure to light at night (LAN) are becoming more evident as the degree of exposure to LAN has grown exponentially over the past 100 years (Gaston et al., 2013). A typical full moon night without any artificial light illuminates the environment from 0.1 to 0.3 lux (Gaston et al., 2013). These are the natural LAN levels we were designed to be exposed to. Before the invention of the electric bulb, humans were exposed to minimal levels of LAN. In contrast, the illumination level of a typical modern urban street is around 5-15 lux, whereas that of an illuminated living room is 100-300 lux (Gaston et al., 2013). With the rampant use of electronic screens nowadays, LAN exposure exceeds the normal levels by a country mile. This exposure impairs our body's ability to distinguish between night

and day. This impairment isn't an exclusive event, and it is accompanied by many psychological and physiological alterations (Bedrosian & Nelson, 2017). In this review, we will look at the evidence that shows the significance of aberrant exposure to LAN in terms of psychological and physiological effects on the human body and animal model systems.

Light Detection and Circadian Rhythm

Most living organisms perceive light with the help of specialized organs such as the eyes in human beings. These organs are made up of specialized cells that respond to light stimuli of varying wavelengths and intensities (Schnapf & Baylor, 1987). In mammals, these special cells are known as photoreceptor cells. There are various kinds of photoreceptor cells. While rod cells and cone cells help in the formation of images, another class of photoreceptor cells, called the intrinsically photosensitive retinal ganglion cells (ipRGCs), are important for circadian phototransduction. The photopigment melanopsin is responsible for this quality of ipRGCs (Berson et al., 2002).

It has been established that even in the absence of rod and cone cells, ipRGCs are capable of producing circadian responses to light exposure (Lucas et al., 1999). Experimentally, it was observed that mice that lacked the melanopsin gene still showed light cycle responses, but only to a lesser degree (Ruby et al., 2002). Based on the spectral characteristics of melanopsin, the photopigment responsible for the activity of ipRGCs, blue light (~480nm), stimulates ipRGCs the most. In contrast, red light (~4600nm) has a minimal effect on these photoreceptor cells (Brainard et al., 2001). It is worth noting that sunlight during the daytime emits more blue wavelength light, whereas sunlight emitted during sunset has more red wavelength light (Bedrosian & Nelson, 2017). This indicates that our circadian response systems are designed to keep pace with the rise and the end of the day, marked by sunlight.

How does this light detection system affect the body? The light detected by ipRGCs plays a key role in setting up the body's "molecular clock." When should the body sleep to get optimal rest? When should the body rise to feel optimal energy levels? These questions are answered by the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is the paramount circadian pacemaker in the mammalian brain. It generates circadian rhythms in rest and activity, core body temperature, neuroendocrine function, autonomic function, memory and psychomotor performance, and a wide variety of other behavioral and physiological processes (Bedrosian & Nelson, 2017). The light detected by ipRGCs winds the molecular clock in the SCN. The molecular clock in the SCN is nothing but a set of transcriptional–translational feedback loops that drive rhythmic 24-h expression of the core clock components (Ko & Takahashi, 2006). The transcriptional–translational feedback loops form the basis of the intrinsic daily circadian rhythm. In the absence of environmental stimuli, the molecular clock will continue to run at ~ 24-h rhythms. This was established experimentally, where *ex vivo* cultures of SCN neurons continued to express near 24-h rhythms for weeks after being removed from the body (Yamazaki et al., 2000).

Since we are looking at sleep and the effects of LAN exposure on sleep, we'll take a closer look at how the SCN helps set the sleeping cycles. The ipRGCs register the light stimuli and send the information to the SCN. The strength of the response depends on the wavelength of the light stimulus and corresponds to the time of the day (since natural conditions mean more blue light during the day, more red light around sunset). The information received by the SCN is then passed on to the pineal gland. The pineal gland secretes a hormone called Melatonin. Melatonin is an indolamine that is secreted in a 24-hour pattern, with production and secretion peaking at night. Melatonin helps set quite a few "clocks" in the body via interactions with the molecular clock mechanism. A greater concentration of Melatonin in the body suggests that it is time to sleep, while lower concentrations indicate it is time to rise. Exposure to light even at dim intensities strongly inhibits the secretion of Melatonin. This could cascade into desynchronization of the central and peripheral clocks of the body (Brainard et al., 1988). The importance of Melatonin is not just observed in humans. In a study, it was observed that Melatonin induced sleep

responses in at least three nonhuman diurnal primate species, namely *Macaca nemestrina*, *Macaca fascicularis*, and *Macaca mulatta* (Zhdanova et al., 2002). It is widely believed that exposure to LAN increases the risk of disease considerably via dysregulation and desynchronization of behavioral and biological daily rhythms (Cho et al., 2015; Karatsoreos et al., 2011).

Psychological Impact of Exposure to LAN

Considering that the structures of the brain and the neurochemicals that control emotions are also responsible for sleep regulation, it is natural that the disruption in sleep patterns by LAN often leads to a disruptive emotional state as well (Palmer & Alfano, 2017). Most mood-related disorders are often characterized by altered sleep patterns, and there is evidence to suggest that this alteration is bidirectional (Bedrosian et al., 2011, 2013; Dumont & Beaulieu, 2007; Fonken et al., 2012). Any disruption in sleep, be it quantitative or qualitative, is linked with a wide variety of negative emotions and fewer positive emotions. For example, after a night of disturbed sleep, couples are not emotionally as adaptive as they are otherwise observed via a lab-based discussion. Medical professionals have also reported a prevalence of more negative emotions in the workplace. This could also be attributed to a high degree of sleep disruption, given the nature of the job (Fonken & Nelson, 2013).

Effects on young minds: The sensitivity of the molecular clock is not really much appreciated by majority of the humankind today. The levels of LAN exposure we're dealing with in this day and age are gargantuan. This exposure begins right from early childhood and is likely to extend through the entirety of an individual's life span. These are chronic levels of LAN exposure. Humans are sensitive to the effects of LAN on mood throughout their lives. Early exposure of children and adolescents to LAN leaves them with disrupted mood patterns right from a very young age. This could have long-lasting consequences as these are the years where brain development is at its peak (Bedrosian & Nelson, 2017). Studies on animal models have also suggested that exposure to LAN can also have long-lasting impacts on learning and memory. A key finding that links LAN exposure to neuronal changes was presented by Bedrosian et al. in their 2013 study. Hippocampus plays a major role in memory and learning and it was observed that in hamsters, LAN exposure led to a marked increase in the expression of hippocampal tumor necrosis factor (TNF) (Bedrosian et al., 2013). Such effects in the formative years of the brain could have long-lasting impacts on not just the memory and learning, but the life the individual is going to lead in the future as well (Fujioka et al., 2011). In a 1999 survey, it was reported that only 1 in 10 individuals between the ages of 2-16 slept in complete darkness (Quinn et al., 1999). The exposure to LAN was initially more of an urban problem than a rural one, but the advent of electronic media has only added to this predicament. This exposure doesn't only affect mood circuits in the brain but also predisposes individuals to develop more night oriented chronotypes which would ultimately result in increased exposure to LAN (Vollmer et al., 2012). Studies on animal models show that exposure to LAN can have long-lasting psychological effects. Young mice that were exposed to dim light at night (dLAN) for the first three weeks of their lives had increased anxiety-like signs as adults (Borniger et al., 2014). It is astounding that the effects are not just limited to direct exposure but can be even passed down to generations. Research conducted on mice by Cissé Y. et al. concluded that when the parents were exposed to dLAN before mating, the offspring developed depressive-like behavior. These changes occurred even when the offspring were being conceived, gestated, and raised under standard light and dark conditions (Cissé et al., 2017).

Effects of the LAN lifestyle: By a general estimation, 15-20% of the adult population in developed societies are engaged in shifts that have unusual working hours, usually at night. The rise of outsourcing of call processes from developed to developing countries is also leading to a rise in night-shift workers in developing countries due to differences in time zones (Bedrosian & Nelson, 2017). The prevalence of major depressive disorder (MDD) is considerably higher in night shift workers than in day shift workers (Scott et al., 1997).

If this work culture is adopted for a long period (20 years or more), it even poses an increased lifetime risk of MDD (Scott et al., 1997), and even a single night shift is sufficient to induce an adverse change in mood (Healy et al., 1993). While reversing the circadian cycle by minimizing light exposure during the day and working in LAN exposed conditions at night has shown to improve depressive symptoms of night shift workers, it is practically impossible to continue living like that due to the social construct of our society being based around regular working hours, and when these workers have their days off (such as on weekends), their circadian cycle will again return to the natural rhythm (Czeisler et al., 1990).

In a longitudinal study conducted on an elderly population, LAN exposure in home settings was underlined as an independent risk factor for depressive symptoms in elderly people. Previous studies with similar limitations have shown moderately reproducible results with respect to LAN exposure (Obayashi et al., 2012, 2014).

A prime example that establishes a correlation between LAN and MDD is that of the old Order Amish living in the US. These folk avoid using electricity. As a result, they are significantly less exposed to LAN compared to the general population of the US. The incidence of MDD in this group of people in the mid 20th century was around the same as that of the general population, i.e., ~1% (Hostetter et al., 1983). Fast forward to the 21st century, the incidence of MDD is still around the same in the old Amish, whereas it has leapt to 15% for the general population (Riolo et al., 2005). While the argument may be a debatable one, but the old Amish also display lesser rates of cancers, obesity, CVD and a multitude of other disorders linked to LAN and night shift work (Stevens et al., 2013; Westman et al., 2010).

Does depressive behavior have a code?

Alterations in the genes of the circadian system and their promoters are often observed in patients with depression. In one study, 113 single-nucleotide polymorphisms in 18 circadian genes were observed in comparison to the healthy subjects (Utge et al., 2010). In their study of the genetic changes in patients with depression and bipolar disorder, Soria and colleagues observed that there were 209 SNPs in 19 circadian genes when compared to healthy controls. Significant SNPs were observed in Cry1 and NPAS2 in patients with depression, whereas in patients with bipolar disorder, they had greater SNPs in CLOCK and VIP (Soria et al., 2010).

Is there a potential way back?

While it is likely that most of us have already faced the psychological effects of LAN given today's "24-hour lifestyle," studies of animal models have shown that these changes can be reversed (Bedrosian et al., 2013). Bedrosian and colleagues have demonstrated that prolonged LAN exposure induced depressive-like responses in hamsters. The activity levels of these hamsters were significantly lower than those maintained in dark night conditions. They displayed more immobility, which is typically interpreted as despair-like behaviour, and also a reduced affinity for sucrose solution, which is similar to anhedonia, a condition where an individual develops an inability to feel pleasure from otherwise pleasurable stimuli. The most hopeful aspect of this study was the finding that within 2 weeks of eliminating LAN exposure, behaviour of the test hamsters resembled that of hamsters maintained in dark night conditions, indicating a reversal of the depressive symptoms (Bedrosian et al., 2013). How long does it take for this reversal of symptoms to manifest in humans? More research is required to investigate it.

Physiological Impact of Exposure to LAN

While the psychological impacts of LAN exposure are serious enough, the physiological impacts of the same raise even more alarms. Over the past three decades, compelling evidence has emerged to establish the relation between LAN exposure and physiological complications (Lucas et al., 2014).

It is important to note that the psychological and the physiological manifestations of LAN exposure are neither mutually exclusive nor independent of each other. In fact, there is sufficient literature that establishes the link

between them. (Untreated Depression and Hippocampal Volume Loss) There is a strong correlation between LAN exposure and depressive symptoms, as discussed above. Prolonged stress or repeated depressive episodes is very likely to affect hippocampal volume by elevating glucocorticoid levels (Sapolsky et al., 1986) and decreasing neurogenesis (Gould et al., 1997). As already observed, LAN affects learning and memory. A study done on mice suggests that constant exposure to light negatively impacted hippocampal cell generation. New neurons of the hippocampus play a key role in memory and learning and hence this impact of LAN. Therefore, the effect of LAN on learning and memory could be, at least in part, be attributed to its effect on hippocampal neurogenesis (Fujioka et al., 2011). Another study on hamsters suggests that dLAN exposure at night is likely to result in the loss of dendritic spine density of hippocampal CA1 pyramidal cells. This loss in dendritic spines also correlates with depressive behavior (Law et al., 2004), further underlining the connection between the physiological and psychological impacts of LAN exposure.

The pathway of CA1 spine reduction most likely involves suppression of Melatonin, as Melatonin is known to counter corticosteroid-induced dendritic spine reduction and promote neurogenesis (Crupi et al., 2010). As reported previously, the offspring of mice that were exposed to dLAN before mating developed depressive symptoms without any external stress stimuli. Another aspect of this study shows that having dLAN exposed parents also led to altered melatonin and glucocorticoid receptor expression in the offspring (Cissé et al., 2017). The effects of LAN exposure are not just limited to changes in the brain. There's considerable evidence that suggests the physiological manifestations are seen across a wide range of systems in the body. An exposure of only 39 minutes to a low-level incandescent bulb can suppress melatonin levels up to 50% in humans (Navara & Nelson, 2007). Melatonin levels play a role in body mass regulation, metabolic rate, nutrient absorption efficiency (Nelson & Drazen, 1999) and even cardiac ATP synthesis (Rodríguez et al., 2007). Thus, suppression of melatonin levels by LAN (Brainard et al., 1988) can have widespread effects on the human body.

Effect on metabolism: Studies on animal models have shown that prolonged light exposure can have strong regulatory effects on the metabolism. Rats that were exposed to constant light showed signs of impaired carbohydrate metabolism in the liver (Mustonen et al., 2002), whereas a constant light environment raised the body fat percentage of female broiler chickens considerably. Male broiler chickens too displayed a rise in their weight, but in this case, constant light exposure increased their appetite and food intake greatly (Robbins et al., 1984).

There are multiple studies that suggest that humans experience similar metabolic effects in response to artificial light at night (ALAN) exposure. For instance, night shift work has been linked with negative effects on carbohydrate and lipid metabolism and insulin resistance, while also being linked to conditions such as hypertension, coronary heart disease and myocardial infarctions (Haus & Smolensky, 2006). LAN's role in human metabolic dysfunction and obesity was studied recently in a study of 5480 adults in Finland. The findings indicated that levels of light exposure were linked to fluctuations in appetite and mood, two factors that contribute to the risk of metabolic syndrome (Grimaldi et al., 2009). In another recent study of 7254 shift workers, it was concluded that night shift work was a significant factor for obesity. It is important to note that these findings were independent of age, BMI, drinking, smoking or exercise (Suwazono et al., 2008).

Role in altered oxidative stress: In living organisms, exposure to LAN exposure can result in the promotion of oxidative stress that could damage their own cells (Reiter et al., 2000). Many studies suggest the possible link between LAN exposure and increased levels of oxidative stress. Rats raised in constant light conditions showed significantly higher levels of lipid peroxidation in the liver, kidney and brain (Bayda et al., 2001) and elevated levels of hepatic oxidative stress (Cruz et al., 2003).

The versatility of Melatonin once again takes the spotlight when its role in antioxidant regulation is considered (Rodriguez et al.,

2004). Melatonin levels are indicators of the total antioxidant capacity of blood in humans. And with LAN exposure, as the melatonin levels drop, the antioxidant capacity of the body drops as well (Benot et al., 1999). Glutathione peroxidase is an important antioxidant enzyme. Rats raised in constant light conditions showed reduced levels of glutathione peroxidase (Cruz et al., 2003) and glutathione (Túnez et al., 2003). It is likely that suppression of Melatonin by LAN is at least in part responsible for the reduced levels of glutathione peroxidase as past studies have shown that Melatonin stimulates the synthesis of glutathione (Túnez et al., 2003). Superoxide dismutase (SOD) is an enzyme that displays antioxidant activity. In patients with peripheral nerve injuries (PNI), SOD levels are known to drop significantly. Studies have shown that Melatonin restores SOD expression in patients with PNI, acting as an antioxidant promoter (Chang et al., 2008). Another proof of Melatonin's importance as an antioxidant promoter is that it has been experimentally observed that the pro-oxidative effects of LAN were preventable via administration of Melatonin (Bayda et al., 2001). Disruption of immune activity: Studies suggest that LAN exposure alters the immune responses of the body as well. An animal model study on cockerels showed that when raised in constant light conditions, they produced low levels of antibodies against sheep RBCs and also displayed delayed hypersensitivity reaction as compared to controls raised in standard light and dark conditions (Kirby et al., 1991).

Another mammalian model study has revealed that LAN exposure correlates with suppression of the cytotoxic activity of natural killer cells (Oishi et al., 2006). Melatonin, a recurring molecule in this review, has appeared once again. When Syrian hamsters were administered Melatonin, their splenic mass, splenic lymphocyte count and macrophage count all showed an upward change. Similar results were obtained when these hamsters were maintained in short photoperiods, naturally increasing their melatonin levels (Vaughan et al., 1987).

Cancer: Current evidence suggests that LAN exposure may be a driving factor in cancer risk. Night shift work has been linked to increased breast cancer incidence (Schernhammer & Schulmeister, 2004). In a nationwide study in Denmark with 7035 women with breast cancer, it was established that even six months of night-shift work raised the risk of breast cancer incidence 1.5-fold (Hansen, 2001).

Another study of 602 colorectal cancer cases among 78,586 women concluded that a rotating night shift for 15 years with at least three nights per month was a factor that increased the risk of colorectal cancer (Schernhammer et al., 2001). Speaking of animal studies, a study on deer mice with increased light exposure duration showed that this increase in light duration increased the likelihood of tumor formation in these mice. In fact, 90% of mice that were subjected to longer light durations developed tumors, whereas those maintained in standard light conditions developed none (Nelson & Blom, 1994).

"The Melatonin hypothesis" proposes that a drop in pineal melatonin levels may increase the risk of breast cancer owing to the interactions between Melatonin and estrogen, a known promoter of breast tissue proliferation (Anisimov et al., 2004). Melatonin has a negative regulatory effect on estrogen in many mammalian species (Reiter, 1980). Melatonin also blocks estradiol-induced proliferation of breast cells (Blask & Hill, 1986). As discussed before, Melatonin is an antioxidant promoter. Thus, it may also protect breast cells against estradiol-induced oxidative damage that could result in cancer (Sánchez-Barceló et al., 2005).

Conclusion

The invention of the electric bulbs has turned out to be a significant moment for the history of humanity. While it has led to a safer and more productive world that functions 24x7, it has also exposed human beings to considerable psychological and physiological threats. In 2007, the World Health Organization declared shift work as a 'probable carcinogen' (Blask et al., 2005; Davis et al., 2001; Kloog et al., 2008; Stevens, 2009). The evidences that have been discussed above indicate that LAN negatively affects mood, induces depressive-like symptoms and is responsible for a host of other health problems. As the human

population rapidly advances towards progress, the impact of LAN should be considered with more seriousness. On a broader front, shift work should be reconsidered, whereas, at a personal level, it is time we consider the control technology has over us. The awareness about the negative impacts of LAN should be imparted across all age groups, especially to children; as technology takes the center-stage in the lives of young individuals, the importance of using the same technology in moderation to avoid its negative effects should be written in bold and underlined. Considering the impact of COVID-19 and how it has confined us in isolation, we have been left with no other choice but to rely heavily on our gadgets. Whether it is to stay connected, study online, or work remotely, we are exposed to screens and light more than ever. Hence, now is a more important time than any to be mindful about our 'screen-time.'

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Review article

Autophagy: A vital regulatory mechanism in cells

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Abstract

Autophagy is a process which maintains cellular homeostasis by recycling intracellular material for reuse by the cell. It also serves the purpose of degradation and removal of unwanted, damaged and toxic entities of the cell. Its prime importance in the physiology of living organisms is displayed by the fact that the process is conserved in all living creatures right from unicellular yeasts to humans. Autophagy imbalance is known to play a crucial function in ageing, immune diseases, cancer and neurodegeneration.

Another principal pathway essential for cellular homeostasis is apoptosis. It is an altruistic pathway of cell death. It is functional during normal growth during embryonic development and morphogenesis and is aggravated under stressed conditions.

Unlike this, autophagy is a cell-survival mechanism, designed to clean up and recycle molecules which is an essential process that is generally seen to be active in cells and also during cell-stressed states. This promotes healthier cell survival.

This article gives a brief overview of the how autophagy pathways are vital in modulating diseases, health and lifespan in animals and humans.

What is autophagy?

Autophagy (ancient Greek for 'self-devouring') is a primary cellular process in which different, non-essential components of a cell are targeted to lysosomes by autophagosomes for degradation. The process has been studied to comprise of 5 major stages which are initiation, elongation, maturation, fusion and degradation. The cellular entity to be degraded is first surrounded by a membrane that further develops into an autophagosome. The developed autophagosome fuses with a lysosome to form an autolysosome. The degradative enzymes of the lysosomes help in degradation of the cargo (Yang and Klionsky; 2009). In simpler words, autophagy can be considered as a recycling system of the cells, which breaks down unwanted proteins, lipids and even organelles to their simple forms to liberate the component biomolecules to be reused by the cell for generation of essential cellular molecules (de Duve, 2005). It also serves as an excellent system for degradation of invading pathogens and toxic misfolded proteins, thus promoting cell survival during infections, onset of disease pathology and even ageing (de Duve, 2005; Ohsumi, 2014).

History and the Nobel Prize

With the invention of electron microscopes, Christian de Duve, in 1949, detected hydrolytic enzymes filled, large pools of membrane bound organelles while studying the effect of insulin on liver cells (de Duve, 2005). By 1955, several hydrolytic enzymes were characterized in these organelles, which de Duve named as 'lysosomes'. After understanding that these 'suicide bags' had the ability to digest and degrade other cellular components, de Duve coined the term 'autophagy' in 1963 (Ohsumi, 2014). More than 70 years later, we understand the role and importance of the lysosomes in the cell, and still many stones are yet to be turned. Lysosomes prove to be one of the fundamental organelles involved in cellular autophagy.

Christian de Duve, Albert Claude and George Palade were awarded the Nobel Prize in Physiology and Medicine in 1974 for deciphering the structural and functional organization of the cell. de Duve was credited for his discovery of lysosomes and peroxisomes. Even if the concept of autophagy was introduced in the early 1960s, the precise mechanism was yet to be elucidated (de Duve, 2005).

Dr. Yoshinori Ohsumi received the Nobel Prize in Physiology or Medicine, in the year 2016, for his discoveries of mechanisms for autophagy.

Ohsumi, in the mid 1990's, studied autophagy in the budding yeast *Saccharomyces cerevisiae*. Ohsumi suspected that if autophagy existed in yeast, inhibition of vacuolar enzymes would bring about accumulation of engulfed cytoplasmic components in the vacuole. To test this speculation, he generated yeast strains that did not have the vacuolar hydrolytic enzymes. He observed accumulation of autophagic bodies in the vacuole after culturing these genetically modified yeast strains in nutrient-deprived medium, producing a strange vacuole under a light microscope. Later, Ohsumi recognized first yeast mutant incapable of accumulating autophagic bodies, by generating random mutations, and named the gene, autophagy 1(*APG1,* later denoted as *ATG*). By 1993, Ohsumi reported 15 different genes responsible for activation of autophagy in eukaryotes (Tsukada and Ohsumi, 1993; Mizushima, 2018). Further investigations revealed that Atg1 forms a complex with Atg13, and this is controlled by the target of rapamycin (TOR) kinase2 (Nakatogawa et al., 2009). TOR was found to be active in nutrient-rich conditions and was inactive during starvation which further lead to autophagy initiation. Later, it was reported that the active kinase involved was indeed a complex of multiple *ATG* genes. By early 2000s, it was known that complexes of multiple *ATG* genes and various other molecules were essential in autophagosome formation (Mizushima, 2018).

Ohsumi and colleagues soon deciphered other *ATG* genes and their precise role in formation and maturation of an autophagosome, and also reported that these genes were evolutionarily conserved in humans. Following this, extensive research was carried out to study the version of the mammalian homologue of yeast Atg8, called light chain 3 (LC3), to be used as a marker of autophagosome formation in mammalian systems. In late 2000s, Ohsumi conducted starvation studies to understand autophagy in mouse models which confirmed the importance of autophagy on mammalian tissues and elucidated the mechanism of autophagy (Mizushima, 2018).

Types of Autophagy

Numerous triggers and conditions initiate autophagy and depending on the degradation mechanism, these are categorized into 3 distinct types, which are macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).

Macroautophagy is the most widely studied type and responds to a majority of cellular stresses and physiological signals. In macroautophagy, a section of the cytoplasm is isolated and engulfed by a delicate membrane called phagophore, following a *de novo* mechanism, which on growing and maturing, forms a double membraned structure called autophagosome. On fusion of the autophagosome with lysosome, the lysosomal enzymes initiate the degradation of the inner autophagosomal membrane and the cellular components enclosed in it (Ichimiya et al., 2020; Mizushima and Levine, 2020).

Macroautophagy was once viewed as only a non-selective process, but is now known to be both selective and non-selective. The non-selective process involves random engulfment of a cytoplasmic portion for degradation (Jin et al., 2013). The selective process degrades particular cargoes which involve specific cargo adaptors for recognition by the receptors (Mizushima and Levine, 2020). The mechanism of macroautophagy includes the combinatorial functioning of different protein complexes encoded by evolutionarily conserved, autophagy related (*ATG*) genes. Till today, there exist 42 different genes which have been discovered and recognised to be the ATG genes (Parzych et al., 2018), 15 of which being the core *ATG* genes (Søreng et al., 2018; Mizushima and Levine, 2020). Different types of macroautophagy processes are known, based on the components to be degraded.

The second type of autophagy is a non-selective process, known as microautophagy which includes the immediate engulfment of cytoplasmic substance by lysosomes, due to invagination or deformation of the lysosomal membrane. It is a simple process which does not involve formation of an autophagosome (Mizushima and Levine, 2020; Schuck, 2020).

Unlike the above-mentioned types, chaperone-mediated autophagy (CMA) has

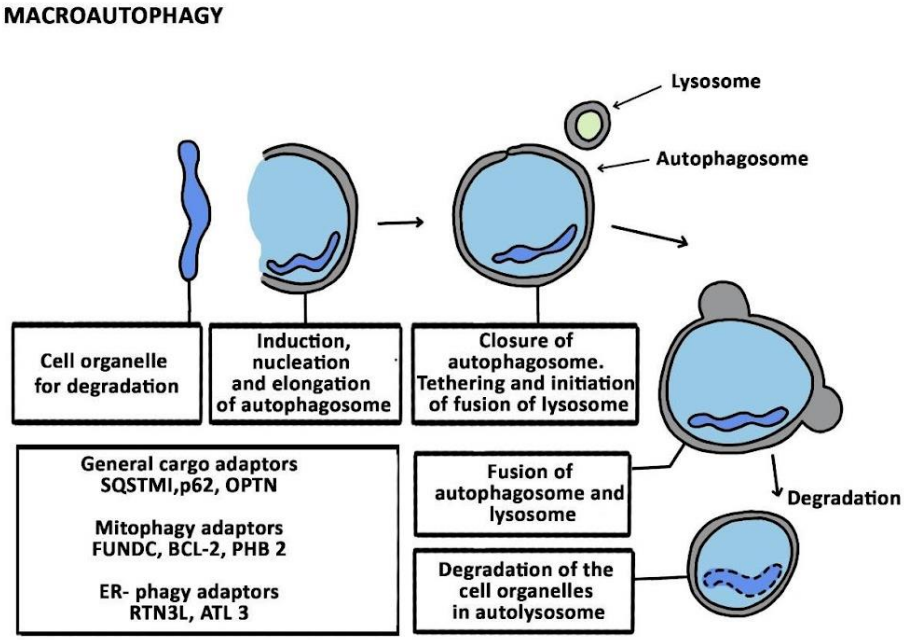

Fig 1: Macroautophagy. A selective process that is mediated by the autophagosome. Different cellular entities are degraded with the help of general or organelle specific autophagy cargo adaptors present on the cargo surface (shown in bottom left box). These adaptors play a crucial role in engulfment of the cargo by the autophagosome on interaction with certain membrane proteins present on the autophagosome. The development of autophagosome consists of different steps including induction, nucleation, elongation and closure. Fusion of lysosome helps in degradation of the cargo in the autolysosome.
Image courtesy: Sachin Modgekar

Table 1: Macroautophagy types and target components.

Name	Target for degradation	Reference
Mitophagy	Mitochondria	(Gatika et al., 2018; Levine and Kroemer, 2019)
ERphagy	Endoplasmic reticulum	(Mizushima and Levine, 2020)
Pexophagy	Peroxisomes	(Ogier-Denis and Codogno, 2003)
Nucleophagy	Nucleus	(Bo Otto and Thumm, 2020)
Allophagy	Paternal organelles	(Rawi et al., 2012)
Lysophagy	Lysosomes	(Gatika et al., 2018; Levine and Kroemer, 2019)
Aggrephagy	Abnormal protein aggregates	(Mizushima and Levine, 2020)
Lipophagy	Lipid droplets	(Cingolani and Czaja, 2016)
Xenophagy	Cellular pathogens	(Gatika et al., 2018; Levine and Kroemer, 2019)
Ribophagy	Ribosomes	(Beese et al., 2020)
NPC-phagy	Nuclear pore complexes	(Gross and Graef, 2020)
DN/RN autophagy	DNA/RNA	(Münz, 2011)

been demonstrated only in mammalian cells and follows a unique pathway. CMA is highly specific and has particular target sequences present on the cellular components to be degraded. Wide variety of substrate proteins like glycolytic enzymes, transcription factors and their inhibitors, proteasome subunits, lipid-binding and calcium-binding proteins and those involved in vesicular trafficking have been reported to be degraded by CMA (Kaushik and Cuervo, 2018). The basic mechanism involves a target protein possessing a CMA-targeting motif which is recognized by a heat shock protein, forming a complex that helps in delivery of the target protein to the lysosomal membrane (Ichimiya et al., 2020). At the membrane, the target protein binds to a CMA receptor on the membrane named LAMP-2A, which aids in translocation of the target protein into the lysosome for degradation (Parzych and Klionsky, 2014).

Autophagy in starvation and exercise.

Many a times, people practise deliberate starvation, either following a traditional ritual or to maintain a healthy lifestyle. Research has proven that these dietary constraints reduce advanced age-related diseases and even increase the lifespan of an individual (Hwangbo et al., 2020). The consequences of these dietary restrictions

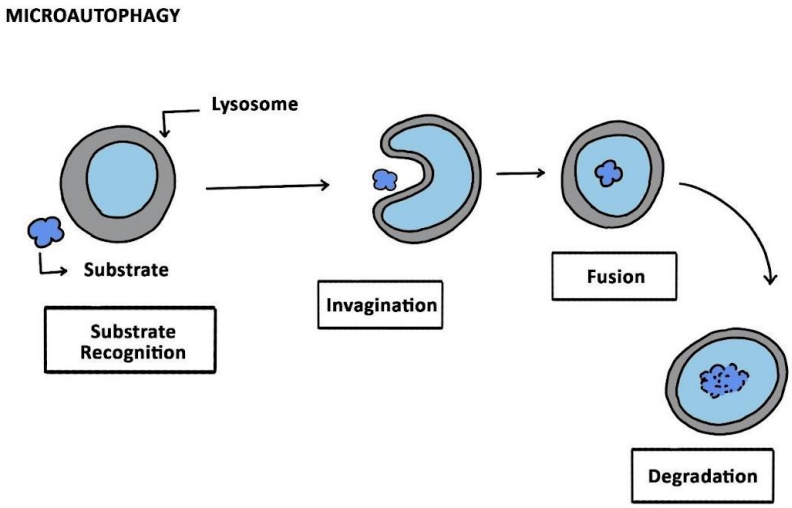

Fig 2: Microautophagy: Non-selective process of cargo engulfment and degradation.
Image courtesy: Shweta Modgekar.

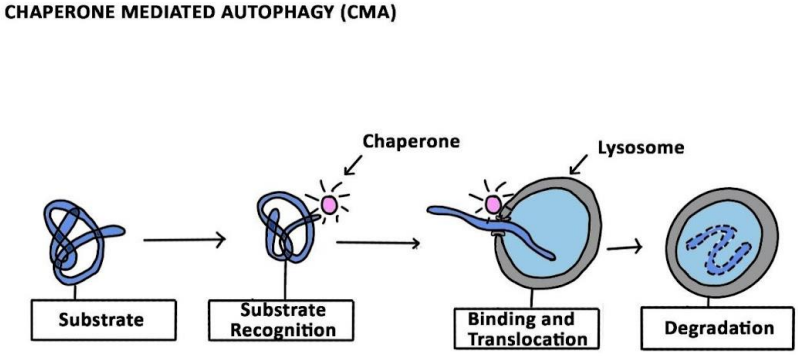

Fig 3: Chaperone-mediated autophagy (CMA). Selective process involving cargo tagging with chaperone for degradation.
Image courtesy: Shweta Modgekar.

are multiple, including enhanced growth factor signalling, activation of stress resistance pathways, energy metabolism, protection against oxidative stress and improved therapeutic response of many drugs (Batatinha et al., 2019). Along with these, starvation has also shown to initiate autophagy, but the mechanism behind this activation is yet to be elucidated. In diabetes, starvation induced autophagy instigates modification of signalling pathways, in liver cells and adipose tissue of mice model, which reduce mTOR signalling, improve mitochondrial function, up-regulation of autophagy pathways which results in reduce blood glucose levels (Singh et al., 2009; He at al., 2012; Choi et al., 2013). Cardiovascular studies conducted in autophagy receptor (LAMP-2A) mutant mice models, subjected to fasting showed severely enhanced myocardial damage as compared to wild type mice (Nishino et al., 2000; Choi et al., 2013). Many studies along with this suggest that starvation induces autophagy and has multiple cardio protective benefits, if autophagy is initiated successfully (Choi et al., 2013; Hwangbo et al., 2020). Evidences for starvation induced autophagy exhibiting neuroprotective function have also been

reported. Mice models of Parkinson's disease (PD), Alzheimer's disease (AD), Huntington disease and Charcot Marie tooth disease have been widely studied to test the effect of starvation-induced autophagy on molecular pathways involved in these diseases (Mhyre et al., 2012; Sun et al., 2015). Even if the exact mechanism by which autophagy safeguards synaptic dysfunction and cognitive defects in these mice models is yet to be known, starvation in these models has been reported to reduce oxidative stress, increase neurotrophic factor signalling and autophagy signalling (Mizushima and Levine, 2020). Autophagic bodies have been observed in neurons of starved AD mice, which have been reported to reduce neurodegeneration. In PD models of mice subjected to starvation, autophagy induction has been reported to alleviate synaptic damage and motor abnormalities (Mhyre et al., 2012; Batatinha et al., 2019).

Along with starvation, exercise can also induce autophagy. Multiple evidences suggest improved autophagy-induced mitochondrial functioning via AMPK activation, thus alleviating reactive oxygen species (ROS) production, after physical training (Romanello and Sandri, 2016; Batatinha et al., 2019). The detailed mechanism of activation of exercise-induced autophagy in a variety of tumour cells is yet to be elucidated. However, studies have reported that exercise can rescue the loss of cellular autophagy control in these cells (Schwalm et al., 2015; Batatinha et al., 2019). Physical training mediated autophagy induction has also been reported to exhibit neuroprotective effect in PD and AD mice models (Wong and Cuervo, 2010; Choi et al., 2013). Elevated levels of abnormal protein clearance, reduced ROS and autophagy defects leading to improved memory performance has been documented. Regular physical exercise has shown to induce mitophagy in degenerating areas of PD mice brain, thus reducing neurodegeneration (Wong and Cuervo, 2010; Choi et al., 2013).

Autophagy and longevity

As mentioned above, starvation possesses some remarkable effects, one of which is autophagy induction. Such dietary restrictions have also been associated with reduced mTOR signalling, that elevate cellular autophagy pathways. This and several other processes, for instance, reduced Insulin/IGF-1 signalling and reduced mitochondrial respiration rates have been associated with augmentation of life span in a variety of models including mice, *Drosophila* and *C. elegans* (Nakamura and Yoshimori, 2018).

Insulin and IGF-1 are known to phosphorylate and inactivate kinase proteins like ULK-1 which are otherwise activated by AMPK mediated phosphorylation, at a different position and initiate autophagy pathways (Kim et al., 2011). Studies in *daf-2* mutants, a gene for insulin family receptor in *C. elegans*, have reported the necessity of mitophagy related genes like kinase *pink1* and E3 ubiquitin ligase *park*, for successful and complete lifespan expansion in the worm (Palikaras et al., 2015). Increased cellular oxidative stress has always been associated with escalation of ageing. ROS production during mitochondrial respiration eventually increases with age and so does the process of ageing in the organism. Studies have shown that mitochondrial mutants of electron transport chain exhibit longevity in *C. elegans*, whereas, mutants of autophagy related genes have been reported to exhibit reduced lifespan in the same model organism (Tóth et al., 2008; Lapierre et al., 2013; Nakamura and Yoshimori, 2018).

Similar genetic manipulation studies in *C. elegans* also suggest premature ageing phenotypes caused due to autophagic inactivation. Investigations in mouse models insinuate postnatal death in whole body Atg gene knockout mice, while tissue specific Atg7 or Atg5 knockout mice have been reported to express inclusion body aggregates in nerve cells, accumulation of certain pigment filled lysosomes, disorganised mitochondria in cells, elevated levels of protein oxidation and reduced muscle mass (Rubinsztein et al., 2011; Nakamura and Yoshimori, 2018). Furthermore, forced activation of autophagy has also been reported to extend life span of organisms. Overexpression of autophagy modulators and lysosomal biogenesis have been documented to extend the life span in

C. elegans (Lapierre et al., 2013), similarly, ATG5 overexpression has shown to enhance the life span in both male and female mice (Pyo et al., 2013). *Drosophila* models with neuron specific expression of ATG8 have shown increased longevity and significant resistance to oxidative stress (Simonsen et al., 2008; Nakamura and Yoshimori, 2018).

Certain pharmaceutical mediators of autophagy have also been associated to aid in lifespan extension in several animal models. For instance, spermidine treatment in *C. elegans*, yeast and mice has shown indirect modulation and transcriptional upregulation of several autophagy associated genes (Eisenberg et al., 2009). Urolithin A exposure in *C. elegans* has been reported to prevent the accumulation of defective mitochondria and increase longevity (Ryu et al., 2016).

How does autophagy differ from apoptosis?

Autophagy is not the only mechanism that can be activated during cellular stress, apoptosis is also a fundamental pathway, which can be activated, depending upon the stress stimulus. Although, both autophagy and apoptosis processes cause degradation of own components, the pathways followed and the basis of these processes differ to a great extent. These are activated by different triggers and the pathway execution involves varied proteins (Coleman et al., 2016). The fundamental difference between autophagy and apoptosis is that activation of autophagy pathway aims at cell, whereas apoptosis pathway activation leads to cell death (Fan and Zong, 2013). However, excess autophagy can also lead to cell death. In autophagy, cellular components are degraded leading to healthy cell survival and balancing energy sources (Fan and Zong, 2013). When it comes to apoptosis, a cell actively kills itself in order to maintain proper functioning in the organism. It is known that excess of autophagy eventually results in cellular death, whereas excess of apoptosis leads to atrophy, hence these mechanisms are tightly regulated in the cell (Martinez et al., 2010). Since both processes in excess would eventually lead to cell death, they have to be tightly regulated regulated in the cell (Martinez et al., 2010).

Another point of difference between the two processes is that lysosome mediated degradation which occurs in autophagy is not observed in apoptosis. Instead, apoptotic stimulus causes leaking of mitochondrial intermembrane space components like Cytochrome C into the cytosol, thus activating the apoptotic pathway proteins, eventually resulting in a systematic fragmentation of the DNA, not observed in autophagy. This degradation can be detected by the TUNEL assay and confocal microscopy (Martinez et al., 2010; Coleman et al., 2016). Along with these, molecular probes like JC-1, rhodamine 123 and mitotracker red have a wide application in detecting defects in mitochondrial membrane potentials (Martinez et al., 2010). Several other markers have also been used to detect apoptosis like annexin V and caspase detection. Annexin V labelling is employed in both light microscopy and flow cytometry to identify and study the mid and late-stages of apoptotic cells.

Detection of Autophagy

Autophagy is majorly detected using primary molecular techniques like electron microscopy, western blotting and fluorescence microscopy. Most commonly, specific antibodies designed against LC3 marker are employed to detect autophagosome formation in western blot and immunofluorescence techniques. LC3 conjugated GFP levels, monitored via flowcytometry have aided in autophagy detection in live mammalian cells (Shvets at al., 2008). Similarly, degradation of p62, a receptor for autophagy which accumulates with aggregation of ubiquitinylated proteins, is being exploited as a marker for autophagy and its levels are determined with the help of western blot (Bjørkøy et al., 2005). Certain stains like acridine orange (AO), a fluorophore, have the ability to accumulate inside acidic vesicular organelles and is used to study development and maturation of autolysosomes using fluorescence microscopy and flow cytometry. A similar staining principle is followed by stains like DAPRed and DALGreen which are useful in detection of autophagosomes and phagosome-lysosome fusion, respectively (Coleman et al., 2016). Additionally, estimating the levels of beclin-1, a protein which forms complex with several

transcription factors and induces autophagy, has also been an efficient marker for quantitative analysis of autophagy (Kang et al., 2011). Although these techniques aid in observing the occurrence of the process, there is still a long way to go before the regulation of autophagy in different tissues of organisms is well understood.

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Review article

Nanoparticles for Smart Delivery of Pesticides and Fertilizers

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Abstract

An increase in the world population has led to an undue upsurge in the production of agricultural goods. To compete with such demands, persistent, unwarranted use of chemical fertilizers and pesticides emanates. This causes damage to the soil along with the microbial flora present in that soil and affects soil fertility. This review focuses on discussing the potential of nanoparticles as a delivery system for fertilizers and pesticides. Due to their unusual fundamental properties, nanoparticles show great potential to be the perfect carriers/ vectors/ encapsulators for many transitory and unstable compounds, like insecticides, pesticides, enhancers, hormones, and fertilizers, thus providing much better release and less damage to the environment.

Introduction

An increase in the population worldwide has forced the agricultural sector to produce more crops to satisfy the needs of billions of people (Rai et al., 2015). It is predicted that there will be around 70% increase in the agricultural yield by 2050 to meet the demands of the growing population (Dr Amanullah, 2020a). To meet such demands, the farmers use excess quantities of inorganic fertilizers, synthetic chemicals and pesticides (Oldfield et al., 2019). These practices affect the soil and water present in the vicinity by causing soil erosion and groundwater contamination. All this also hampers the nutritional value of the food (Gudovan, 2017; Dr Amanullah, 2020). Thus, there is an urgent need for developing pesticides and fertilizers that will be released into the soil in a controlled and proportionate manner.

Usually, pesticides and fertilizers are sprinkled or sprayed on the plants and soil in an unquantified manner. They settle on the soil and contaminate it causing soil pollution, a decrease in fertility, and groundwater pollution. It also gives rise to resistant varieties of organisms (Oldfield et al., 2019; Srivastav, 2020). Sometimes the residues from these antimicrobials and anti-insecticides persist on the vegetables and may get ingested by humans. Also, direct contact with these chemicals affects the farmers and other workers that work with them (Coronado et al., 2004; Hudson et al., 2014). With the use of Nanotechnology and Nanomaterials, it is possible to control the release of these materials into the soil and on the plants. This will increase the efficiency of these active ingredients while ensuring adequate usage (Yusoff et al., 2016; Ghorbanpour et

al., 2017; Kumar et al., 2019) which, will guarantee comparatively less soil and groundwater pollution (Camara et al., 2019; Kah et al., 2013)

It is hoped that nanotechnology will aid in the following processes.

- It will increase the solubility of pesticides and fertilizers
- Permit the slow release of these chemicals
- Prevent the early degradation of pesticides and fertilizers, providing extended stability.

Various technologies including electroporation and ultrasound are available for transporting agricultural constituents to seeds or cells in culture. However, of all of these, nano-carriers hold great promise to carry agricultural ingredients, due to their capability to transport complex bundles of ingredients across biological barriers and target specific tissues (Karny et al., 2018).

The use of Nanotechnology has also been explored in hormone delivery, nanosensors to detect plant diseases, nanobarcoding and controlled release of agrochemicals.

Thus, here in this review, various types of nanoparticles that are used for encapsulation and release of agrochemicals are discussed.

Advantages of Nanoparticles as efficient drug delivery system:

The term Nanoparticles is a derivative of the term 'nano' which means submicroscopic or small particles that come in the size range of 10^{-9} m. (Wilczewska et al., 2012). Any material when reduced to a submicroscopic size range depicts properties that differ from the bulk material and can be efficiently used for drug delivery of agrochemicals (Couvreur, 2013; Yusoff et al., 2016). The very first nanosystem developed for drug delivery was the liposomes, then the mesoporous silicon nanoparticles followed and others later (Sur et al., 2019; Wu et al., 2011).

Pesticides that are utilized extensively in agricultural fields have certain properties that make them, undesirable. Some of these are:

1. Deterioration of plant tissues by herbicide like 'Paraquat',
2. Extensive and intense, unintentional effects on non-targeted arthropods, such as damsels, spiders and bugs by 'Permethrin',
3. Impact on non-target pests, including mammals by Thiocarbamates, an organosulphur compound.
4. Other than this some agrochemicals are inept and get accumulated in the environment instead of reaching the target due to leaching, immobilization, erosion, and volatilisation. (Yusoff et al., 2016).

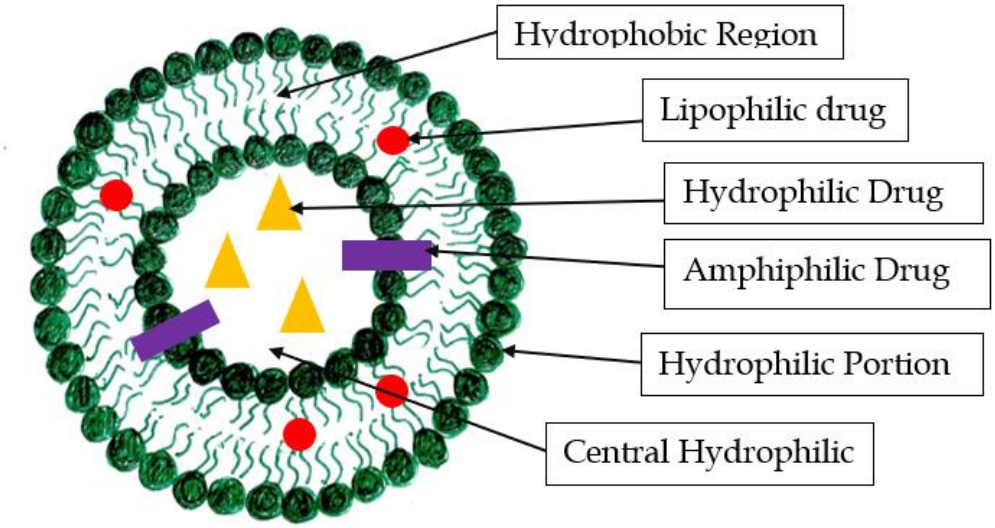
Nanoparticles can overcome these shortcomings. Due to the small size, they can enter into small capillaries which helps the desired molecule to reach the target. These molecules have a large surface area which makes it possible for them to display a large number of surface groups (Sur et al., 2019; Wu et al., 2011). The Nanoparticles prepared from synthetic polymers like poly(d,l- lactide-co-glycolide) (PLG), poly(d,l-lactic-coglycolic acid)(PLGA) are acknowledged for their exceptional biocompatibility and biodegradability (Zhao et al., 2014). The MSNs (Mesoporous Silica Nanoparticles) are well known for having resistance to microbial attack and chemical steadiness (Yusoff et al., 2016). The most relevant property of nanoparticles is that they allow the controlled and precise release of the encapsulated material (Wu et al., 2011).

Types of potential Nanoparticles that can be used as a delivery system

1. Liposomes:

Liposomes were first introduced in the 1960s by British haematologist Dr Alec D. Bangham and collaborators at the Babraham Institute, University of Cambridge (Bangham & Horne, 1964). They are vesicles that are made up of a lipid bilayer. They can be defined as colloidal sphere-shaped structures formed by the self-assembly of amphiphilic lipid molecules in solution, such as phospholipids (Sebaaly et al., 2016).

They consist of hydrophilic molecules as the internal core and hydrophobic molecules within the bilayer & amphiphilic molecules at the water/lipid bilayer interface **(Fig. 1)**.



The drug can be loaded either in the hydrophobic region or hydrophilic region or amphoteric region. They are also biocompatible, biodegradable, non-toxic and non-immunogenic (Mathiyazhakan et al., 2018). This makes them the most versatile carrier system for easy drug delivery.

Apart from being able to accommodate various types of molecules, the liposome easily fuses with the plasma membrane by endocytic pathways (Guimarães et al., 2021). These liposomes can also be personalized by modulating the physicochemical properties of the lipid bilayer, for example, the phase transition temperature (Tm) or enriching them with sterols (Karny et al., 2018). Liposomes provide great assistance with the delivery as they are stable in aqueous environments due to their amphiphilic nature and their stability can be varied by using phospholipids with extended tails, and low degrees of tail unsaturation and ether linkages, (Guimarães et al., 2021). For example, liposomes having phospholipids with saturated hydrocarbons that have longer chains form rigid bilayer structures and shorter unsaturated hydrocarbon chains form disordered bilayers (Kapoor et al., 2017; Rawicz et al., 2000). Due to the underlying properties, they can be altered and modified easily according to the needs of the recipient entity.

Fig 1: Liposomes and the drug encapsulation (adapated and modified from Guimarães et al., 2021)

Many phospholipids can be used for making liposomes. Some of them are phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE),

phosphatidylinositol (PI), phosphatidylglycerol (PG) and phosphatidylserine (PS) (Guimarães et al., 2021). Amongst these, the most common and naturally found phospholipids are PC and PE. They are abundantly present as phosphatides in plants and animals. Hence, if plant-derived liposomes are utilised, they are assumed to be a familiar entity and absorbed into the plant parts without any hypersensitivity reactions or rejection. This has been substantiated when nano-liposomes made from soy-derived phospholipids like PEGylated liposomes were successfully used to deliver plant nutrients like Mg & Fe etc (Karny et al., 2018).

Even though liposomes hold a lot of promise in providing a solution for drug delivery in plants they have not yet been commercially manufactured in large quantities. The major reason for this is a lack of stability, reliable sterilization methods, batch-to-batch reproducibility, low drug entrapment, particle size control, production of large batch sizes and short circulation half-life of vesicles.

Chemical and physical instability are both major concerns when it comes to liposomes, the chemical stability may alter because of hydrolysis of ester bond and/or oxidation of unsaturated acyl chains of lipids. Certain unsuitable drugs may leak out of the liposome and thus hamper the physical integrity of the liposomes (Sen et al., 2014). This issue can be solved by lyophilization of liposomes but this will affect the production cost tremendously. Sterilization of phospholipids is difficult because of the thermolabile and sensitive nature of phospholipids to sterilization procedures related to the use of heat, radiation and/or chemical sterilizing agents. The liposome design of a drug can only be industrialized if the encapsulation efficiency is such that the dosages could be carried in an amount of lipid that will not cause toxicity or alter the non-linear (saturable) pharmacokinetics of that liposomal drug preparation. Another factor affecting liposomal drug delivery is the lysosomal disintegration that occurs due to

Table 1: Soybean phospholipid content (adapted and modified from Chew & Nyam, 2020).

Oil	**Phospholipids (%)**	**Phosphorous (mg/kg)**
Cottonseed	1.0 – 2.5	400 – 800
Peanut	0.3 – 0.7	100 – 250
Corn	0.7 – 2.5	250 – 700
Palm	0.04 – 0.1	15 – 30
Soyabean	**2.0 – 5.0**	**600 – 1500**
Sunflower	0.5 – 1.0	200 – 400

the enzymes present in the soil or the plant tissues (Sharma & Sharma, 1997).

2. Mesoporous Silica Nanoparticles:

These are the latest and most promising types of nanoparticles studied to be used as drug delivery systems for plants. The first claim that Mesoporous Silica Nanoparticles (MeSNPs) (MSNs) can be efficiently used as drug delivery nanoparticles was in 2001 (Tang et al., 2012). MSNs, are chemically and thermally stable mesoporous structures.

They also have large surface areas (0.800 m^2g^{-1}), tunable pore sizes (2–10 nm in diameter) and distinct surface properties **(Fig. 2)** (Serna, 2010). These qualities ensure that materials can be easily compatible with loading in MSNs. (Torney et al., 2007) The drugs entrapped in these molecules can be released using capping agents (chemicals that cut the bonds attaching the caps to the MSN) that can trigger the release of the drug entrapped (Torney et al., 2007; Karny et al., 2018). In addition, the ability to control the release as per the need of the environment gives them an edge over other drug loading materials. Recent development suggested that some of the gatekeepers like, PEGylation of MSNs (Gao et al., 2020; Palanikumar et al., 2015), curcumin coating (Xu et al., 2016), temperature-sensitive poly (ethylene glycol)/poly (ε - caprolactone) (PEG/PCL) multiblock copolymer (MBC-MSN), which has a dense, crystalline structure, (Cho et al., 2017) calcium chloride and sodium chloride together (MON-CaC) (Gao et al., 2020), amine cross-linked fluorescein isothiocyanate (MSN-APTES-FITC) if added to the conventional MSN molecules, increase the rate of absorption of the MSN's within the cell **(Fig. 3)** (Rastegari et al., 2021).

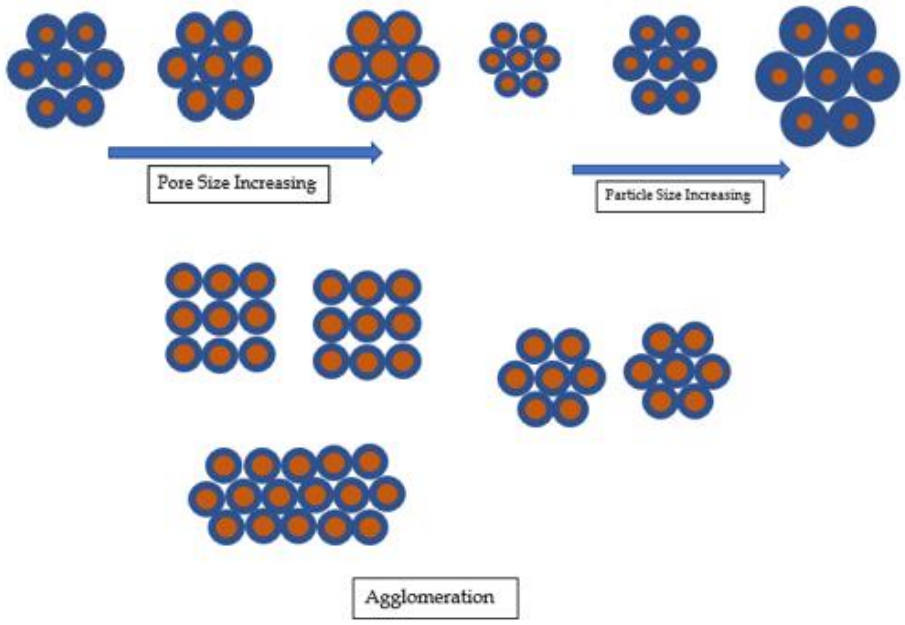


Fig 2: Tunable physicochemical properties of MSNs (adapted and modified from Rastegari et al., 2021).

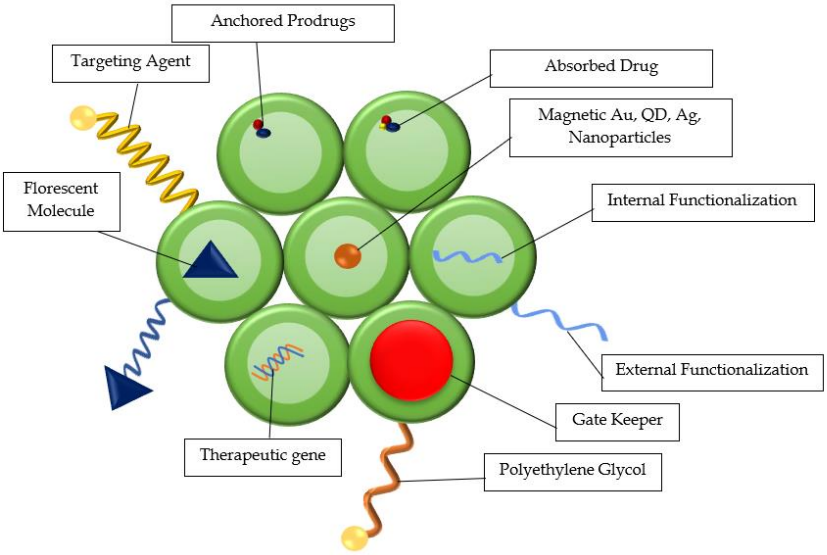


Fig 3: MSN pores with different surface modifications allow the construction of smart MSNs with controlled drug delivery and release, required for various therapeutic and diagnostic applications (adapted and modified from Rastegari et al., 2021).

These particles can be also be modified into any shape and size and morphology like a sphere, rod, to wormlike structures, by tailoring the molar ratio of silica precursors and surfactants, pH control using base catalysts addition of co-solvents or organic swelling agents, and by introducing oraganoalkoxysilane precursors during the co-condensation reaction (Tang et al., 2012).

MSNs are known to load a high concentration of drugs and protect the loaded drug from enzymatic damage by loading it within the porous structure (Palanikumar et al., 2015; Yiu et al., 2007). They also show potential for targeted and stimuli-responsive drug delivery (Rastegari et al., 2021)

3. Plant-Derived Nanoparticles (PDNPs)

Plant-derived nanoparticles are nanoparticles that are released from plants like ginger, broccoli, lemon, grapefruit, neem, clove, etc. (Gahukar & Das, 2020; Yu et al., 2020). These molecules being plant-derived can prove to be the most eco-friendly, simple, safe, low-cost, and relatively less toxic option for drug delivery (Iravani & Varma, 2019). Nanoparticles that have been isolated from diverse edible plants (grapefruits, grapes, ginger, carrots and citrus lemon) have been characterized. Plant-derived nanoparticles are usually prepared by differential centrifugation followed by a density gradient centrifugation step using plant juices as starting material (Negula et al., 2017).

Plant-derived natural pesticides, insecticides, and fungicides have been reported to be more efficient than chemical products Plant-based versions depends on the action of flavonoids, alkaloids, and terpenoids, present in plants. By modulating the molecule size and quality they can be effectively used as oviposition deterrent, repellent, antifeedant, insect growth regulator and toxic substances (Gahukar & Das, 2020). Plant-derived Nanoparticles should be adapted for use on a large scale since products like crude oil, water extracts, essential oils, seed cakes, etc. derived from plants and their waste are used by farmers on small scale and prove to be effective. Conventional PDNP's have been verified to be as effective as marketable/formulated formulations (Isman, 2017; Lee et al., 2013).

Table 2: Comparison chart between different types of MSN particles

Mesoporous silica/ Nanoparticle MSN	Gate keeping agent	Abbreviation	Abilities	Treiggering molecule	Reference	Mesoporous silica/ Nanoparticle MSN
MSN	Aminopropyl triethoxy silane	MSN-APTES	Absorbed by plants	-	(Hussain et al., 2013)	MSN
MSN	Aminopropyl triethoxy silane-fluorescein isothicyanate Introduction	MSN-APTES-FITC	Absorbed by plants	-	(Hussain et al., 2013)	MSN
MSN	Polyethylene glycol	PEG-MSN	Used for DNA transfer in plants.	DTT (dithiothreitol)	(Torney et al., 2007)	MSN
MSN	Polyethylene glycol lipids	PEG-LipoMSNs	Increased bioapplicability with more than 10 times prolonged half-lives and decreased distribution in reticuloendothelil system-related organ	-	(Tang et al., 2012)	MSN
MSN	Large pores-curcumin	MSNs (LPCC)	Curcumin could be entrapped in the pores with nearly no leakage and is triggered by GSH	GSH (Glutathione)	(Xu et al., 2016)	MSN
MSN	Large pores-curcumin/ F127	MSN-LPCC-C-F127	Curcumin could be entrapped in the pores with nearly no leakage and is triggered by GSH	GSH (Glutathione)	(Xu et al., 2016)	MSN
MBN- MSN (multiblock)	Poly (ethylene glycol)/ poly (ε-caprolactone)-multiblock	PEG/ PCL-MBC-MSN	Can release the drug in heat sock conditions and will not release unless heat shock is present	Heat shock	(Cho et al., 2017)	MBN-MSN (multiblock)

Likewise, plant-based essential oils (EOs) showed better performance than extracts and allelochemicals, in bio-efficiency (Yu et al., 2020). Clove oil is used against *T. castanuem* the nanoparticles of clove oil protect the crop for more duration than the clove oil itself (Ikawati et al., 2021). These particles are not yet used for plant pesticides or fertilizers, instead, they have been used for the delivery of drugs for cancer (Iravani & Varma, 2019; Yu et al., 2020).

Conclusion

Use of Nanoparticles is a new, emerging field that holds great promise in providing solutions for many problems like drug delivery, cargo loading of drugs and targets specific releases. They also show

more resistance against damage from UV radiation and an aqueous environment. Nanoparticles thus provide a more stable product that can be used more efficiently than the traditional available products. Liposomes, Mesoporous silica/ organosilica Nanoparticles and Plant-derived nanoparticles are some developing subfields of nanoparticles that can be explored to deliver plant fertilizers and pesticides for agricultural use.

Liposome nanoparticles can be made comparatively more easily than Mesoporous silica/ organosilica Nanoparticles and Plant-derived nanoparticles but the latter pose advantage over liposomes as they are more stable when exposed to the environment. Mesoporous Silica/Organosilica Nanoparticles are a new approach but they are more stable and can be adapted than the Liposomes for drug delivery of insecticides, fungicides and pesticides. These molecules can be modified using gate-keeper polymer updates, which allows them the ability to be activated and release the drug only when required. However, more research is required to ascertain its effect on the environment and plants when exposed for a long time. PDNP's on the other hand are eco-friendly as they are created from plant materials and exudates. PDNPs can be used to entrap, various other therapeutic agents, like plant secondary metabolites (Curcumin), lemon nanovesicles proteins, siRNAs, miRNAs, chemotherapy drugs and DNA expression vectors.

There are many innovative emerging Nanoparticles like carbon nanotubules, PEG polymers, and Plant Exosome nanoparticles that are understudied to be used as effective delivery systems for delivering plant pesticides, insecticides and fungicides. More research is required on these nanoparticles to study the effects on the environment.

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Nobel Prize in Science 2021

Nobel Prize in Physiology or Medicine 2021: Unravelling the enigma of sensing temperature and pressure

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Abstract

The sensations such as heat, cold and pressure are all important for perceiving and reacting to our environment. It is crucial to understand how these work in order to treat chronic pain and other ailments. When neural signals from the terminals of nociceptors (pain receptors) reach second-order neurons in the spinal cord or brainstem, they are transmitted to specific higher-order brain areas, resulting in the perception of pain at locations throughout the body. Some of the molecular mechanisms underlying the transduction of noxious stimuli have begun to be elucidated in recent studies. Many stimuli have been shown to activate ion channels on nociceptor terminals, which function as molecular transducers to depolarize these neurons and cause nociceptive impulses to move through pain pathways. Members of the transient receptor potential (TRP) family are among these ion channels. Two such channels of the TRP family, namely - TRPM8 and TRPV1 were discovered by one of the Nobel laureates in Physiology or Medicine 2021, David Julius. The other Nobel laureate sharing the prize is Ardem Patapoutian. Many physiological processes, such as touch and pain sensation, hearing, and blood pressure regulation, are influenced by mechanical stimuli. Ardem Patapoutian and his team discovered two such mechanosensitive pressure receptors – Piezo 1 and Piezo 2. These are multipass transmembrane proteins found in vertebrates and invertebrates, as well as plants and protozoa.

About the Research

Our ability to detect heat, cold, and touch is essential for life and governs how we interact with the environment. David Julius (University of California, San Francisco) and Ardem Patapoutian (Scripps Research in La Jolla, California) received the Nobel Prize for Physiology or Medicine 2021 for their outstanding work in identifying the gene and understanding the mechanism by which our bodies perceive temperature and pressure. David Julius has been a longtime faculty member at UC- San Francisco, whereas Ardem Patapoutian, who is a memorable alumnus of the same institute (UC- San Francisco) has done his postdoctoral research in the lab of Louis Reichardt from 1996 to 2000.

Prior to the discoveries of David Julius and Ardem Patapoutian, the topic of how temperature and mechanical stimuli are converted into electrical impulses in the nervous system was essentially unanswered. David Julius identified a

heat-sensitive sensor in the skin's nerve endings using capsaicin, a strong chemical found in chilli peppers that generates a burning sensation, whereas Ardem Patapoutian identified a novel sensor that responds to pressure caused due to mechanical stimulation in the skin and the internal organs.

Our ability to perceive touch and temperature, particularly unpleasant temperature, is vital to our survival and defines how we interact with our internal and external environments. Chronic pain occurs when the response to pain fails. David Julius identified the potential for major advancements in the late 1990s by studying how the chemical compound capsaicin causes the burning sensation experienced when one comes into contact with chilli peppers (Caterina et al., 1997).

After Julius had characterized the heat-sensing capsaicin receptor, Julius and Patapoutian began working independently on the body's mechanism for sensing cold in the early 2000s. While their methods differed, they both used menthol in their experiments, which led to the discovery of the same receptor -- TRPM8 (Transient Receptor Potential cation channel subfamily M8). TRPM8 belongs to the Melastatin TRP channel subfamily. TRPM8 was the first cold-activated ion channel discovered and the role of TRP ion channels in thermosensation was established thereafter (Peier et al., 2002; McKemy et al., 2002).

Capsaicin was known to activate nerve cells but the mechanism of generation of pain was ambiguous. Dr. Julius and his colleagues compiled a database including millions of DNA fragments corresponding to genes expressed in sensory neurons that respond to pain, heat, and touch. They hypothesized that the library would contain a DNA fragment encoding a capsaicin-reactive protein. Individual genes from this collection were expressed in cultured cells that do not normally react to capsaicin. After long research, a single gene was discovered that could make cells sensitive to capsaicin.

Following up on the discovery, it was determined that the identified gene produced a unique ion channel protein known as TRPV1 (Transient Receptor Potential cation channel subfamily V1), which was named after the previously discovered capsaicin receptor. TRPV1 belongs to the Vanilloid TRP channel subfamily. It is an ion channel that opens in response to heat in cell membranes. When Julius studied the protein's ability to respond to heat, he discovered a heat-sensing receptor that gets activated at temperatures that are perceived as painful (Tominaga et al., 1998).

The TRPV1 receptor is the most studied member of the TRP family to date. This is due to the fact that it is the only one activated by the vanilloid capsaicin. This activation causes an influx of calcium and sodium, which depolarizes the cell at negative holding potentials. Capsaicin can activate TRPV1 in isolated membrane patches lacking the intracellular signaling machinery. The binding of at least two capsaicin molecules is required for complete activation of this channel, according to results from binding assays (Szallasi et al., 1999) and electrophysiological recordings (Hui et al., 2003).

Additionally, inhibition of TRPV1 has been shown to be therapeutically useful (DiMarzo et al., 2002). TRPV1 channel is thought to comprise of six transmembrane domains, with a short hydrophobic stretch between the fifth and sixth transmembrane domains that form a pore. It is activated by heat (>43°C), low pH (Tominaga et al., 1998), voltage (Gunthorpe et al., 2000; Piper et al., 1999), and various lipids (Julius & Basbaum, 2001; Caterina & Julius, 2001; Clapham, 2003; Prescott & Julius, 2003) in addition to the vanilloid capsaicin (Caterina et al., 1997). TRPV1 is inactivated in cells by binding to PIP2. It is unblocked by PIP2 hydrolysis which is mediated by PLC (Prescott & Julius, 2003).

The discovery of TRPV1 marked a turning point in the research of temperature-sensing receptors, paving the path for the discovery of others. Additional TRPV1 and TRPM8-related ion channels that are triggered by a wide range of temperatures have been found. Many laboratories pursued research programmes to investigate the roles of these channels in

thermal sensation using genetically manipulated mice that lacked these newly discovered genes (Caterina et al., 2000).

Dr. Patapoutian discovered a new class of mechanical sensors that respond to pressure on the skin and internal organs (Coste et al., 2010), as well as touch perception and proprioception (the ability to feel the position and movement of our body parts) using pressure-sensitive cells (Ranade et al., 2014).

Ardem Patapoutian went on to investigate whether mechanical stimuli can activate these receptors. When individual cells were poked with a micropipette, Patapoutian and his collaborators discovered a cell line that gave off a measurable electric signal. The mechanically activated receptor was considered to be an ion channel, and as a following step, 72 candidate genes encoding potential receptors were found. To find the gene responsible for mechanosensitivity in the cells studied, these genes were inactivated one by one. After a long experimentation, Patapoutian and his colleagues discovered a single gene that, when silenced, rendered the cells immune to poking with the micropipette. Piezo1 is a novel mechanosensitive ion channel that was discovered; it was named so after the Greek word for pressure (píesi). A second gene, named Piezo 2, was also discovered due to its similarity to Piezo 1. Piezo 2 was found to be overexpressed in sensory neurons, and further research confirmed that Piezo 1 and Piezo 2 are ion channels that are activated directly by the application of pressure to cell membranes (Coste et al., 2010).

Piezo1 channels are generally found in non-sensory tissues, such as the lungs, bladder, and skin, with high expression in these tissues; on the other hand, Piezo2 channels are mostly found in sensory tissues, such as Merkel cells found in the skin and mucosa and Dorsal Root Ganglion sensory neurons of the spinal cord. The sequences of Piezo 1 and Piezo 2 are unlike those of other known ion channels or protein groups. Piezo 1 and Piezo 2 have a large number of anticipated transmembrane domains, similar to voltage-activated sodium channels, which have 24 transmembrane domains made up of a 4-fold repeat of 6-transmembrane units (Hanlon & Wallace, 2002). Piezo proteins, on the other hand, have no pore-containing or repeating domains. Piezo 1 is also located in the endoplasmic reticulum, indicating that Piezos can function on the plasma membrane as well as intracellular compartments (Satoh et al., 2006; McHugh et al., 2010).

In somatosensory neurons, Piezo 2 is important for rapidly adapting mechanically stimulated currents. Piezo 2 may so play a function in touch and pain perception (Basbaum et al., 2009; Lewin & Moshourab, 2004). Piezo 1 and 2 are expressed in a variety of tissues, and homologs of these proteins may be found in mammals, plants, and protozoa, suggesting that Piezo proteins may have a broad function in mechanotransduction. Furthermore, Piezo 2 has been demonstrated to play an important part in proprioception, the vital feeling of body position and motion. Piezo channel mutations have also been linked to a number of hereditary human diseases, including autosomal recessive congenital lymphatic dysplasia, hereditary xerocytosis, and an autosomal recessive muscular atrophy with perinatal respiratory distress.

In recent years, Patapoutian's brilliance has been rewarded with the kind of prestigious honours that often pave the way to a Nobel Prize, which include- Memberships in the National Academy of Science, The American Academy of Arts and Sciences. Patapoutian is also a laureate of the Kavli Prize for Neuroscience.

Future Prospects

The discovery of pain receptors and the biological mechanisms that underpin them has piqued the interest of pharmaceutical companies, as they could be utilised to develop novel treatments. Though there are obstacles to overcome before such drugs can be clinically useful, it is hoped that newer approaches will one day overcome them. The groundbreaking discoveries of the Nobel laureates of 2021 have given us a better knowledge of how heat, cold, and mechanical force can activate nerve impulses that allow us to

perceive and adapt to our surroundings. This understanding is being used to develop treatments for a wide range of illnesses, including chronic pain.

TRP channels were discovered to be molecular detectors of thermal stimuli, which answered a fundamental question in sensory transduction: How are thermal stimuli converted into neuronal activity? TRPM8's discovery and subsequent studies elucidating its properties have shed light on the molecular mechanisms underlying cold sensation. TRPM8's biological importance in mediating sensory signaling, as well as its role in non-neuronal tissues, will undoubtedly be investigated further in the future. Although some regions and residues linked to TRPV1 function have been identified, more research into the structure-function relationship of this channel is needed. The upcoming years will be crucial in expanding our understanding of the molecular mechanisms underlying our perception of noxious stimuli.

A better understanding of the structural details, kinetic properties, and structure-function relationships of Piezo channels heralds a new era of research into the pathological and physiological processes of mechanotransduction. Despite this, many new questions have arisen, and new perspectives are beginning to emerge. It's uncertain whether Piezo2 and Piezo1 have identical structures or mechano-gating processes. Some questions that remain unanswered are: Do Piezo channel posttranslational modifications affect their activation? Would a therapeutic approach that targets Piezo channels be effective in treating human diseases caused by Piezo channel mutations? The mechanism by which Piezo channels maintain their open state is also unknown. In order to better understand the structure-function relationship of Piezo channels, future research efforts should focus on obtaining the structures of Piezo channels in various conformational states, as well as a more in-depth investigation of structure-guided functional characterization.

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Nobel Prize in Science 2021

Nobel Prize in Physics 2021: They Found Hidden Patterns in Complex Systems and Predicted Global Warming!

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Abstract

The Nobel Prize is an International award given to a person or organization for their contribution to "the greatest benefit to mankind", in six fields of studies. Several scientists have been given the opportunity to accept this distinguished award over the years. Marie Curie, Albert Einstein, Sir Alexander Fleming, and others are a few amongst them. In the year of the pandemic 2021, Syukuro Manabe, Klaus Hasselmann, and Giorgio Parisi were awarded the Nobel Prize in Physics for their seminal contributions to understanding complicated physical systems. While Parisi received half of the prize for uncovering the interaction of disorder and fluctuations in physical systems ranging from the atomic to the planetary scales, Manabe and Hasselmann together shared half of the award for physical modeling of Earth's climate, quantifying variability, and reliably predicting global warming. Parisi's contributions to the theory of chaotic and random processes were used to forecast global warming. Over the years, climate change and global warming have posed to be serious issues that have gained massive recognition. There can be a positive change if we just begin by doing our part in fighting against the same.

Introduction

Nobel Prize committees over the years have acknowledged the global challenge of climate change and therefore climate modelling has been receiving a great deal of attention. This year three scientists who began to break down and simplify the complexity of the global climate by laying the groundwork for predictions about the planet's future, were given the Nobel Prize in Physics (Rincon, 2021). The laureates discovered strategies to account for the chaos and randomness present in everything from materials to atmospheric motion while still making valuable predictions.

The Nobel Prize in Physics for the year 2021 was shared by Syukuro Manabe, Klaus Hasselmann, and Giorgio Parisi for their revolutionary achievements in understanding complex physical systems. While Parisi bagged his half of the prize for discovering the interplay of disorder and fluctuations in physical systems from atoms present in planetary scales. The other half was shared by Manabe and

Hasselmann for the physical modelling of Earth's climate, measuring its variability, and predicting global warming (Castelvecchi & Gaind, 2021).

The work of the laureates, although very different, circulated around a common understanding of the working of complex systems. A complex system is a system of potentially different elements that interact in different and "seemingly" random ways that is difficult to predict or model theoretically (Zeng et al., 2017). These systems can include a variety of different interplaying components and are seemingly governed by chance and chaos like financial and biological systems or even climate ecosystems, and weather. In the case of weather, even a small deviation can lead to huge differences in the later pedestals (Newberry, 2021). This year's Nobel Laureates for physics have provided a better understanding about such systems and how they develop over the long period.

Syukuro Manabe, who is referred to as the pioneer of the development of physical models of the earth's climate studied how the balance of the energy that was absorbed and released by the Earth interacted with the vertical movement of air masses caused due to convection as well as latent heat of vapour (Rafferty, 2021). This in turn formed bases of multiple climate models. His work, along with his colleague Richard Wetherald published in 1967, correlated doubling of carbon dioxide concentrations in the atmosphere with a 2 °C increase in temperature at the surface of the earth (Manabe & Wetherald, 1975).

Ten years down the line Klaus Hasselmann came up with a model that linked the weather and the climate by postulating a way to outplay the chaotic weather changes that were troublesome for calculations (Mitch Jacoby, 2021). This helped in answering a major question as to how climate models can be relied on, while the weather changes frequently and can be chaotic. He also developed techniques that could prove that anthropogenic activities play a major role in the increasing temperature of the planet and have a direct association with the emission of carbon dioxide. Hasselmann's techniques play an important role in identifying the effect of or fingerprints that natural phenomena and human activities create on climate data (*Nobel Prizes 2021*, n.d.).

Over the years Climate models have been intricately refined with the help of satellite measurements and weather observations that help in mapping complicated interactions. They clearly show increased carbon dioxide levels in the atmosphere and an increased greenhouse effect. It is studied that the earth has heated up by 1°C over the past 150 years (*World of Change: Global Temperatures*, n.d.). Syukuro Manabe and Klaise Hasselmann have contributed to the greatest benefit for humankind by providing a solid physical foundation for our knowledge of the Earth's climate.

While the physics of climate change was studied by the two scientists the discovery of hidden patterns in complex systems was accredited to Giorgio Parisi. In 1979 Parisi discovered a hidden structure in the replica that helped in solving the disorder in the system (Mezard et al., 1988). His discoveries played foundational roles in the understanding of multiple complex systems that explained the many different and "apparently" random phenomena, not only in physics but also in other fields such as neurosciences, mathematics, and even machine learning (Catanzaro, 2021). Professor Parisi's research threw light on what happens when a complex and disordered system is under frustration. He noticed that complex systems can memorize their trajectories and get stuck in a substandard state such that it remains constant for a long period. He studied these patterns by using the replica trick where the disordered system is replicated multiple times and each time the replicas are compared to study the different behaviors of the system. After a point of time, he knew that there would be some repetitive patterns occurring (Mezard et al., 1988). This helped give a better understanding of the complex system by deducting the noisy data. The same concept was used by the meteorologists in order to study the patterns that could be seen in the change of climate by deducting the chaotic weather. Who knew that simple research in theoretical physics would prove to be the foundation of what we now use as a reliable

model to predict something as threatening and destructive as global warming.

The Laureates' and Their Careers

Giorgio Parisi was a physicist who was born in Rome in 1948 and graduated from the University of La Sapienza in 1970 with a Ph.D. in high-energy physics. He studied the Higgs mechanism along with Nicola Cabibbo (Johnston, 2021). Later on, he worked on the theory of collisions of electrons and positrons at the Laboratori Nazionali di Frascati. After working at the University of Rome Tor Vergata, he returned to La Sapienza in 1992. (Nuzzo, 2006). He has been decorated with multiple awards for his work on theoretical physics shedding some light on his discoveries in disordered systems, particle physics, and statistical physics for which he won the Wolf prize in 2021.

Syukuro Manabe was born in Japan in 1931. In 1958, he received his Ph.D. from the University of Tokyo. Then he moved to the United States to work for the US Weather Bureau until 1997. He subsequently returned to Japan to work as the director of the Global Warming Research Division at the Frontier Research System for Global Change. He returned to the United States in 2002 and is now a senior meteorologist at Princeton University (Nuzzo, 2006). Manabe was the inaugural recipient of the Asahi Glass Foundation's Blue Planet Prize in 1992, and he received the Franklin Institute's Benjamin Franklin Medal in 2015. He and Susan Solomon, his colleague, were bestowed with the Crafoord Prize in Geosciences in 2018 for their important contributions to understanding the role of trace gases in the atmosphere and how they affect the Earth's climate system. (*Nobel Prizes 2021*, n.d.).

Klaus Hasselmann was born in Hamburg, 1931. He received his Ph.D. in physics from the University of Göttingen in 1957 after which he transferred to the Institute of Naval Architecture at the University of Hamburg, where he worked until 1961 (Rafferty, 2021). After this, he went to the United States and worked at the Scripps Institution of Oceanography before returning to Germany in 1964 to teach at the University of Hamburg. In 1975 he became a director of the Max-Planck Institute of Meteorology in Hamburg before retiring in 1999. As a young doctoral student way back in 1950 studying physics in Hamburg, Germany he studied fluid dynamics and began noting observations about the ocean waves and current**s**. (Johnston, 2021) Continuing with his oceanography he moved to California where he met his colleague Charles David Kelling (The keeling curve suggests the changes in the carbon dioxide level). Little did he know that his research would be centered around his colleague.

Giorgio Parisi, of Sapienza University in Italy, received the prize for his work quantifying randomness and relating the movement of atoms to the movement of the entire planet (Johnston, 2021) at the age of 73.

The Origin of the Thought

Climate research has evolved significantly in recent years, but the foundations were established many decades ago, even before the Nobel Prizes were presented in 1901. John Tyndall, an Irish physicist, began calculating how much heat various gases in the atmosphere absorb in 1859 (Irfan, 2021). He compared the atmosphere to a dam, suggesting that was the obstacle for the terrestrial rays. He said that just like the river could cause an increase in the local depth of the stream, even the atmosphere can cause a rise in the temperature of the Earth's surface (Manabe & Broccoli, 2020). Svante Arrhenius, a Swedish physicist, computed how much carbon dioxide warms the earth in 1896 and subsequently postulated that increasing carbon dioxide in the atmosphere would cause the planet to heat (Arrhenius, 1896). However, determining how these broader tendencies play out on smaller, more palpable dimensions proved difficult. The amount by which a specific location would warm if greenhouse gas emissions were increased is determined by the local conditions such as the tree cover, rainfall, heat conditions, wind patterns, and how much heat the atmosphere traps.

Why Is the Study of Complex Climate Systems the Need of The Hour?

Global warming and Global climate change has been often depicted as unmitigated disasters for humanity and the environment, but the truth is more complicated. Experts believe that the climate is changing and that anthropogenic CO_2 rises are to blame (Moore, 2008). Climate models have become more complex in the decades since this early study, and computational power has begun to catch up. However, the greatest uncertainty in climate estimates is what mankind will do – whether nations, organizations and people will opt to dramatically reduce harmful emissions. And, as experts explain, this is still an active field of study. It is up to us to work on the findings of the three Laureates and protect the planet from a sad and drastic end. They have given us the knowledge and legacy to understand the process of global warming. Now it is our duty to make the world a better and safer place to live in. The findings that have been recognized this year depict that the understanding of climate as a system is built on a solid scientific foundation that is built of years of careful studies and observations. The Nobel Laureates have not only built a path to intricately study the complex system but have also directed the world by predicting global warming using these refined studies and methods.

Yes, the temperature of the earth is increasing over time but the conditions can be ameliorated using the profound findings of Syukuro Manabe, Klaus Hasselmann, and Giorgio Parisi.

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Nobel Prize in Science 2021

Nobel Prize in Chemistry 2021: Revolutionizing Green Chemistry by Asymmetric Organocatalysis

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Abstract

Building of molecules has always been a vital and challenging area of research, until the development of a new precise tool for molecular construction was envisioned and promoted by last year's Nobel laureates. The Nobel Prize in Chemistry 2021 was awarded jointly to Benjamin List and David MacMillan, for their innovative and substantial work in the development of a third type of catalysis method called asymmetric organocatalysis which is a greener approach towards chemistry. Its thriving applications in the field of pharmaceutical research are noteworthy with remarkable benefits to humankind.

About the Research

Chemists can develop new compounds by connecting simple molecular structures together, such that they bind in the desired and appropriate way. But it is tricky to handle compounds since they are not visible to the eye. To perform this arduous task efficiently, development of a new precise tool by last year's Nobel laureates has made a great impact. The Nobel Prize in Chemistry 2021 was awarded jointly to Benjamin List, one of the directors of the Max Planck Institute for Coal Research in Mülheim, Germany and David MacMillan, University Professor of Chemistry at Princeton University and former chair of the Department of Chemistry (Hargittai, 2021). This award was conferred for their development of asymmetric organocatalysis, a new and innovative method for molecule construction. It has aided in the development of novel medications as well as made chemistry environmentally more sustainable. It has applications in pharmaceuticals, plastics, perfume and food flavouring industries. The Laureates' individual and substantial work in 2000 envisioned and promoted the development of the field of organocatalysis. Organocatalysis accompanies biocatalysis and transition metal catalysis, and has now become the third pillar of catalysis (Brzezinski et al., 2021). Catalysts are substances that speed up processes without being consumed, and are therefore essential tools for chemists. Importantly, the catalysts discovered by the duo can distinguish between left and right-handed forms of molecules, synthesizing different asymmetric molecules that are non-identical from their mirror image. They developed catalysts that can promote asymmetric catalysis, wherein a reaction produces more of the right handed version

of a molecule than the left handed one, or vice-versa (Castelvecchi & Stoye, 2021).

In principle, all the catalysts that were discovered before the year 2000 belonged to one of two groups, namely either metals or enzymes. Metals provided special structural stability that offered excellent catalysing property to them. However, their sensitivity towards water and oxygen limited its application and efficiency in large scale industries. The second form of catalysts is the biocatalysts-enzymes specialised in driving asymmetric catalysis. This group of catalysts generally forms one mirror image out of the two that are possible. Moreover, they also work side by side; such that when one enzyme is done with a reaction, another enzyme takes over. Thus, enzymes can build complex molecules with incredible precision (Deutsche, 2021). Due to their high efficiency, researchers in the 1990s attempted to build new enzyme variants to accelerate the chemical reactions. Enzymes are massive complexes made up of hundreds of amino acids. Do amino acids need to be a part of an enzyme to catalyze a chemical reaction? Or is it possible that single amino acid, or other similar small compounds, could perform the same function? That was Benjamin List, at the Scripps Research Institute in Southern California, who had an out-of-the-box question. He was aware that an amino acid called proline had been employed as a catalyst in research dating back to the early 1970s. Several tests were performed by him without any actual expectations to test whether proline could catalyze aldol reaction. Aldol reaction involves binding of carbon atoms from two distinct molecules. It was a straightforward approach that, miraculously, worked right away. Benjamin List's tests not only proved that proline is an effective catalyst, but also that it can stimulate asymmetric catalysis (Castelvecchi and Stoye, 2021). It favoured the formation of one of the two possible mirror images to develop over the other. Proline is a chemist's dream tool when compared to metals and enzymes. It is a relatively simple in structure, low-cost, and environment friendly compound (Brzezinski et al., 2021). However, Benjamin List was not the only one who felt this way. David MacMillan, who worked at the University of California, Berkeley, likewise was aiming for the same objective.

David MacMillan began by designing basic organic compounds that, like metals, could provide or receive electrons momentarily. Organic molecules are the molecules that make up all living organisms. They have a stable carbon atom structure which is coupled to active chemical groups that often contain phosphorus, sulphur, nitrogen, or oxygen. Organic molecules have complicated properties depending on how they are assembled. MacMillan's understanding of chemistry suggested that, to catalyse an organic molecule in the Diels–Alder reaction in which he was interested, formation of an iminium ion was needed. This would contain a nitrogen atom, possessing an inherent affinity for electrons. He chose several organic molecules with the required properties and examined their ability to carry the Diels–Alder reaction, which is commonly used by chemists to form carbon atom rings Castelvecchi & Stoye, 2021). It worked the way he had hoped and believed, exceptionally well. Several organic compounds demonstrated the property of asymmetric catalysis, with one of the two possible enantiomers accounting for more than 90% of the final outcome. He coined the term Organocatalysis for this technique (Brzezinski et al., 2021).

Since 2000, there has been something akin to a bonanza in this field, with List and MacMillan leading the way. They have identified many low-cost, long-lasting organocatalysts that can catalyze a large array of reactions. Earlier, either each intermediate product had to be isolated and purified in chemical manufacturing operations, or the proportion of by-products was too high. As a result, some of the material was wasted at each phase of the chemical process (Deutsche, 2021). Organocatalysts are significantly more flexible since numerous phases in a manufacturing process can often be conducted in a continuous sequence. This is known as a cascade reaction, and it can help minimize waste in chemical production significantly. Researchers were able to produce strychnine in just 12 stages using organocatalysis and a cascade reaction in 2011 (instead of 29 using

previous methods), and the technique is 7,000 times more productive (Brzezinski et al., 2021). Pharmaceutical research, which often involves asymmetric catalysis, has benefited greatly from organocatalysis. Until the discovery of asymmetric catalysis, a large number of pharmaceuticals comprised both the mirror versions of a molecule; one was active, while the other could occasionally cause certain side effects. With the utility of organocatalysis researchers can now produce vast amounts of diverse asymmetric compounds rather easily and more efficiently (Brzezinski et al., 2021). Moreover, organocatalytic approaches are now collectively being employed with supramolecular catalysis, transition-metal catalysis, photoredox catalysis, and so forth, transforming the method of molecular construction with otherwise unattainable precision and efficiency (Ooi & Crudden, 2021). Benjamin List and David MacMillan were able to look past these prejudices and come up with an ingenious solution that scientists had been pondering for decades. Thus, Organocatalysts have indisputably proved to be one of the most useful research tools in today's scientific world, and are currently offering the greatest benefits to the environment and mankind.

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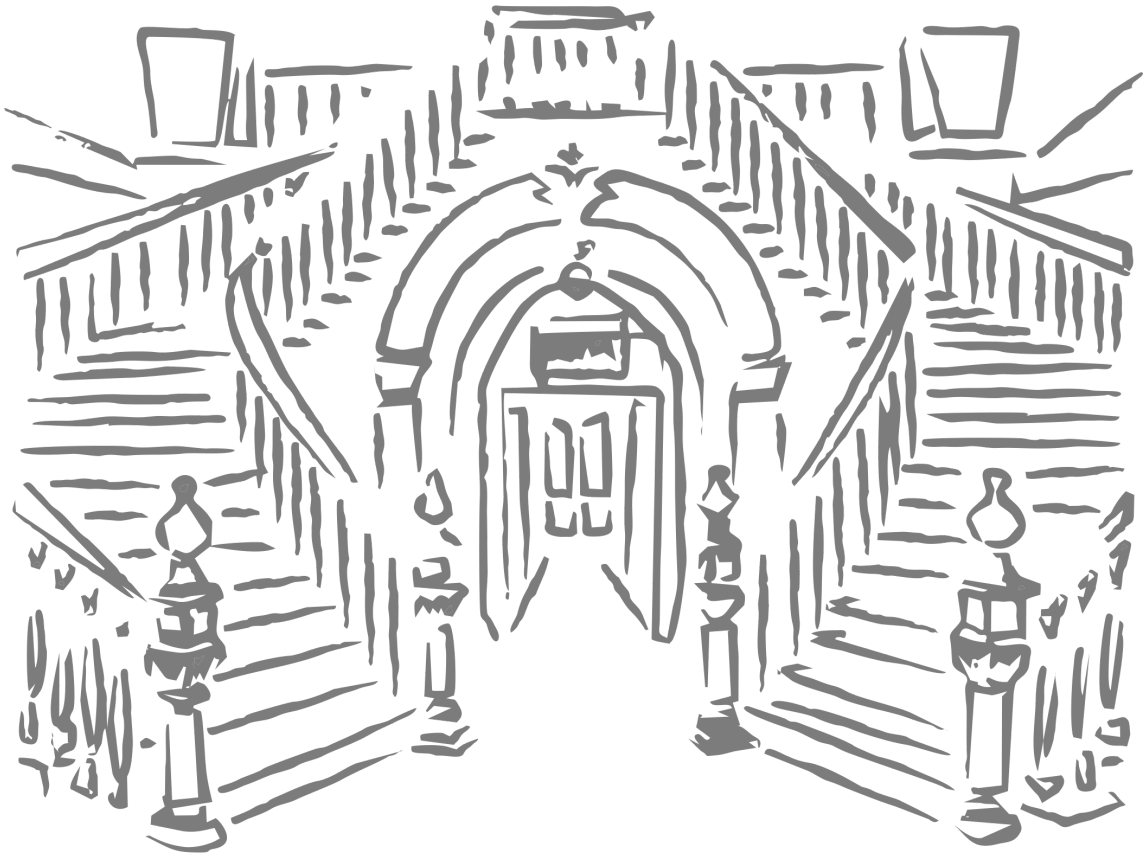

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