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MESSAGE FROM THE PRINCIPAL

Brevity in writing is the best insurance for its perusal.

— Rudolf Virchow

As we move further into the 21st century, it is increasingly clear that science and technology will play an ever-growing role in shaping our world. From climate change to artificial intelligence, the challenges we face require robust scientific research and innovative solutions.

At the same time, the importance of scientific literacy has never been more apparent. In a world where misinformation can spread rapidly through social media, it is essential that the public has access to accurate and trustworthy scientific information.

As a science journal, it is our duty to provide a platform for the latest research and ideas in the field. But we also recognize that we have a responsibility to go beyond publishing research. We must also engage with the public and policymakers to ensure that scientific knowledge is translated into action. This means communicating our findings clearly and effectively and advocating for evidence-based decision-making in all areas of society.

Some of our students, under the guidance of our faculty members, have also actively participated in this research endeavor. This clearly indicates the unique teamwork of our faculty members and students. I strongly believe that this research culture will continue and there will be many more contributions from all the Departments of the College in the years to come.

I congratulate the contributors and editorial board for the successful publication of the journal.

May God bless you in all your endeavors!

Dr. Sr. Lalitha Thomas

Principal

EDITORIAL

SCIENTIA - A Student Journal of Science published by the Research publication and Development Cell, Jyoti Nivas College Autonomous, Bangalore annually.

SCIENTIA is an outcome of the joint efforts of faculty and undergraduate students of physical and Life sciences to help the students to build systematic and effective research techniques, to become knowledgeable about any topic that they need to analyze and write about.

Objective

The ***Student Journal of Scientia*** is devoted to research carried out by students at the undergraduate level. The journal provides a platform for young students to explore their creativity, originality, and independence in terms of research articles. The articles will be judged for suitability of publication in the following two broad categories:

1. Project-based articles
   These articles are based on research projects assigned and guided by Faculty and carried out predominantly or entirely by the student.
2. Articles based on original ideas of studentThese articles are born of student's ideas, and developed by him/ her, withpossible guidance from a faculty. The Journal proposes to provide a platform for such ideas with the hope that this will promote a culture for young students to generate new ideas and expand their potential for creative, original, and independent thinking.

** **It will be an E-journal with no cost to the authors.**

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DEVELOPMENT OF A COST-EFFECTIVE VENTILATOR USING ARDUINO

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ABSTRACT

This project work explains the construction and working of a low-cost ventilator. The motivation for constructing this kind of ventilator comes from the worldwide shortage and cost of ventilators for treating COVID-19 patients. The COVID-19 pandemic has been striking worldwide. This low-cost portable ventilator delivers breath by compressing the Ambu bag or generally known as a manual resuscitator or self-inflating bag, with the help of motor drive mechanism means it will be operated without the help of humans. The low-cost, easy-to-build ventilator performs similarly to a high-quality commercial device.

Keywords: low-cost ventilator, ambu-bag, Arduino, servo motor, COVID-19 patients.

INTRODUCTION

A medical ventilator is a machine that helps lungs work. It can be a lifesaving machine if one has a condition that makes it hard for them to breathe properly or when one can't breathe on their own at all. A ventilator helps to push air in and out of the lungs so the body can get the oxygen it needs.

Mechanical ventilation is a life-saver in the development of modern ICUs. This paper provides an idea of the origin of modern mechanical ventilators. Based on

reviewed literature, a simple, easy-to-use and easy-to-build design of low-cost portable ventilator is proposed in this paper. The proposed ventilator prototype is

assumed to have better working performance than already available in the market at a very low cost. This ventilator will help in the situation like COVID-19 when the whole world is facing difficulties related to ventilators. The last few months have seen an increased demand for ventilators in the treatment of patients with COVID-19, a fact that led to a ventilator shortage worldwide.

This low-cost portable ventilator delivers breath by compressing the Ambu bag or generally known as a manual resuscitator or self-inflating bag, with the help of motor drive mechanism means it will be operated without the help of humans.

In[8] the authors have described the materials and the mechanical, electrical, and electronic aspects used to implement the SURKAN mechanical ventilator, which was developed in Ecuador during the COVID-19 pandemic for some health centers in the country. They have used a POSIFA Flow Sensor that allows the measurement of airway pressure.

In[9] the authors have developed a low-cost ventilator in accordance with guidelines from the US Food and Drug Administration (FDA) for emergency use. This device works by using pressurized medical-grade gases, with the flow rate and tidal volume controlled through time-limited interruptions in the flow. This simplified approach reduces the number of components required compared to traditional ventilators, resulting in faster deployment in clinical settings, increased reliability, and life-saving ventilation support.

METHODOLOGY

This machine supports 500-600ml tidal volume with a continuous working ability for several days. It will provide 12 Respiratory rate (RR/min) that can provide the required amount of tidal volume for pneumonia patients. This work provides a solution to tackle the shortage of ventilators by introducing the low-cost portable ventilators. This Low-Cost Portable Ventilator as shown in figure 1, is an inexpensive and reliable device that can be used to replace bulky and expensive

hospital ventilators. These ventilators can be brought by an individual or by small clinics at much cheaper cost and can be readily available in emergencies. One of

the major advantages of this kind of ventilator is that they do not need a specialized person to operate.

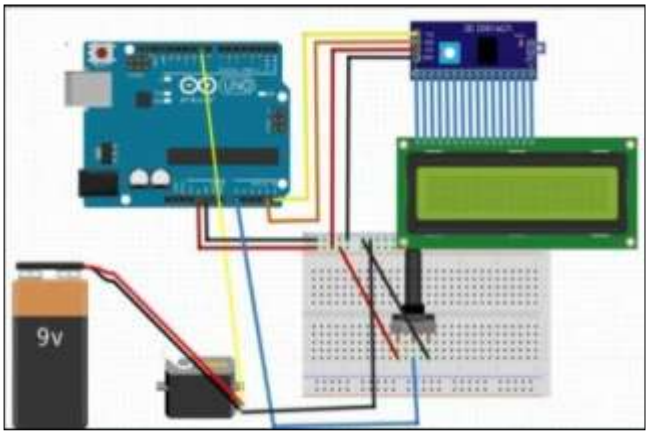

Figure 1: Circuit Diagram of Ventilator

Block Diagram of ventilator

The block diagram of the proposed ventilator consists of Arduino Nano, servo motor, ambu bag, LCD, and a potentiometer as shown in Figure 2.

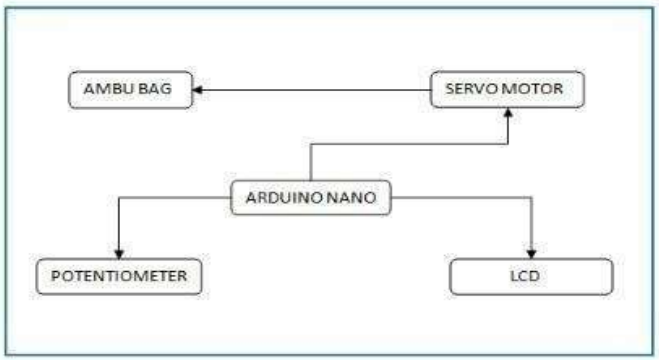

Figure 2: Block Diagram of Ventilator

Components Description

A). Arduino Nano

Arduino Nano is one type of **microcontroller board**, and it is designed by Arduino. cc. It can be built with a microcontroller like Atmega328. It is a small-size board and also flexible with a wide variety of applications. The Arduino Nano is equipped with 30 male I/O headers, in a DIP-30-like configuration, which can

be programmed using an Arduino Software integrated development environment (IDE), which is common to all Arduino boards and running both

online and offline. The board can be powered through a type B mini-USB cable or from a 9 V battery.

The devices required to start our projects using the Arduino Nano board are Arduino IDE and mini USB. The Arduino IDE software must be installed on our respective laptops or desktop. The mini USB transfers the code from the computer to the Arduino Nano board.

Figure 3: Arduino Nano

B). <u>LCD Display and Servo Motor</u>

A liquid-crystal display (LCD) is a flat-panel display or other electronically modulated optical device that uses the light-modulating properties of liquid crystals combined with polarizers. Liquid crystals do not emit light directly, instead using a backlight or reflector to produce images in color or monochrome.

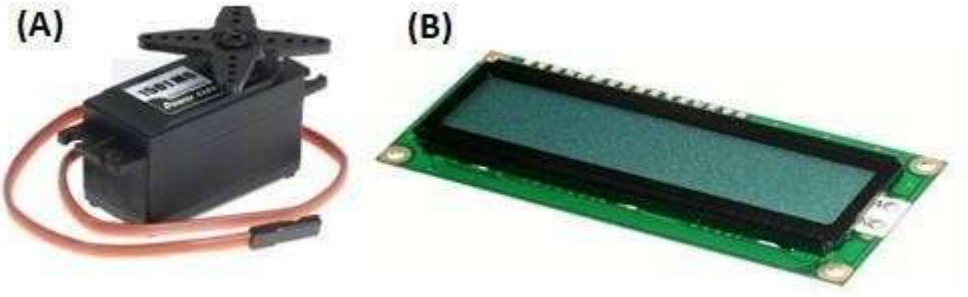

Figure 4 : (A) Servo Motor (B) Servomotor

A servo motor is a type of motor that can rotate with great precision. Normally this type of motor consists of a control circuit that provides feedback on the current position of the motor shaft, this feedback allows the servo motors to

rotate with great precision. If we want to rotate an object at some specific angles

or distance, then you use a servo motor. It is made up of a simple motor which runs through a servo mechanism.

C). Bread Board, Connecting Wires And USB

A **breadboard**, or protoboard, is a construction base for the prototyping of electronics.

Figure 5: (A) Breadboard (B) Connecting wires (c) USB

Connecting wires allows an electrical current to travel from one point on a circuit to another because electricity needs a medium through which to move. In the case of computers, wires are embedded into circuit boards, carrying pulses of electricity that are interpreted as binary signals of zeros and ones. The **Universal Serial Bus (USB)** is the technology that allows a person to connect an electronic device to a computer. It is a fast serial bus.

D). Ambu Bag

A bag valve mask (BVM), sometimes known by the proprietary name Ambu bag or generically as a manual resuscitator or "self-inflating bag", is a hand-held device commonly used to provide positive pressure ventilation to patients who are not breathing or not breathing adequately. The device is a required partof resuscitation kits for trained professionals in out-of-hospital settings (such as ambulance crews) and is also frequently used in hospitals as part of standard equipment found on a crash cart, in emergency rooms, or other critical care settings.

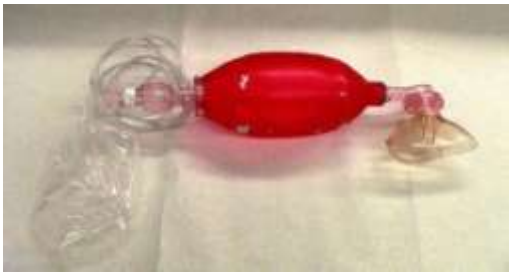

Figure 6 : Ambu bag

E). Potentiometer and resistor

A potentiometer comprises a long wire with a uniform space of the cross area. Normally the wire is comprised of manganin or constantan. Sometimes the wire might be cut into certain pieces and each piece is associated toward the end focuses through a thick metallic strip.

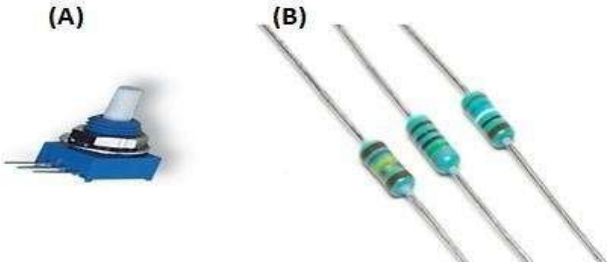

Figure 7: (A)Potentiometer (B) Resistor

A resistor is a **passive two-terminal electrical component that implements electrical resistance as a circuit element**. The current through a resistor is in direct proportion to the voltage across the resistor's terminals.

RESULTS AND DISCUSSION

a) Project Description

After the whole component is set, we upload the code to the Arduino nano. It will show the breath cycle and simultaneously the servo motor starts rotating at an angle of 180 degrees. A wire is used to connect the wooden board to the servo motor. When the servo motor rotates, it pulls the wire and the wooden board slightly presses the ambu-bag. The reservoir bag is connected to an oxygen cylinder and then the ambu bag sucks the oxygen and passes it through the mask.

We can control the breath cycle by rotating the potentiometer.

The code is to control lcd with servo motor

We create servo object to control a servo. We introduce integer of pot pin and val. The analog pin is used to connect the potentiometer and Val is the variable to read values from the analog pin my servo attach(9) it attaches the servo on pin 9 to the servo object

cd. begin(16,2) it used to display 16 letters with 2 rows

Then we print 'Emergency Vent' in first row and ‘MICROCONTROLLER’ on second row of the lcd... Then we delay it for 4000s.

Then we are going to set the potentiometer using the variable valve according to it we are setting the scale of the servo motor

If valve <=30 then we set the position of the servo motor from 0° to 180° using the code for(pos=0 ;pos<=100;pos+=1) and from 180° to 0° using the code for(pos=100 ;pos>=0;pos-=1) for breath cycle of 4 seconds

If valve >=31 && valve<=60 then we set the position of the servo motor from 0° to 180°and from 180° to 0° for breath cycle of 4.43 seconds

If valve >=61 && valve<=90 then we set the position of the servo motor from 0° to 180°and from 180° to 0° for breath cycle of 3.53 seconds

If valve>=91 && valve<=120 then we set the position of the servo motor from 0° to 180°and from 180° to 0° for breath cycle of 5 seconds

If valve>=121 && valve<=150 then we set the position of the servo motor from 0° to 180°and from 180° to 0° for breath cycle of 5.5 seconds

If valve>=151 && valve<=180 then we set the position of the servo motor from 0° to 180°and from 180° to 0° for breath cycle of 6 seconds

If valve<=90 its position from +1 to - 1 and if valve>=91 its position from +0.6 to -0.6

We divide the valve into 6 parts so it can be used to control the servo motor. For each breath cycle the servo motor is kept at a different angle.

b) Implementation of code

```
#include <Servo.h>
#include  <Wire.h>
#include <LiquidCrystal.h>
const int rs = 12, en = 11, d4 = 5, d5 = 4, d6 = 3, d7 = 2;
LiquidCrystal lcd(rs, en, d4, d5, d6, d7);
Servo myservo; // create servo object to control a servo
int potpin = 0; // analog pin used to connect the potentiometer
int val;    // variable to read the value from the analog pin
float pos = 0;
void setup() {
myservo.attach(9); // attaches the servo on pin 9 to the servo object
lcd.begin(16, 2);
lcd.setCursor(0,0);
lcd.print("Emergency Vent");
lcd.setCursor(0,1);
lcd.print("MICROCONTROLLER");
delay(4000);
}
void loop() {
val = analogRead(potpin);            // reads the value of the potentiometer (value
between 0 and 1023)
val = map(val, 0, 1023, 0, 180);     // scale it to use it with the servo (value between
0 and 180)
myservo.write(val); // sets the servo position according to the scaled value
lcd.println(val);
delay(15); // waits for the servo to get there
if (val <=30 ) {
```

```
lcd.setCursor(0,0);          //sets the cursor at row 0 column 0
```

```
lcd.print("Spd:Fast Ang:100 "); // prints 16x2 LCD MODULE
lcd.setCursor(0,1);          //sets the cursor at row 1 column 2
lcd.print("Breath cycle 4 sec ");
for (pos = 0; pos <= 100; pos += 1) { // goes from 0 degrees to 180 degrees
// in steps of 1 degree
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
}
for (pos = 100; pos >= 0; pos -= 1) { // goes from 180 degrees to 0 degrees
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15); // waits 15ms for the servo to reach the position
}}
else if (val >=31 && val<=60  ) {
lcd.setCursor(0,0);          //sets the cursor at row 0 column 0
lcd.print("Spd:Fast Ang:110 "); // prints 16x2 LCD MODULE
lcd.setCursor(0,1);          //sets the cursor at row 1 column 2
lcd.print("Breath cycle 4.43 sec ");
for (pos = 0; pos <= 110; pos += 1) { // goes from 0 degrees to 180 degrees
// in steps of 1 degree
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
}
for (pos = 110; pos >= 0; pos -= 1) { // goes from 180 degrees to 0 degrees
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);// waits 15ms for the servo to reach the position
}}
else if (val >=61 && val<=90  ) {
lcd.setCursor(0,0);          //sets the cursor at row 0 column 0
```

```
lcd.print("Spd:Fast Ang:120  "); // prints 16x2 LCD MODULE
```

```
lcd.setCursor(0,1);          //sets the cursor at row 1 column 2
lcd.print("Breath cycle 3.53 sec ");
for (pos = 0; pos <= 120; pos += 1) { // goes from 0 degrees to 180 degrees
// in steps of 1 degree
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
}
for (pos = 120; pos >= 0; pos -= 1) { // goes from 180 degrees to 0 degrees
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
}}
else if (val >=91 && val<=120  ) {
lcd.setCursor(0,0);          //sets the cursor at row 0 column 0
lcd.print("Spd:Slow Ang:100 "); // prints 16x2 LCD MODULE
lcd.setCursor(0,1);          //sets the cursor at row 1 column 2
lcd.print("Breath cycle 5 sec ");
for (pos = 0; pos <= 100; pos += 0.6) { // goes from 0 degrees to 180 degrees
// in steps of 1 degree
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
}
for (pos = 100; pos >= 0; pos -= 0.6) { // goes from 180 degrees to 0 degrees
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
/// / waits 15ms for the servo to reach the position
}}
else if (val >=121 && val<=150  ) {
lcd.setCursor(0,0);          //sets the cursor at row 0 column 0
```

```
lcd.print("Spd:Slow Ang:110  "); // prints 16x2 LCD MODULE
```

```
lcd.setCursor(0,1);          //sets the cursor at row 1 column 2
lcd.print("Breath cycle 5.5 sec ");
for (pos = 0; pos <= 110; pos += 0.6) { // goes from 0 degrees to 180 degrees
// in steps of 1 degree
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
}
for (pos = 110; pos >= 0; pos -= 0.6) { // goes from 180 degrees to 0 degrees
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
// waits 15ms for the servo to reach the position
}}
else if (val >=151 && val<=180  ) {
lcd.setCursor(0,0);          //sets the cursor at row 0 column 0
lcd.print("Spd:Slow Ang:120 "); // prints 16x2 LCD MODULE
lcd.setCursor(0,1);          //sets the cursor at row 1 column 2
lcd.print("Breath cycle 6 sec ");
for (pos = 0; pos <= 120; pos += 0.6) { // goes from 0 degrees to 180 degrees
// in steps of 1 degree
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
}
for (pos = 120; pos >= 0; pos -= 0.6) { // goes from 180 degrees to 0 degrees
myservo.write(pos);              // tell servo to go to position in variable 'pos'
delay(15);
// waits 15ms for the servo to reach the position
}}
}
```

c) Future Enhancement

The project has a very vast scope in future. This project can be implemented on intranet in the coming years by updating and when requirement for the same arises, as it is very flexible in terms of expansion. The project can be IOT based as well. This smart technology in the area of medical is of great impact. Not only medical experts but common people can increasingly take the benefits of this technology, thus generating a significant improvement in medical care. In this recent covid-19 pandemic, this low-cost medical system is of great benefit to a mass population.

CONCLUSION

The low-cost, easy-to-build non-invasive ventilator performs similarly to a high-quality commercial device, with its open-source hardware description, which will allow for free replication and use in LMICs, facilitating the application of this life-saving therapy to patients who otherwise could not be treated.

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9. How to Make Ventilator Using Arduino & AMBU Bag by **Tech Undefined**

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RESEARCH POTENTIAL AND FEATURES OF CERIUM OXIDE NANOPARTICLES

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ABSTRACT

Nanomaterials are defined as materials with any external dimension in the nanoscale or having an internal structure or surface structure in the nanoscale, with nanoscale defined as the "length range approximately from 1 nm to 100 nm". Excellent catalytic activities derived from mutation of the oxidation state between Ce^{4+} and Ce^{3+} have drawn attention to cerium oxide nanoparticles (CeONPs). Cerium(IV) oxide nanoparticles due to the self-regeneration of their particles exhibit antioxidant properties both in vitro and in vivo states. They are synthesized by the hydroxide-mediated method. The generation of reactive oxygen species (ROS) leads to its excellent exhibition of antibacterial activity against both Gram-positive and Gram-negative bacteria.

Keywords: Cerium oxide nanoparticles (CeONPs), self-regeneration, hydroxide-mediated method, ROS.

INTRODUCTION

Nanoparticles of any material exhibit unique optical, magnetic, mechanical and electronic properties which makes the play a crucial role in wastewater treatment and pollution reduction. Cerium(atomic number-58) being a member of the lanthanide group is the most abundant rare metal. The chemistry of rare earth is different from that of main group elements and transition metals, the reason being the nature of the 4*f* orbitals. Due to the shielding of 5p and 4d electrons in the 4f orbital [1], it exhibits excellent catalytic properties and shows a wide bandgap along with excitation energy [2]. Studies have shown that cerium oxide nanoparticles mimic the features of the enzyme superoxide dismutase (SOD)[3]. Cerium oxide nanoparticles (CeONPs) find applications in corrosive protection, solar cells and catalyst for fuel oxidation [4]. Biosynthesized nanoparticles have

gained a lot of importance as they are simple, efficient, and cost-effective. The biomedical domain considers the diagnostic and therapeutic aspects of this

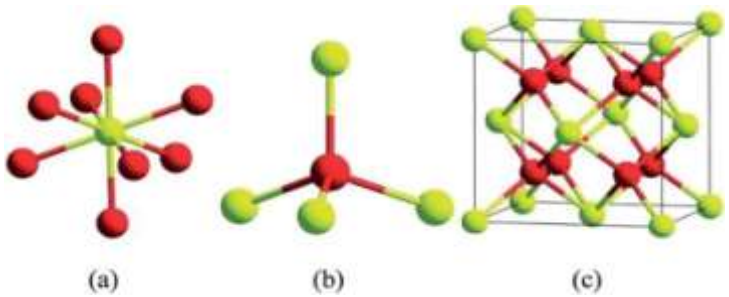
nanoparticle. CeONPs are found to have multi-enzyme, mimetic properties that produce various biological effects. CeONPs are lucrative material in biological fields such as bioanalysis, bio scaffolding and drug delivery.

SYNTHESIS OF CeONPs

Numerous techniques such as flame spray[7], hydrothermal, aqueous precipitation, solvothermal, reversed micelles[8] and thermal decomposition have been reported to synthesize CeONPs while maintaining its size and properties. The synthesized CeONPs can be bare or covered with a coating of protective substances that can be hydrophilic or hydrophobic. Synthetic methods are important because they determine the solubility, size, structural arrangement and morphology of nanoparticles, thus affecting many of their properties. For biological use, biocompatible CeONPs is synthesized in pure water [9].

PROPERTIES

The cerium oxide nanoparticle (Ce_4O_8) is a face-centred cubic (fcc) fluorite lattice comprising eight oxygen atoms bonded to the cerium atom with the complete unit cell (Ce_4O_8) measuring 5.1 Å on an edge [5].



Structurally analyzed ceria crystals Ce_4O_8 (unit cell); in (a) and (b) yellow colour represents eight-folds of cerium atoms and red represents four-fold oxygen atoms in the ceria crystal structure; (c) basic fcc fluorite lattice structure of Ce_4O_8 (taken from K. Reed et al., Environ. Sci.: Nano, The Royal Society of Chemistry, 2014* [6]*).

The Oxygen storing Capacity (OSC) value of cerium dioxide in the gas phase is 1452.47 µmol per O_2 per g and can be explained by the below-mentioned equilibrium reaction:

$$CeO_2 \leftrightarrow CeO_{2-x} + x/2O_2 \text{ for } x = 0.5 \text{ then } CeO_{1.5} (Ce_2O_3)$$

Films of cerium oxide show excellent optical properties thus finding applications as electro-optical and optoelectronic devices[10]. These films have a high

refractive index(range of 1.6–2.4) and transparency in visible and IR (near- and mid-) regions. Cerium oxide nanoparticles have low 3+/4+ ion ratios. Thus they

exhibit high catalyse mimetic activity, which is responsible for the decomposition of a potentially harmful oxidizing agent known as H_2O_2. It produces H_2O and O_2[11]. Cerium oxide nanocrystals ferromagnetic behaviour. Magnetic analysis suggests that Ce^{3+} ions have their magnetic moment, unlike Ce^{4+} ions. But synthesized cerium nanoparticle shows the insignificant influence of Ce^{3+} ions on ferromagnetism.

APPLICATIONS

CeONPs have potential usage in brain tumour-related treatment. According to researchers, CeONPs protect healthy cells from damage and killed malignant glioma cells. CeONPs' antioxidant effects are an approach to treating obesity in Wistar rats. CeONPs hindered the accumulation of triglyceride by causing interference in the adipogenic pathway. CeONPs lessen the plasma levels of triglycerides, glucose, leptin, and insulin. CeONP is also being used in the treatment of **Retinitis pigmentosa and Hepatic ischemia[12].**

CONCLUSION AND FUTURE PROSPECTS

Cerium oxide nanoparticles have been making progress excessively in the field of all sciences especially biomedical sciences as they are being used in the treatment of various deadly diseases. These applications are flourishing in the industry and proving themselves essential to human beings. Advances being made in this field are providing solid proof for its various properties and applications.

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TOXIC EFFECTS OF CADMIUM

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ABSTRACT

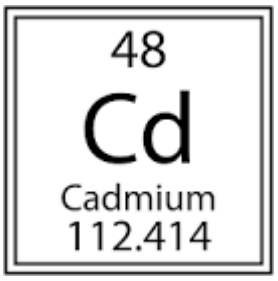
This paper emphasizes cadmium toxicity. It includes Cadmium distribution pattern in our environment, industrial production and uses, exposure and modes of incorporation into the human body and its toxic effects.

The findings revealed that Cadmium is mined and then released into the environment through the air, water and soil during smelting. Due to the widespread nature of its occurrence, it is present in measurable amounts in almost everything that we eat, drink, and breathe. It appears that within the past two decades there has been increased industrial production and use of the metal.

The acute (short-term) effects of cadmium in humans through inhalation exposure consist mainly of effects on the lung, such as pulmonary irritation. Chronic (long-term) inhalation or oral exposure to cadmium leads to a build-up of cadmium in the kidneys that can cause kidney disease. Cadmium has been shown to be a developmental toxicant in animals, resulting in fatal malformations and other effects.

Key words: Cadmium, toxic effects, environment, industrial production, exposure.

INTRODUCTION



Cadmium is a chemical element with the symbol Cd and atomic number 48 and belongs to Group 12 (IIb, or zinc group) of the periodic table. It a soft, malleable, metal found in zinc ores, and to a much lesser extent, in the cadmium mineral

Greenockite.

About three-fourths of cadmium is used in Ni-Cd batteries, most of the remaining one-fourth is used mainly for pigments, coatings and plating, and as stabilizers for plastics. Cadmium has been used particularly to electroplate steel where a film of cadmium only 0.05 mm thick will provide complete protection against the sea. Cadmium has the ability to absorb neutrons, so it is used as a barrier to control nuclear fission.

Cadmium can mainly be found in the earth's crust. It always occurs in combination with zinc. It also consists in the industries as an inevitable by-product of zinc, lead and copper extraction. After being applied it enters the environment mainly through the ground, because it is found in manures and pesticides.

CADMIUM DISTRIBUTION IN THE ENVIRONMENT

Cadmium has been widely dispersed into the environment through the air by its mining and smelting as well as by other man-made routes like usage of phosphate fertilizers, presence in sewage sludge, and various industrial uses such as Ni-Cd batteries, plating, pigments and plastics.

The most important sources of airborne cadmium are smelters. Other sources of airborne cadmium include burning fossil fuels such as coal or oil and incineration of municipal wastes.

When released into the atmosphere by smelting or mining or some other processes, cadmium compounds can be associated with respirable-sized airborne particles and can be carried long distances. Cadmium metal and cadmium salts have low volatility and exist in air primarily as fine suspended particulate matter. It is deposited onto the earth below by rain or falling out of the air. Once on the ground, cadmium moves easily through soil layers and is taken up into the food chain by uptake by plants. Measurements of atmospheric cadmium up to 7 μg/m3 have been reported in these industrial types of areas in the United States.

Cadmium enters drinking water directly from pollution-source releases to surface water and groundwater or from deposition from air to surface water, from soil runoff to surface water, or from leaching from rocks and soils into groundwater. The concentration of cadmium dissolved in the open ocean is less than 0.005 μg/L. The concentration of cadmium in drinking water is generally reported to be less than 1 μg/L, but it might increase to 10 μg/L as a result of industrial discharge and leaching from metal and plastic pipes

UPTAKE OF CADMIUM IN PLANTS

Plants are contaminated with cadmium via two routes—uptake of cadmium in soil through the roots and deposition of cadmium in air onto leaf surfaces followed by

translocation to other plant parts. Cadmium residues in plants are typically less than 1 μg/kg.

CADMIUM IN SOIL (SOIL INGESTION AND DERMAL UPTAKE)

Human intake of cadmium in soil occurs through soil ingestion that results from hand-to-mouth activities. Such intake is typically less important than the inhalation, water-intake, and food-consumption pathways associated with the same soil. Cadmium concentrations in soil vary widely. In nonpolluted areas, they are usually below 1 μg/g; in polluted areas, concentrations of up to 800 μg/g have been detected.

CADMIUM IN FOOD CHAIN

From the soil, certain plants (tobacco, rice, other cereal grains, potatoes, and other vegetables) take up cadmium more avidly than they do other heavy metals such as lead and mercury. Cadmium is also found in meat, especially sweetmeatssuch as liver and kidney. In certain areas, cadmium concentrations are elevated in shellfish and mushrooms. Cadmium can also enter the food chain from water.

The WHO “safe” level for human ingestion of Cd has been estimated at 500 μg/week. Absorption of oral Cd tends to be erratic, with continuing presence of unabsorbed radio-Cd in the gut lumen for 3–5 weeks after a test meal in human subjects. Dietary Cd intake estimates in European countries were 10–30 μg per day, with increased risk of consumption with certain foods such as shellfish, offal, and rice.

In non-Cd-polluted areas, the most significant human route of Cd intake is cigarette smoking, with various estimates of 0.2–1.0 μg Cd assimilated with each cigarette smoked, accounting for approximately half the total human Cd intake

TRANSPORTATION OF CADMIUM IN HUMAN BODY

After absorption, Cd is transported to the liver, bound to albumin, where it induces the synthesis of metallothionein (MT), a class of small cysteine-rich heavy metal binding proteins. Following release from the liver, MT-bound Cd enters the plasma. Cd is eliminated in the urine. MT-bound Cd appears in the glomerular filtrate, from where it is re-absorbed intracellularly by renal tubule cells. In the latter, the Cd is cleaved from the MT by lysosomal action, and Cd^{++} ions are re-excreted into the tubular fluid. **The ability of Cd to induce hepatic and renal lesions exacerbates its toxic effects, and compounds its propensity to accumulate over years.**

TOXIC EFFECTS OF CADMIUM ON HUMAN BODY

Cadmium affects cell proliferation, differentiation, and apoptosis. These activities interact with DNA repair mechanism, the generation of reaction oxygen species (ROS) and the induction of apoptosis. Cadmium binds to the mitochondria and can inhibit both cellular respiration and oxidative phosphorylation at low concentration. It results in chromosomal aberrations, sister chromatid exchange, DNA strand breaks, and DNA- protein crosslinks in cell lines.

Renal damage in cadmium toxicity: Cadmium predominantly accumulates in kidney and liver, but it can be found in other tissues such as bone and placenta. Occupational and environmental exposures to cadmium have implicated renal dysfunction.

Cadmium bone and Itai-itai disease: Several studies mentioned cadmium can affect the skeletal system. Exposure to cadmium caused skeletal demineralization, whereby it may directly interact with bone cells, diminish mineralization, also inhibit procollagen C-proteinases and collagen production.

Itai-itai disease is the most severe form of chronic cadmium intoxication. Cadmium intoxicants cause femoral and low back pain in initial manifestation, the further pain spread to the other areas of the body. Moreover, skeletal deformities can cause bone fractures.

Cadmium and reproductive system: Several previous studies found that cadmium has the potential to affect reproduction and development in several mammalian species, and recent studies have also confirmed these findings. It causes defects in spermatogenesis, sperm quality, and secretory functions of accessory glands. Besides, it decreases libido, fertility, and serum testosterone level. In female reproductive system, the function of ovary and development of oocytes may be inhibited. It has also been reported that the rate of spontaneous abortion and time of pregnancy are increased and the rate of live births decreased.

Cadmium and carcinogenicity: Cadmium compounds were categorized as carcinogenic in humans by International Agency for Research on Cancer (IARC). It may be considered as lung carcinogen, also inducer of prostatic or renal cancers. Cadmium may also be a potential risk factor for breast cancer. Another study suggested that cadmium exposure may be involved in pancreas cancer because of

inducing increased risk for neoplasia.

CONCLUSION

The heavy metal cadmium (Cd) is a pollutant associated with several modern industrial processes. Cadmium poisoning has been reported from many parts of the world. It is one of the global health problems that affect many organs and, in some cases, it can cause deaths annually. Long-term exposure to cadmium through air, water, soil, and food leads to cancer and organ system toxicity such as skeletal, urinary, reproductive, and respiratory systems.

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APPLICATION OF MATHEMATICS IN DATA SCIENCE

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ABSTRACT

Interpretation of data and decision-making includes meaningful insights using data engineering, visualisation and computation. In recent times, Data Science is making its wayinto several discussions and is also being considered a golden opportunity in one's career making. But Data Science as a subject is not complete without the application of various Mathematical concepts to it. Mathematics plays an important role in this field. The purposeof this paper is to present how Mathematical concepts are applied to the field of Data Science.

Keywords: Data Science, Mathematics, Parametric modelling, Regression, Probability Theory

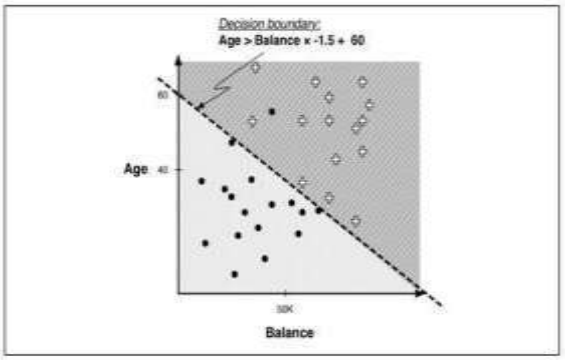
INTRODUCTION

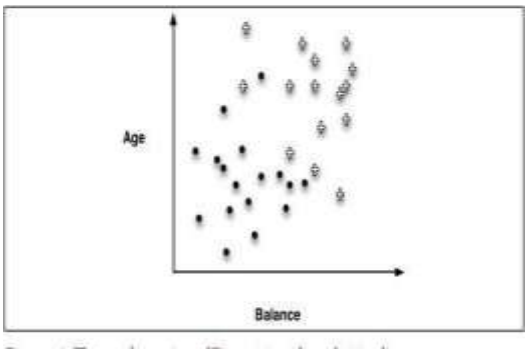
How does Netflix know what movie to recommend to you? How is the price of a property in terms of the land decided? How are certain emails put under the spam section? It is by applyingMathematics to Data Science. How are algorithms created and patterns identified? Again the answer is Mathematics. It is a vast field with multiple notions like Maximum Likelihood Estimation, Regression (Linear, Logistic, Multiple, Polynomial), Bayes' Theorem and so on. A simple example of regression is determining the relationship between sales of ice-cream and the temperature(celsius)[1].

METHODOLOGY

Parametric modelling is helpful to fit our model to the given data[2].

The equation of a straight line is y=mx+c where m indicates the slope of the line





Age is considered along the y axis and balance along the x-axis. The relation between age andbalance can be expressed through mathematical modelling as

Age=(-1.5)*Balance +60

Classification function

Class (x)= {+ if 1.0*Age-1.5*Balance+60>0

={* if 1.0*Age-

1.5*Balance+60<=0General linear

model:

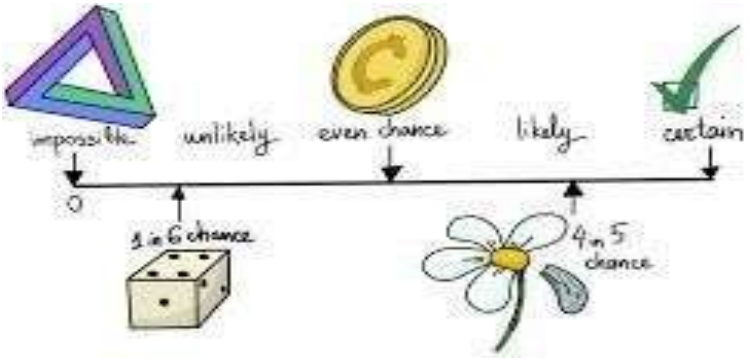
f(x)=w0+w1x1+w2x2+…….

Here

f(x)=60+1.0*Age-1.5*Balance [3]

PROBABILITY THEORY

Any data scientist must know how to analyse random phenomena to handle the data present. This is where the Probability Theory comes into play. Almost all phenomena allow for analysisof the likelihood of an event occurring with "0" indicating that there is no chance for the eventto occur and "1" indicating that the event shall certainly occur.



For example, if we toss a coin there is a 0.5(50%) probability of flipping a head or a tail by tossing it a single time. The actuaries of an insurance company study the probability of occurrence of an uncertain event and determine the amount of money that will be required to recover from the losses. We can also test the efficacy of a new vaccine by collecting data and using the probability theory [4]. A present-day example could be the study of the relationship between exposure and illness due to coronavirus by epidemiologists[5]. Weather forecasting serves as another example. Users desire weather forecasts which are nothing but probability forecasts which use numerical expressions for temperature, humidity, precipitation and so on.To infer statistical data we can use classical probability, relative frequency probability or subjective probability.

Now let us try and apply probability to find a solution for the collected data. Suppose there are50 people in a room with 365 seats, we shall find the probability of at least two of them havingthe same birthday (we shall assume a non-leap year). There are 365 possible scenarios for eachof these 50 people.

All possible outcomes=365*365*365*……..*365 (50 times)=365^{50} (1)

Since the number of favourable outcomes is much larger than the number of all possible outcomes, we find the number of unfavourable outcomes.

Favourable outcomes=All possible outcomes-Unfavourable outcomes (2)

Unfavourable outcomes are when none of the 50 people has the same birthdays.

Each person sits on a chair numbered with his/her birthday. Person 1 will have 365 options, person 2 will have 364 options (because we have assumed that no

two people have the same birthdays) and so on. This gives us $^{365}p50$ as the number of unfavourable cases.

Unfavourable outcomes=$^{365}p50$ (3)

Substituting (1) and (3) in (2)

Favourable outcomes=365^{50} -

$^{365}p50$

So, the probability of 2 out of 50 people in a room having the same birthdays is

$$\text{Probability}=\frac{365^{50} - {}^{365}p50}{365^{50}}$$

RESULT AND DISCUSSION

These methods stated above can be similarly used to study various other forms of data. For example, the probability of how many people in a room possess the same mobile device or how many people are watching the same show among a given list of shows and so on. Mathematical modelling can be used to study relationship between any two quantities in the given data.

CONCLUSION

In a nutshell, Data Science helps in finding and identifying patterns, creating statistical modelsand algorithms by using various mathematical notions. There are numerous other examples aswell which help give us a deep insight on the application of Mathematics to Data Science. The purpose of this paper is to provide a proof to this fact and to develop interest in learning Mathematics to anybody interested in pursuing Data Science as a career.

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ARTIFICIAL INTELLIGENCE

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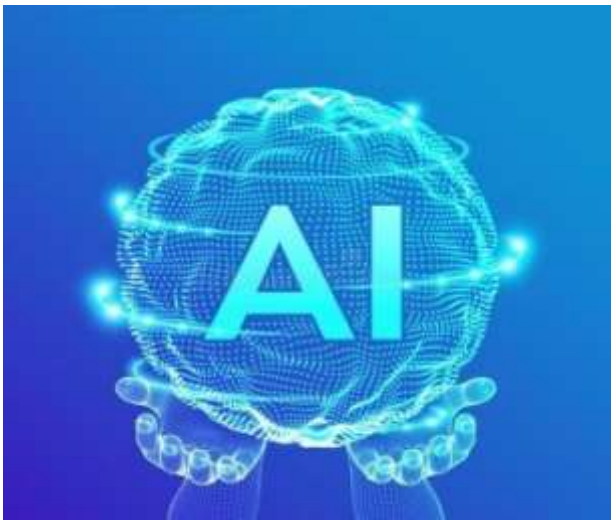


ABSTRACT

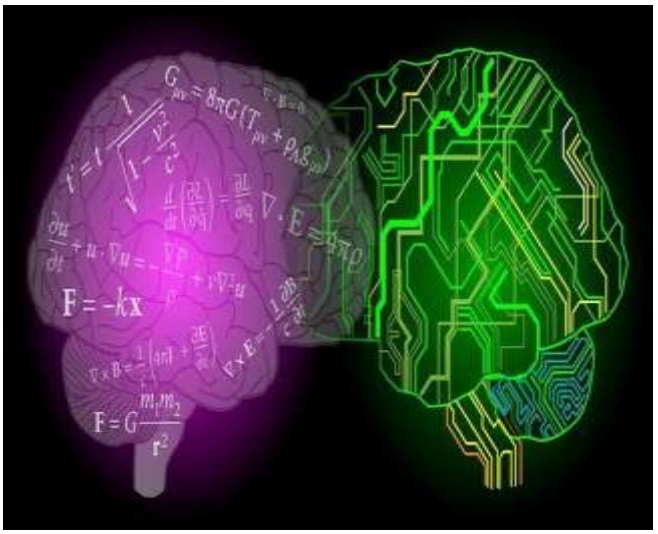
Artificial intelligence is shaping the future of humanity across every industries. The purpose of Artificial intelligence in the field of science is to solve greatest problems that are difficult, time consuming and impossible to be solved by Humans. Physics provide us the fundamental knowledge that are required for the technology. Physics is the mother of all Data problems. Physics boosts the artificial intelligence in lot of ways for the development. The purpose of this paper is to understand the purpose of physics in Artificial intelligence.

INTRODUCTION

Artificial intelligence refers to the simulation of human intelligence in machines that are programmed to think and act like Humans. It involves the development of algorithms and computer programs that can perform tasks that typically require Human intelligence such as visual perception, speech recognition, decision making, and language translation. Artificial intelligence has the potential to revolutionize many industries and has a wide range of applications, from virtual personal assistants to self-driving cars. Artificial intelligence (AI) is humanity's most powerful technology. Software that solves problems and turns data into insight is transforming our lives at an accelerating pace.

Artificial intelligence is all around us. People in developed economies interact with AI systems many times every day without being aware of it. The most obvious example is smartphone. It has more processing power than the computers that NASA used to send Neil Armstrong to the moon in 1969. It uses AI algorithms to offer predictive text and speech recognition services. Many of the apps we download to our phones also employ AI to make themselves useful to us. The AI in our phones becomes more powerful with each.

THE ROLE OF PHYSICS IN ARTIFICIAL INTELLIGENCE



Artificial intelligence driven frameworks are accelerating a diverse array of critical areas of physics research. From protein structures to climate modeling, detecting gravitational waves to understanding the universe, these breakthroughs demonstrate the lasting impact. The impact of using AI to create new models for solving hard physics problems has the potential to dramatically quicken the pace of progress for scientific discovery across the most fundamental fields of knowledge that explain and shape the world and Universe we live in. The application of artificial intelligence in atmospheric physics involves the use of algorithms like neural networks, decision trees and fuzzy logic. One of the biggest discoveries, the Higgs boson particle or “god particle”, was discovered using the neural network. Feynman diagram calculations and gauge theory calculations also take help of the AI. Bending of light due to Gravity called Gravitational lensing can also be found out using neural networks. It uses across physics has seen rapid growth with advances in its use across the scientific process from modelling to data analysis. From quantum theory to materials science condensed matter physics covers a large number of Quantum fields in physics, as it deals with different scales of time

and length depending on the molecular details and the type of substance being analyzed.

APPLICATIONS OF ARITIFICIAL INTELLIGENCE

The traditional goals of AI research include reasoning, knowledge, representation, planning, learning, natural language processing, perception and the ability to move and manipulate objects. The main applications of artificial intelligence are in military, healthcare and computing, data analysis, data modeling, statistical model, Astrophysics and machine learning. Artificial intelligence technology is used to create engines. Artificial intelligence has helped the increase productivity among faculties and helped them concentrate more on students than office or administration work.

CONCLUSION:-

Artificial intelligence and the technology are one side of the life that always interest us and surprise us with the new ideas, topics, innovations, products, etc. Artificial intelligence is not implementing as the films representing it (i.e. intelligent robots), however there are many important tries to reach the level and to compete in market, like sometimes the robots that they show in television.

At the end we have been in this research through the Artificial intelligence, role of physics in artificial intelligence and applications of AI, ethics of AI. This is not end of AI, there is more to come from it, who knows what the AI can do for us in future, may be it would be a robotic society.

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PERCENTAGE OF POLLEN GERMINATION

(COLLECTED FROM DIFFERENT LOCALITIES)

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ABSTRACT

The aim of this experiment is to determine pollen grains germinated in different localities. pollen germination is a method to test the pollen viability.Pollen viability is an important factor in successful hybridization.The pollen grains are obtained in the flowering plant , the growth of pollen tube depends on various factors and is different in different locations. The 10% of sucrose solution is prepared which help the pollen grain to germination when kept in sunlight. Hanging drop method is used to aid the pollen germination process. pollen grains germinate on stigma and pollen tubes grow into the ovary through transmitting tract.

Key words : Pollen germination, *Hibiscus rosea sinensis*, pollen grains.

INTRODUCTION

Pollen grains are also called microspores. They are microscopic structures, which bear male reproductive organ of a flower – androecium. The function of pollen grain is to carry or transfer the male gametes of plant to the female reproductive structure of plant for fertilisation to occur.

Structure

Pollen is a mass of microspores in a seed plant appearing as a fine dust. Inner layer - intine, outer layer - exine . The exine is extremely strong and is extremely resistant to disintegration; it's unaffected by extreme heat, acids or strong

bases. Exine is made up of sporopollenin . The intine is made of cellulose or hemicellulose .

The intine will emerge as the pollen tube out of one of the pores of the exine in a germinating pollen grain. Pollen grains are not only minute, but usually light as well so they are able to be easily transported by water, wind, or pollinators.

The development of pollen grains takes place using meiosis division in the pollen cells. Microspores are then separated from the tetrads and these separations are what form pollen grains. The morphological structure of pollen grain consists of one or more vegetative cells and the male gamete cell. The mature pollen grain develops at different levels and so has different numbers and kinds of cells. A matured pollen grain consists of a pollen tube cell and a generative cell – only two cells. When germination occurs, the pollen tube cell will become the pollen tube and the generative cell will go to the ovaries of the plant.

The vegetative cell produces the pollen tube, a tubular protrusion from the pollen grain which carries sperm cells within its cytoplasm. The germinated pollen tube must then enter in the nutrient-rich style and curl to the bottom of the ovary to reach the ovule. Once the pollen tube reaches the ovule, it burst to deliver the two sperm cells.

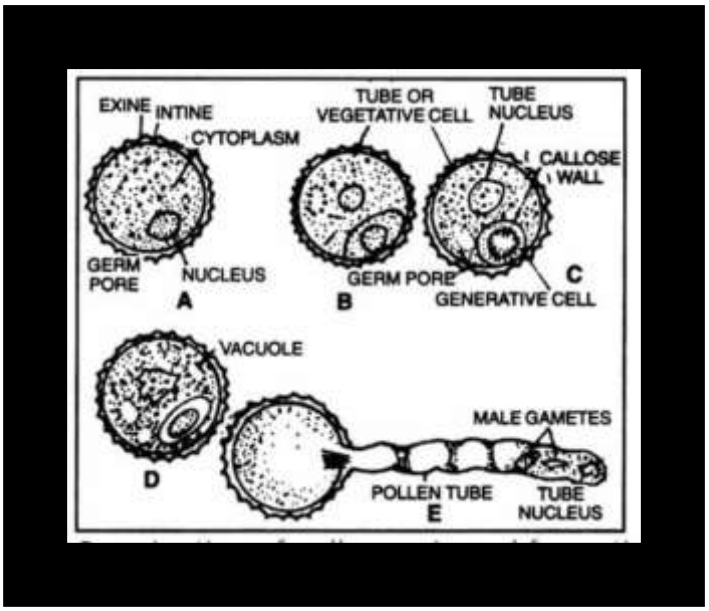

Fig 1 : Structure of a pollen

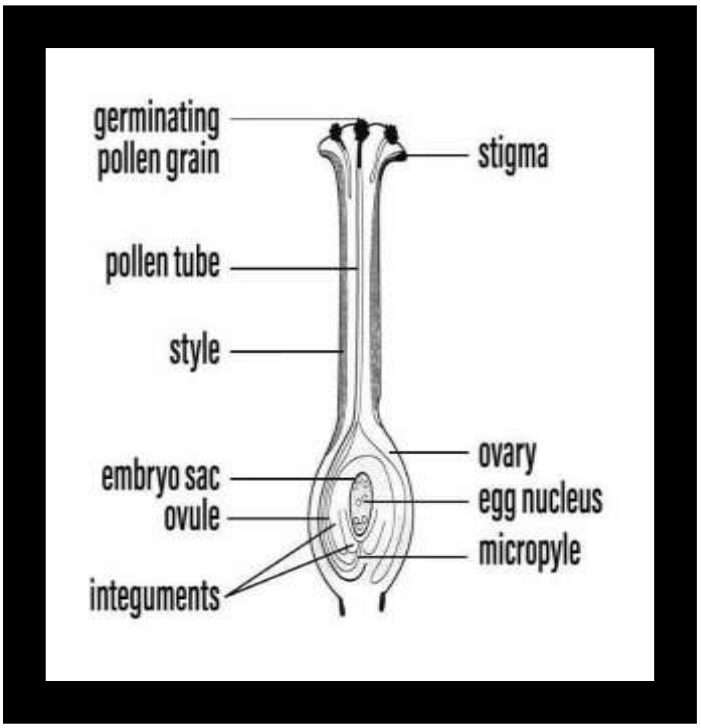

Fig 2: Germination of pollen on stigma

HANGING DROP METHOD

The hanging drop technique is a well-established method for examining living, unstained, very small organisms.

Pollen germination and pollen tube growth can be determined by placing a small drop of germination medium on a cover glass; pollen grains are speckled on that drop with a clean brush, and the cover glass are then inverted and rested on the cavity slide.

METHODOLOGY

Aim : To calculate the percentage of pollen germination of one particular type of pollen grain collected from different areas/locality under different conditions.

Principle: In nature, pollen grains germinate on the compatible stigmas of the carpel. Pollen grains can also be induced to germinate in a synthetic medium. During germination, intine (inner wall) of pollen grain emerges out as pollen tube through one of the germ pores in exine (outer wall).

Requirements : 10% sucrose solution, Petri dish, concavity slides, coverslips, brush, needle, simple microscope, matured pollen grains of *Hibiscus rosa-sinensis.*

Procedure:

(i) Prepare the pollen germination medium by taking 10% sucrose solution in a beaker.

(ii) Take a drop of 10% sucrose solution on a cover slip and sprinkle mature pollen grains of *Hibiscus rosa-sinensis*. on the drop.

(iii) Invert the cover glass on to a concavity slide.

(iv) After 10 minutes, observe the slide under microscope.

(v) Count (a) total number of pollen grains seen in the microscope field, and (b) the number of pollen grains that have germinated.

RESULTS AND DISCUSSION

Several pollen grains germinated and put forth pollen tubes.

The total number of pollen grains and the number of germinated pollen grains in the observed microscope field were observed.

Name of the plant used as source of pollen :

Hibiscus rosea-sinensis

Fig 3: Geo tagged image of flower 1

Fig 4: Geo tagged image of flower 2

Fig 5: Geo tagged image of flower 3.

Fig 6: Geo tagged image of flower 4

Fig 7: Geo tagged image of flower 5

For flower 1:

Number of pollen grains in a field of microscope (N) = 15

Number of germinated pollen grains in a field of microscope (n) = 5

Percentage of pollen germination = (n/N) 100 or 100n/N

= (5/15) 100

= 33.33%

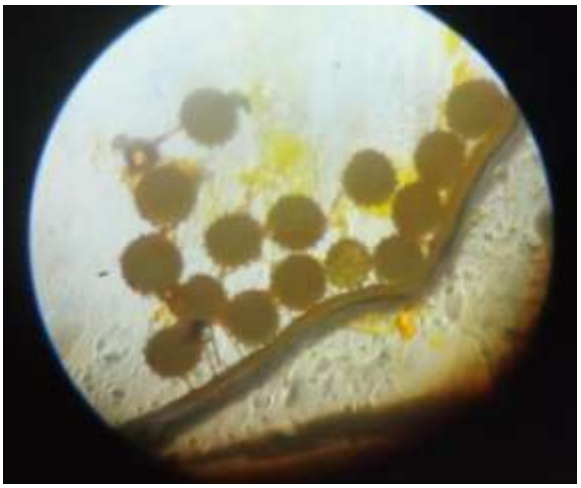

Fig 8: Locality –Koramangala

For flower 2 :

Number of pollen grains in a field of microscope (N) = 7

Number of germinated pollen grains in a field of microscope (n) = 4

Percentage of pollen germination = (n/N) 100 or 100n/N

= (4/7) 100

= 57.14%

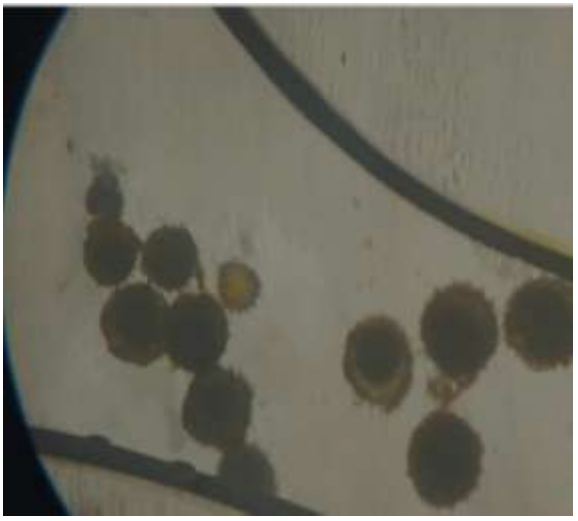

Fig 9: Locality – Indiranagar

For flower 3 :

Number of pollen grains in a field of microscope (N) = 10

Number of germinated pollen grains in a field of microscope (n) = 4

Percentage of pollen germination = (n/N) 100 or 100n/N

= (4/10) 100

= 40%

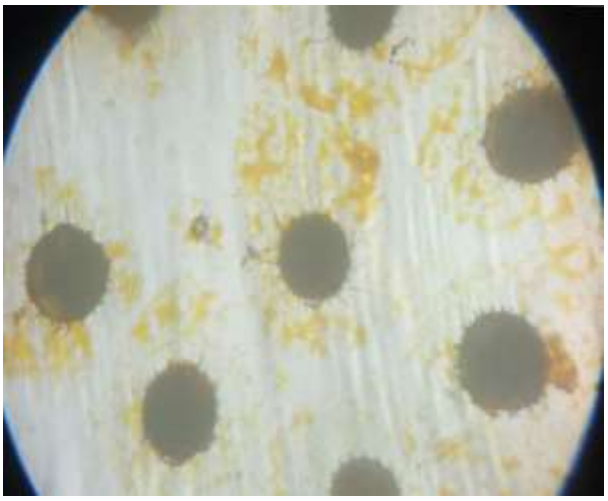

Fig 10: Locality - Ejipura

For flower 4 :

Number of pollen grains in a field of microscope (N) = 16

Number of germinated pollen grains in a field of microscope (n) = 9

Percentage of pollen germination = (n/N) 100 or 100n/N

= (9/16) 100

= 56.25%

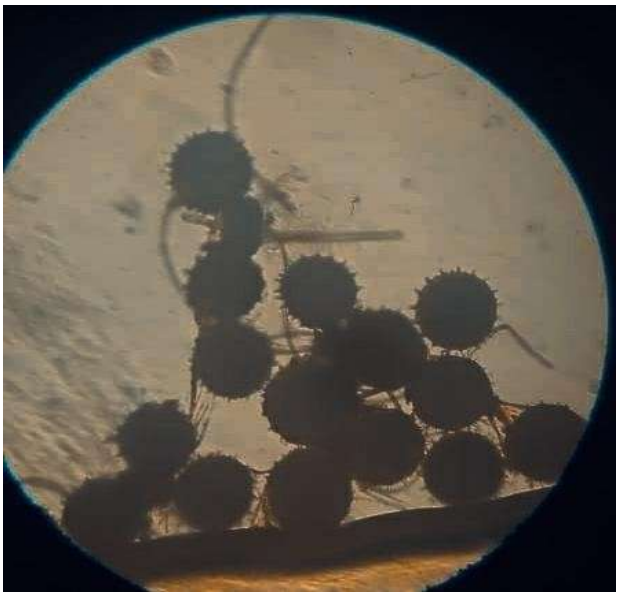

Fig 11: Locality - Panthur

For flower 5 :

Number of pollen grains in a field of microscope (N) = 4

Number of germinated pollen grains in a field of microscope (n) = 1

Percentage of pollen germination = (n/N) 100 or 100n/N

= (1/4) 100

= 25%

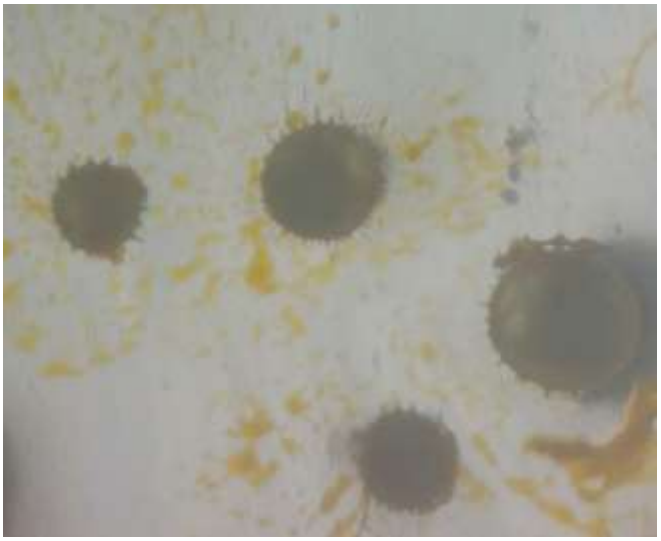

Fig 12: Locality – Marathahalli

Location	Percentage of germination
1. Koramangala	33.33%
2. Indiranagar	57.14%
3. Ejipura	40%
4. Panthur	56.25%
5. Marathahalli	25%

Table 1 : Interpretation of pollen tube growth in different localities

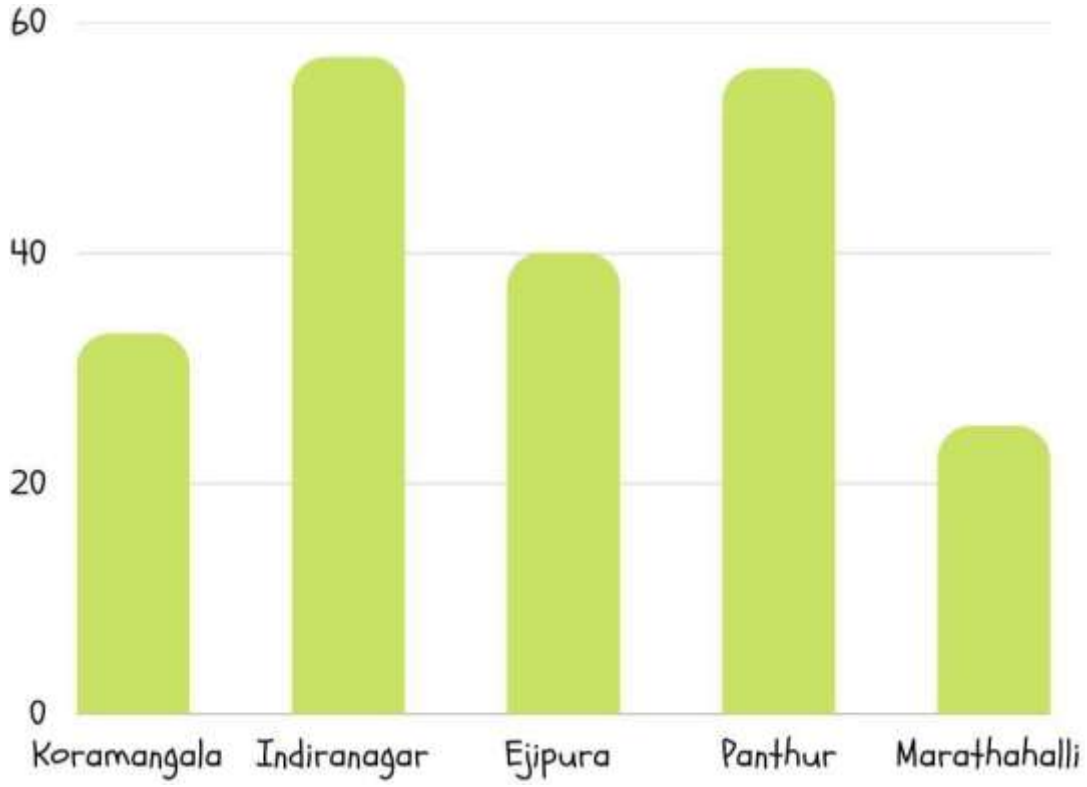

Fig: Graph showing the percentage of pollen germination in different localities

RESULT

From the above data, we found that the pollen grains collected from the locality of Indiranagar, Karnataka have germinated the highest (57.14%) and those collected from Marathahalli, Karnataka have a lower germination rate (25%)

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THE ROLE OF DIETARY ANTIOXIDANTS AGAINST EARLY AGEING AND AGE-RELATED DISEASES – A REVIEW

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ABSTRACT

Antioxidant enzymes serve a critical function in protecting cells of the biological system from oxidative damage that is caused by free radicals. The free radical theory of ageing proposes that age-related damage at the cellular and tissue levels is caused by oxygen-derived free radicals in the mitochondrial space. According to current scientific understanding, the excessive creation of free radicals in the body, as well as the imbalance between their concentrations and antioxidant defences, may be linked to ageing and a variety of diseases.
Reactive oxygen species (ROS) cause intracellular and extracellular oxidative stress, which accelerates skin ageing and causes wrinkles and abnormal pigmentation, and exposure to UV radiation, increases the production of reactive oxygen species (ROS) in cells. The mechanisms of ROS formation and its removal from the body are summarized in this review. The impact of ROS produced in the skin, as well as ROS functions in skin alteration, are also discussed. Antioxidants are an excellent way to avoid the symptoms of photo-induced skin ageing. Antioxidant-rich foods and a stress-free lifestyle will aid in the reduction of oxidants and the prevention of ageing.

Keywords: Antioxidants, Ageing, Reactive oxygen species, Free radicals

INTRODUCTION

Ageing is a complex process in which an organism's physiological changes occur with time, eventually leading to death. The ageing process is characterized by a steady build-up of DNA mutations and misfolded proteins, altering the capacities of the antioxidative system and affecting a wide range of enzyme functions. Nutrition, lifestyle, genetic background, and smoking all have an impact on the ageing process, and as a result, there are a plethora of ideas attempting to explain the causes of ageing. Furthermore, oxidative stress has

been linked to the ageing process as well as some serious neurological illnesses(Voss & Siems, 2006).

Humans have the greatest life span of any mammalian species and require more energy per pound of body weight than any other mammalian species. These two distinct biological traits represent an unusually modest inherent rate of physiological function ageing.

Ageing appears to be the result of normal developmental and metabolic processes in mammalian species. Despite the tremendous complexity of ageing mechanisms, simpler systems such as longevity determinant genes (LDGs) may influence the rate of ageing. Laboratory's comparative and evolutionary research has revealed that ageing is pleiotropic, resulting from long-term harmful side effects of normal metabolic and developmental processes.

Oxidative stress is a complex process that is characterized by an imbalance between the production of the free radicals and the ability of the body to eliminate these free radicals by using exogenous or endogenous antioxidants. Free radicals are the by-products of biochemical reactions occurring in the biological system that when present in higher amounts can cause oxidative stress. There are two types of free radicals (oxidants): reactive oxygen species (ROS) and reactive nitrogen species (RNS). Antioxidants are substances that are generally seen in foods that help in delaying or preventing lipid peroxidation caused due to oxidative stress in the biological system.

Our body has inbuilt antioxidants that help in eradicating these free radicals and the antioxidants can be of majorly two types - enzymatic antioxidants (superoxide dismutase (SOD), catalase, glutathione peroxidase) and non-enzymatic antioxidants (Reduced glutathione, Vitamin C, etc).

REDOX IMBALANCE IN AGEING

Comparative studies were made and they have indicated that ageing might be the result of pleiotropic effects of two major biological processes - differentiation and developmental processes and energy metabolic pathways. Ageing generally depends upon the developmental rate and when this rate of development is delayed the genes responsible for ageing will prolong the life of biological systems. Ageing rate is related to metabolic rate or the rate at which oxygen is being utilized per unit weight of tissue as the oxygen metabolism rate is interlinked with the rate of oxygen radical production, the active oxygen species may be important as a causative factor in ageing (Cutler, 2018).

The “oxidative stress theory” of ageing says that the ROS that emerges from the mitochondria will cause progressive damage resulting in the functional decline that causes ageing in humans. The level at which oxidative stress contributes to

ageing may vary between organisms, tissues, and distinct cell types, and around 4% of the oxygen consumed by the mitochondria gets converted into ROS. Superoxide anion which is a ROS gets converted by SOD to hydrogen peroxide, which can react with free iron to produce the highly reactive hydroxyl radical, which in turn will readily damage biomolecules including DNA, protein, and membrane lipids (Cui *et al*., 2012).

Studies have shown that SOD is localized in the mitochondrial intermembrane space and that hydrogen peroxide formed from SOD gets converted to water by catalase or glutathione peroxidase. There are a range of various protective mechanisms against ROS that include chemical antioxidants such as vitamins C and E. Reducing the activity of catalase or SOD results in a greater sensitivity to oxidative stress and reduced longevity (Balin & Allen, 2018).

Studies of oxidant-associated damage during ageing were found to have an oxidative modification of intracellular macromolecules, primarily lipids, proteins, and DNA. In DNA, oxidative damage to mitochondrial and nuclear nucleic acids was seen to increase significantly in all the major tissues in humans and other organisms. Higher levels of lipid peroxidation products like MDA, 4-HNE, and F2-isoprostanes were observed in tissues of aged and young organisms of kidneys, brain, liver, lung, and muscle. Scientists have discovered age-related oxidative modifications to a large variety of proteins, including changes in structural proteins, enzymes, and proteins that play a major role in signal transduction pathways.

OXIDANTS AND SKIN

The skin as the largest organ in the human body, has a protective role and has lipids, proteins, carbohydrates, DNA making it highly susceptible to oxidative stress of the dermal cells. It has many defence molecules in order to increase oxidative stress and it is said that during evolution, skin adapted itself in such a way that it could fight against the increased concentration of oxygen and oxygen metabolites as it is constantly being exposed to them.

One of the major defence mechanisms of skin is related antioxidants and certain enzymes that can react directly with ROS, preventing them from reaching their biological target. Oxidative stress plays an important role in the modulation of the extent of inflammatory response and subsequent tissue damage, and the appearance of different structural and functional changes in skin proteins, e.g., in collagen and elastin.

In the skin, oxidative stress plays a major role in the intrinsic and extrinsic ageing of the skin. Extrinsic ageing occurs because of the oxidative stress that is caused by photons produced by the UV irradiation and gets accelerated by the influence

of environmental conditions. Intrinsic ageing can be seen as a result of genetic factors and corporal changes that appear during the normal ageing process.

The structure of the skin is quite complex since it is composed of several layers, each having a specific role and function but the ageing processes in it can thinner the epidermal as well as dermal skin layers and the skin loses its sensibility, getting dryer, and gradually loses its function to serve as the first line of defence. The disruption of the extracellular matrix in the dermis region of the skin occurs due to intrinsic and extrinsic ageing which leads to wrinkling appearance as the collagen, elastic fibres, and hyaluronic acid gets reduced which plays a role in thickening of the skin with the help of glycoproteins that reacts with hyaluronic acid to form a thick network with the collagen fibrils which is anchored to the dermal-epidermal junction.

In intrinsic ageing, the collagen and elastic fibres remain intact with each other but when being exposed to the free radicals that are produced by the enzymatic as well as non-enzymatic, they disrupt the proteins present in the skin and can lead to many skin disorders. Most of the anti-oxidants show a concentration in the epidermis rather than in the dermis which gives information that ROS load is higher in the epidermis than in the dermis.

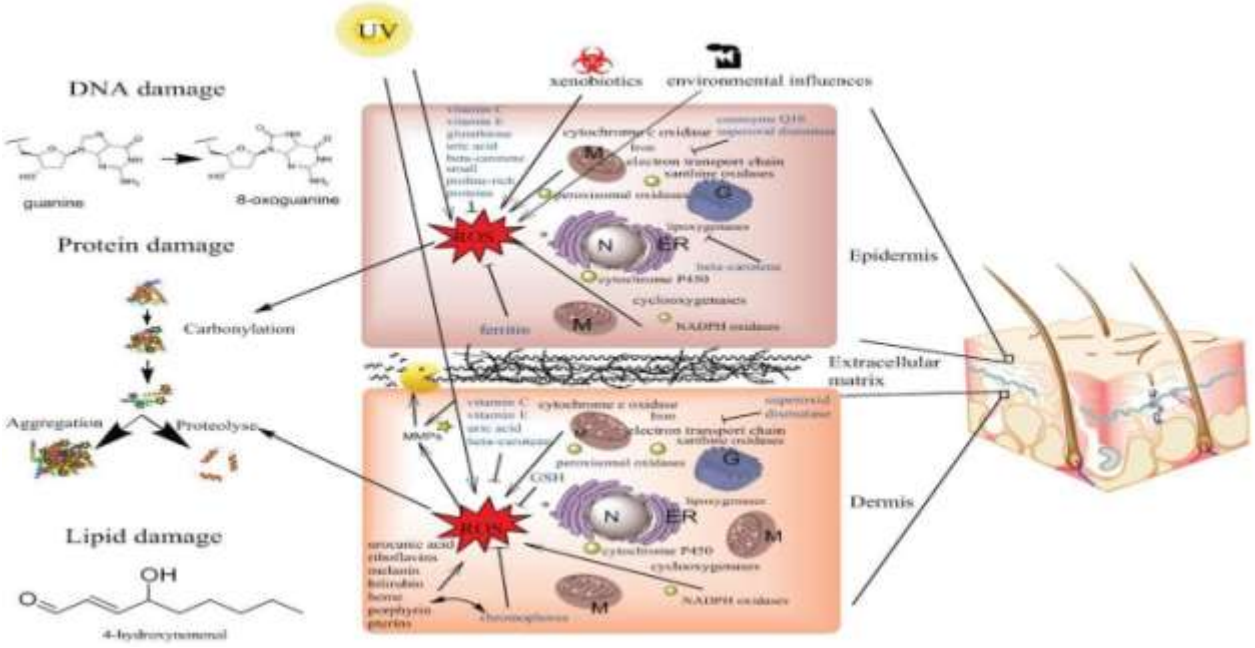

Figure 1: Schematic of the interplay between different ROS sources and the anti-oxidative systems in the skin. All ROS sources discussed in this manuscript are exemplified in black letters. Examples of anti-oxidative systems are given in blue letters. Possible outcomes of oxidative damage to the cells (damage to DNA, proteins, and lipids) are shown on the left-hand side of the figure. (Courtesy: Rinnerthaler *et. al*, 2015)

MECHANISM OF SKIN AGEING

The lipid peroxidation process led to changes in the fluidity of the plasma membrane and caused the molecules to leak out from the mitochondrial membrane this process gets intensified in the dermis with ageing and the phospholipase activity led to the disruption of the membrane integrity of cell membranes. ROS can inactivate the enzymes and can cause protein degradation on DNA leading to base loss, base modifications, and single and double DNA breakage events, which could result in adverse processes and even cancer. The antioxidants have free radical scavenging activities that might have great significance in the protection and therapeutics of age-related diseases caused by ROS. Morphological changes of the skin were observed that accelerated as age advanced. Histopathological loss of epidermal polarity and physiological disorders of keratinocyte maturation were observed.

THE INTERLINK BETWEEN FREE RADICALS (OXIDANTS), VITAMINS AND ANTIOXIDANTS

A vast body of epidemiological evidence implies that eating a diet high in vitamin sources protects against illness development. Longevity can be increased if the disease can be avoided or delayed. Poorer food intake has been linked to lower calcium, iron, zinc, B complex vitamins, and vitamin E intake in the elderly. Diets with a low-calorie content or a poor nutrient density may raise the risk of diet-related illnesses. Fifty percent of older persons consume fewer vitamins and minerals than the RDA, and 10% to 30% have vitamin and mineral levels that are below normal. Elderly folks, vegans, alcohol-dependent people, and those with malabsorption are all at risk for poor vitamin consumption. As people get older, their daily food and beverage consumption decrease (Thomas, 2004).

Dietary antioxidants lipophilicity could play a crucial part in the ageing process since it allows the chemical to accumulate within the human body for longer effects, which is important for longevity research. Many dietary intervention studies on longevity have focused on lipophilic antioxidants such as vitamin A, vitamin E (alpha-tocopherol), flavonoids, resveratrol, and coenzyme Q10. Longer-living people have higher plasma levels of vitamin A and E (non-enzymatic lipophilic antioxidants) (Mecocci *et al*., 2000). The normal population's plasma vitamin A and E levels decreased with age, according to the study (Chong-Han, 2010).

Vitamin E is the major antioxidant in the skin, and it gives an electron to reactive oxygen species while being oxidized in the process. Vitamin E must restore its lost electron from vitamin C, a secondary antioxidant, in order to antioxidant function. Vitamin C must also be replaced, either through new dietary sources

or through the use of a tertiary antioxidant such as vitamin A. The human lifespan is extended by this transfer of electrons, however, it requires the absorption of vitamins for skin health (Draelos, 2013).

Primary antioxidant: Vitamin E (Tocopherol)

Vitamin E is the most powerful antioxidant. Vitamin E, a fat-soluble antioxidant found in plasma, membranes, and tissues, comes in eight different forms. Singlet oxygen, superoxide, and hydroxyl radicals are just a few of the free radicals that vitamin E can scavenge. The plasma vitamin E levels appeared to decline as people became older (Shapiro & Saliou, 2001). Vitamin E, in combination with vitamin C, has been shown to improve the UV-induced erythema threshold and prevent sunburn in two recent independent investigations (Shapiro & Saliou, 2001).

Secondary antioxidant: Vitamin C (Ascorbic acid)

Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin that scavenges and quenches free radicals while also rebuilding vitamin E from its radical state. Because vitamin C is a cofactor for lysyl and prolyl hydroxylase, which stabilizes collagen's triple-helical structure, it is widely known that it is required for wound healing. (Draelos, 2013)**.** Some of the important sources of vitamin C are citrus fruits, fresh leafy vegetables, strawberries, melons, tomatoes, broccoli, and peppers (Thomas, 2004).
Ascorbic acid is used in a variety of cosmetics that promise to protect the skin from environmental aggressors and photo-ageing (Shapiro & Saliou, 2001).

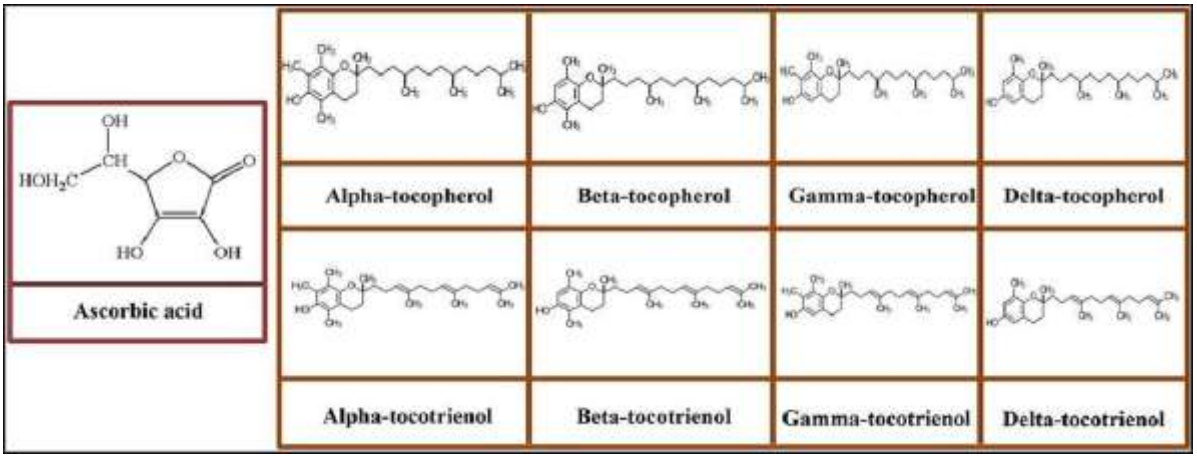

Figure 2: The molecular structures of vitamin C (ascorbic acid) and vitamin E congeners including tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol) and tocotrienols (α-tocotrienol, β-tocotrienol, γ-tocotrienol, and δ-tocotrienol).
(Courtesy: Tan *et al*., 2018)

Tertiary antioxidant: Vitamin A (Retinoids)

Other compounds that can act as antioxidants in the body besides vitamins E and C are known as tertiary antioxidants. Vitamin A, for example, can quench singlet oxygen. Tomatoes, carrots, sweet potatoes, strawberries, mango, watermelon, and cantaloupe are among the orange, red, and yellow fruits and vegetables contain vitamin A. Vitamin A is required for vision and has a well-studied skin receptor, indicating that it is important for skin health (Draelos, 2013).

Retinoids, which are found in nature, play an important role in the differentiation and function of epithelial tissue. These chemicals have also been utilized at pharmaceutical levels to treat a variety of dermatologic conditions, including acne, photodamage, and keratinization disorders, such as psoriasis. Retinol, retinal (and its isomers), all-trans retinoic acid, 13-cis retinoic acid, 9-cis retinoic acid, and 3,4-didehydroretinoic acid are the most abundant natural retinoids. (Shapiro & Saliou, 2001). Vitamin A deficiency weakens immunity and, as a result, light-sensitive receptors are damaged. Individuals who are deficient in vitamin A may develop xerophthalmia or lifelong blindness (Tan *et al*., 2018)

In cosmetic applications, vitamin A and its analogs are among the most important substances utilized in the treatment of skin. They've been used to treat psoriasis both orally and topically. They've been used topically to treat acne and photodamage, and they're currently being studied for striae, cellulite, and wound healing (Shapiro & Saliou, 2001)

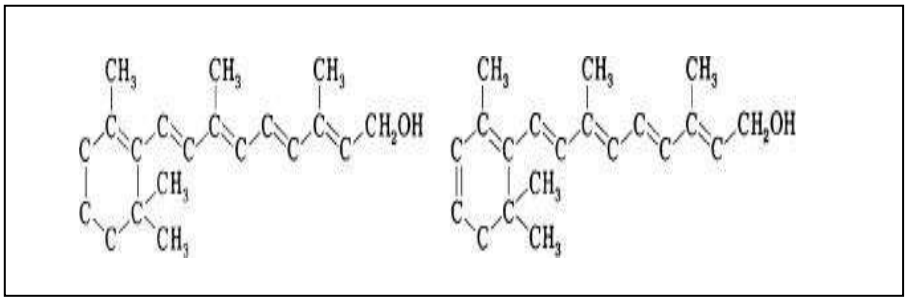

Figure 3: Molecular structure of vitamin A [Vitamers A1 and A2. Left: All-trans-vitamin A1 (retinol1). Right: All-trans-vitamin A2 (retinol2).] (Courtesy: Patwardhan, 1945)

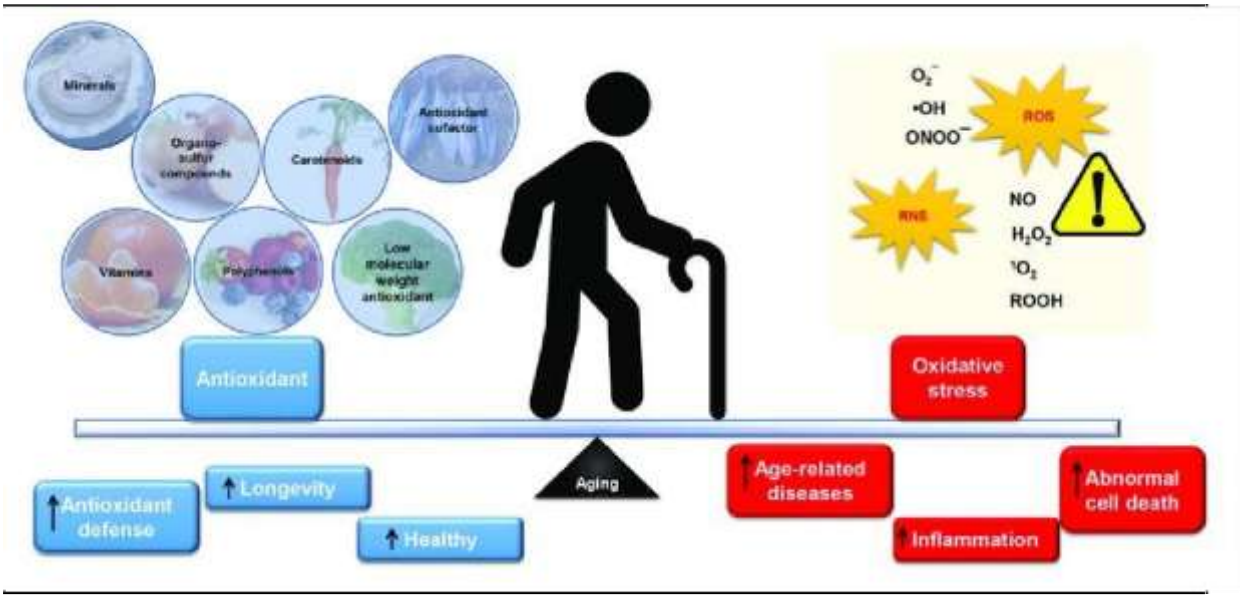

Figure 4: Balancing of the antioxidants and oxidative stress in ageing. Antioxidants may be able to neutralize excess ROS/RNS in a regularly functioning cell. Overproduction of reactive species such as superoxide (O2), hydroxyl radical (•OH), peroxynitrite (ONOO), hydrogen peroxide (H2O2), hydroperoxides (ROOH), singlet oxygen, reactive lipid aldehydes, and reactive nitric oxide (NO) combined with low antioxidant levels in the body can cause oxidative damage to cellular constituents (protein, lipids, and DNA). This phenomenon affects the aged, causing aberrant cell death, inflammation, and, as a result, contributing to age-related disorders. Antioxidants are important for scavenging ROS/RNS and thereby protecting cells from oxidative damage. Exogenous (minerals, organosulfur compounds, vitamins, carotenoids, polyphenols) and endogenous (antioxidant cofactors such coenzyme Q10; and low molecular weight antioxidant: glutathione) antioxidants have been demonstrated to maintain antioxidant defense and lead to healthy longevity. (Courtesy: Tan et al., 2018).

GLUTATHIONE

Glutathione is an important antioxidant found in bacteria, plants, and animals. Glutathione protects cells from the effects of ROS, such as lipid peroxides, peroxides, free radicals, and heavy metals. Through non-enzymatic and enzymatic processes, glutathione can scavenge ROS. The free thiol group of glutathione contributes to glutathione's non-enzymatic antioxidant action. Enzymatic processes involving glutathione reductase, glutathione peroxidase, and glutathione-S transferase help glutathione detoxify oxidants and electrophiles. Glutathione regulates the cell's redox status, specifically by modulating the correct tertiary structure of proteins via a thiol-disulfide exchange, which occurs simultaneously with glutaredoxin and protein disulfide isomerases.

Because it can be made in the body from amino acids like L-glutamic acid, L-cysteine, and glycine, glutathione is classified as a non-essential nutrient for

humans. Between the amine group of cysteine and the carboxyl group of the glutamate side-chain, glutathione has a gamma peptide bond linked to a tripeptide. (**Figure 5**)

CAROTENOIDS

Based on their chemistry, carotenoids are split into two categories: carotenes and xanthophylls. Carotenes (-carotene, -carotene, and lycopene) are hydrocarbon-only carotenoids, whereas xanthophylls are oxygenated derivatives .

The majority of carotenoids are tetraterpenoids, which are made up of eight isoprene molecules and 40 carbon atoms. The polyisoprene structure of all carotenoids is composed of a long conjugated chain adjacent to multiple double bonds with near symmetry on the central double bond. Oxygen-rich functional groups can change the basic acyclic structure. Carotenoids are involved in the modulation of the cell cycle, apoptosis, and cell differentiation, as well as the enhancement of the immune system, the regulation of cell signaling pathways, and the promotion of growth factors. Carotenoids are lipophilic substances that live inside cells and protect the membrane from oxidative damage. Carotenoids are well-known for their eye-sight-protecting properties. These carotenoids are known as pro-vitamin A.

Many degenerative diseases caused by oxidative stress, such as Alzheimer's disease and dementia, are prevented by carotenoids. Carotenoids slow disease progression by suppressing oxidative stress, promoting A peptide synthesis, and inhibiting pro-inflammatory cytokines, among other things. Raw tomato (*Solanum lycopersicum*) consumption protects against malignancies of the esophagus, stomach, colon, and rectum, as well as cardiovascular disease and Alzheimer's disease. Certain antioxidants, such as carotenoids, can lessen the incidence of age-related disorders, according to research. In terms of nutrition, the effects of various carotenoids in the diet promote healthy ageing. (Tan *et al*., 2018)

POLYPHENOL

The multiples of phenol structural units distinguish polyphenols (also known as polyhydroxy phenols). Polyphenols, which are abundantly found in fruits and vegetables, are secondary metabolites produced by plants and are thought to protect against UV radiation and pathogen invasion. Polyphenols also affect the flavour, colour, and odour of food, all of which influence how we perceive it. The flavonoid class is the most frequent of all polyphenolic chemicals.

Flavonoids are the polyphenol group that has been studied the most. Flavonoids are separated into subgroups based on the carbon content and degree of unsaturation (flavanols, flavones, flavanonols, flavanones, catechins, anthocyanins, and chalcones.

Flavonoids have favourable biochemical and antioxidant effects against oxidative stress-related disorders such as cancer, diabetes, cardiovascular disease, Alzheimer's disease, and dementia. Flavonoids from cocoa, green tea, and citrus fruit have been shown in numerous studies to have favourable effects on the brain (Birt & Jeffery, 2013).

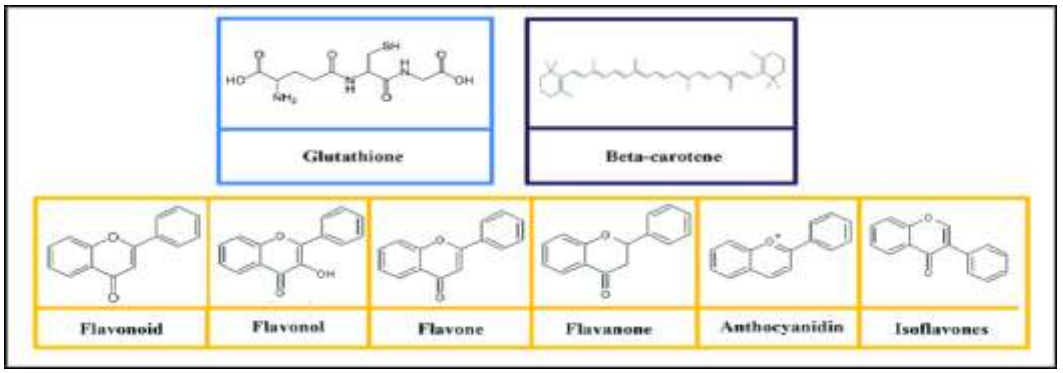

Figure 5: Glutathione, polyphenols (flavonoid, flavonol, flavone, flavanone, anthocyanidin, and isoflavones) and beta-carotene have different molecular structures.
(Courtesy: Tan *et al*., 2018)

ABERRATIONS

ROS - Reactive Oxygen Species
RNS - Reactive Nitrogen Species
DNA - Deoxyribonucleic acid
CAT - Catalase
SOD - Sodium oxide dismutase
GPX - Glutathione peroxidase
LDG - Longevity Determining Genes
RDA - Required Dietary Allowance
UV - Ultra Violet radiation
MDA - Malondialdehyde
4-HNE - 4-Hydroxynonenal
F2-isoprostanes - prostaglandin like compounds
H2O2 - Hydrogen peroxide
O 2 - Superoxide
(•OH) -Hydroxyl Radical
ONOO - Peroxynitrite

ROOH - Hydroperoxides
O2 - Singlet Oxygen
NO - Nitric Oxide

CONCLUSION

Ageing is a metabolic process that occurs at the cellular and tissue level of every living organism but early ageing can be seen due to the high production of free radicals in the body and hence there comes a need to prevent or overcome it. This can be done by our body's system by production of antioxidants when not present adequately the dietary antioxidants come in handy and play an important role in the biological system. The antioxidant-rich food and stress-free life will help in reducing the oxidants and prevent ageing.

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TYPES OF PLACENTATION

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ABSTRACT

Different types of placentation are observed. For flowers, it is done by dissecting the ovule of a flower and observing it under a dissecting microscope. For fruits or vegetables it is done by simply taking a section of it and observing with the naked eye. The number of locules in different types of placentation were observed.
Key words- Placentation, locules, septa, ovules

INTRODUCTION

Flowers are the reproductive part of a plant. They are not only involved in reproduction but are also a source of food for other living organisms. They are a rich source of nectar.
Flowers can be complete or incomplete.
A complete flower consist of sepals, petals, stamen and pistil. On the contrary, an incomplete flower lacks one or more of the structures.
A complete flower consists of two different parts:

I. Vegetative
II. Reproductive

Here we are discussing only about the female reproductive part called gynoecium.

Ovary, stigma, style are the three parts of a gynoecium.

Ovary: It is the swollen basal portion containing ovules.

Stigma: It is the portion which receives pollen grains during pollination.

Style: It is the portion which connects stigma and ovary.

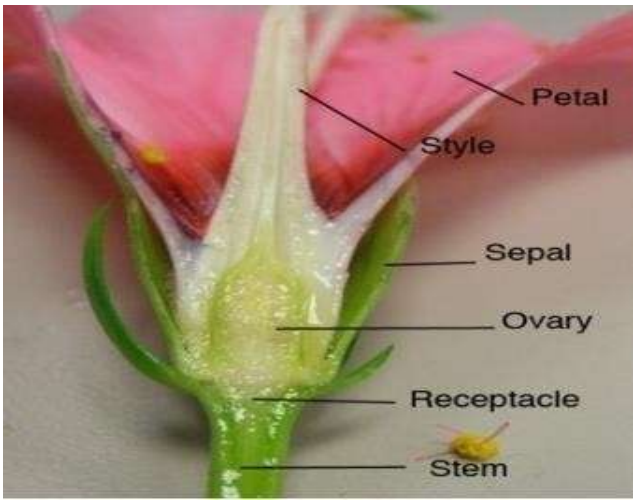

Figure 1: Structure of a Flower

STRUCTURE OF A OVARY

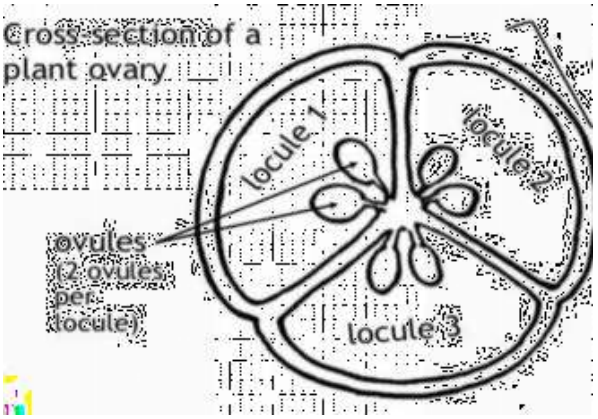

Figure 2: Cross-section of a plant ovary

The ovary of the flower is divided into chambers called locules. Based on the number of chambers, the ovary can be unilocular, bilocular or multilocular.The carpels are separated from each other by the formation of the septum in some plants, the number of carpels and locules may be same or different, depending on the presence of septum.The ovules in the ovary are attached to the inner ovary walls by the placenta. The placenta can be placed in various positions depending upon the carpel and ovary.

Eg: Cucumber

Figure 3: Placentation in Cucumber

As shown in the figure 3, the ovary consists of locules, septa and ovules. The septa is separating the locules into chambers. It consists of three chambers in which the ovules are attached to it so it is trilocular.

PLACENTATION

It is defined as the arrangement of ovules inside an ovary. The placenta is found in mono to multi carpellary, syncarpous ovary. Usually a single ovule is attached to the base of the ovary.

There are six types of placentation based on the arrangement of ovules in the ovary. It will be further discussed in the experiment below.

TYPES OF PLACENTATION

- Marginal Placentation
- Axile Placentation
- Parietal Placentation
- Basal Placentation
- Free Central Placentation

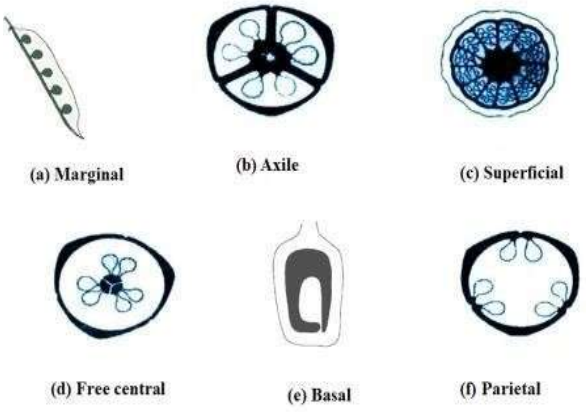

Figure 4: Types of Placentation

EXPERIMENT

AIM: To observe different types of placentation.

MATERIALS REQUIRED: Flowers, fruits, vegetables, sharp blade, slide, dissecting microscope, water, dropper.

PROCEDURE:

(i) Take a flower and remove its sepals, petals and stamen.

(ii) Separate the pistil from the flower. With the help of a sharp blade separate the ovary from its base.

(iii) By using another sharp blade make multiple thin sections of the ovary and place it in a petri dish containing water. Choose the thinnest section.

(iv) Take a clean glass slide. Place the thin section of ovary on the slide.

(v) Place the slide under a dissecting microscope.

(vi) For a fruit or vegetable, take a cross section and observe the placentation.

(vii) Record the observations.

OBSERVATIONS

a) MARGINAL PLACENTATION

This placentation takes place in monocarpellary, unilocular ovary. The ovules are borne along with the junction of the two margins of the carpel.

In marginal placentation, the placenta forms a ridge along the ventral surface of the ovary and the ovules develop on it making two separate rows. This type of placentation is found in the Fabaceae or Solanaceae family.

Figure 5: Pea plant (Fabaceae family)

Figure 6: Placentation in Pea

As seen in the above picture the seeds are attached to the ovary wall. The development of ovules takes place on the ridge. Thus two separate rows are formed.

b) AXILE PLACENTATION

It is a type of placentation that can be seen in bi or multicarpellary, syncarpous ovary. The walls of the carpel meet in the centre of the ovary, where the placenta are formed like a central column. The ovules are borne at or near the centre on the placenta in each locule.

Figure 7: Hibiscus Plant

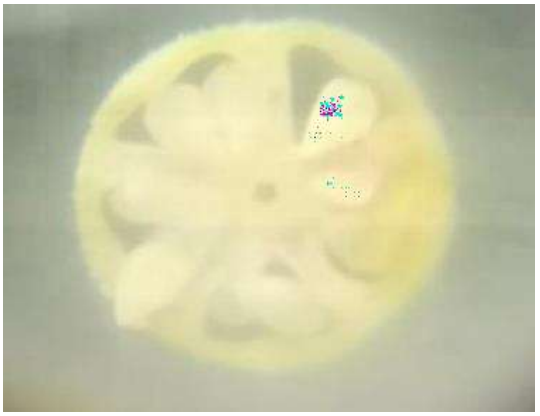

Figure 8: Placentation in Hibiscus

As seen in the picture the ovules meet in the centre of the ovary. Thus, it is axile placentation.

c) PARIETAL PLACENTATION

This type of placentation occurs in multicarpellary, syncarpous, unilocular ovaries. The carpels are only fused by the margins. The placenta that bear ovules develops at the places where the two carpels are fused.

Eg: Cucumber, Muskmelon, and Tomato.

Figure 9: Placentation in Cucumber

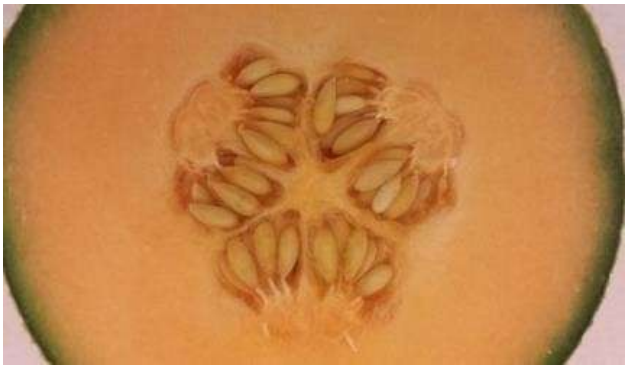

Figure 10: Placentation in Muskmelon

d) BASAL PLACENTATION

This type of placentation can be seen in bicarpellary, syncarpous, unilocular ovary. The placenta develops directly on the receptacle that bears a single ovule at the base of the ovary. It can be seen in the Asteraceae family.

Figure 11: Sunflower seeds

Figure 12: Placentation in Sunflower seeds

In sunflower seeds, a single ovule is attached to the base of the ovary wall. So it is called basal placentation.

e) FREE CENTRAL PLACENTATION

In free central placentation, ovules are borne around a central column, which is not connected with the ovary wall by any septum.

Eg: Dianthus, Primrose.

(Materials for this could not be found)

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IRISN: A WHITE FAT BURNING HORMONE AS AN ANTICANCER AGENT

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ABSTRACT

Irisin, is a myokine/adipokine, plays a vital role in converting white adipose tissue into brown adipose tissue. Irisin obtained by the proteolytic cleavage and glycosylation of the FNDC5 protein. Irisin has been seen to regulate many types of cancer, namely breast cancer, lung cancer, prostate cancer and pancreatic cancer. This regulation is done mostly through the PI3K/AKT pathway which controls the epithelial mesenchymal transition. Irisin levels have demonstrated to be high during cancers related to hormone imbalance like breast cancer and prostate cancer, mostly related to adipose tissue, whereas the levels have found to be low in cancers like lung and pancreatic. It helps in establishing the link between obesity and cancer, which can be seen from marked decrease in breast cancer risk with regular exercise. Most of the research done on the regulation of this hormone is through cell lines and has been *in vitro*. Serum Irisin levels can act as novel biomarkers for cancers whose prognosis is still poor.

Keywords*:* Irisin, cancer, prognosis, regulation, Fndc5, adipokine

INTRODUCTION

The Globocan 2020 estimated repot indicate that there were 19.3 million new cases of cancer and almost 10 million deaths from cancer in 2021 irrespective of region and level of development **(Sung *et al.*, 2021)**. The lack of a definite treatment for various types of cancer is the reason for its increasing frequency and encourages the intensive ongoing research pertaining to the field of oncology. Irisin is an adipokine/myokine, initially detected in mouse skeletal muscle fibres, which upon subjecting mice to physical activity converts white adipose tissue into brown adipose tissue and is key in reducing obesity. Irisin is formed by the proteolytic cleavage of a peptide of 94 amino acids out of the 206 amino acids of the fibronectin type-3 domain-containing protein. Peptide chain obtained as a result of this cleavage is glycosylated to form active form of hormone **(Bostrom *et al.*, 2012).** The excretion of the hormone is widespread but is mainly reported in muscles, white fat tissue, testes, epididymis, rectum and many others **(Huh *et al.*, 2012)**. Irisin has also been detected in brain-spinal cord fluid, human breast milk, saliva and parkinje cells in cerebellum and in the testicular tissue **(Suna Aydin *et al.*, 2014).** Irisin's role in fate metabolism regulation makes is a key factor in development of obesity, insulin resistance accompanying obesity, diabetes and other disorders **(Aydin *et al.*, 2016; Zhu *et al.*, 2018)**. Irisin increases levels of Uncoupling Protein-1 (UPC1), facilitating conversion of white adipose tissue into brown adipose tissue, hence lowering levels of obesity **(Bostrom *et al.*, 2012).**

Over the past few years the clear association of Irisin with cancer has been highlighted, as obesity increases disposition to cancer. The increase in energy consumption during cancer formation leads to an increase in the metabolic rate of an organism inducing release of Irisin **(Kuloglu *et al.*, 2019)**. Elevated serum Irisin levels can be observed in some gastro-intestinal cancer, some benign

tumours and endometriosis tissue **(Kuloglu *et al*., 2016)**. However, in cancers associated with obesity and hormones such as prostate cancer and breast cancer, a significant decline in Irisin levels can be observed in patients in comparison to control. Irisin can also be seen to regulate division and cell proliferation in prostate cancer **(Tekin *et al*., 2015).**

IRISIN: A PGC1*α*-DEPENDENT MYOKINE

PGC1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) is an exercise induced transcriptional factor which stimulates secretion and increased expressions of FNDC5 (Fibronectin type III domain-containing protein 5). This membrane protein is then proteolytically cleaved in order to secrete the hormone Irisin which acts on white adipose tissue to release UCP1 **(Bostrom *et al.*, 2012)**. Originally described as a coactivator of PPAR which is a modulator of UPC1 and production of brown fat, PGC1α can also control mitochondria biogenesis, angiogenesis and fibre type switching **(Handschin and Spiegelman, 2008).** Upon examination of the adipose tissue of transgenic mice with PGC1α gene, the subcutaneous fat layer which is prone to 'browning' showed significant levels of UCP1 and Cidea mRNA. An increase in UPC1 expression was also observed in visceral, epididymal fat after three weeks of wheeling. This effect of the PGC1α gene is indirect as it induced the expression of several other fat browning genes in vitro suggesting that the gene causes myocytes to secret molecules that induce thermogenic properties **(Bostrom *et al.*, 2012).**

Out of the five genes that seem to have to up regulated by PGC1α, Fndc5 promoted 7fold induction of UCP1, depicting its role in activation of browning and thermogenic genes **(Chen *et al*., 2011).** Similar genes were observed in Human subjects after subjection to vigorous exercise **(Vind *et al.*, 2011)**. Upon examination of the immunohistochemistry of treated cells higher density of

mitochondria and higher levels of oxygen consumption helped prove the potent induction of thermogenesis by Fndc5. The peptide was observed to act mainly during differentiation of cells committed to adipocyte lineage. An important transcription factor PPARα induced by Fndc5 results in a significant increase in UCP1 expression highlighting the working of Fndc5 via this nuclear receptor **(Seale *et al.*, 2011)**. Fndc5 has one signal peptide, two fibronectin domains and one hydrophobic domain. The signal peptide is cleaved, the mature protein is then proteolytically removed and glycosylated to release the 112 amino acid long hormone Irisin.

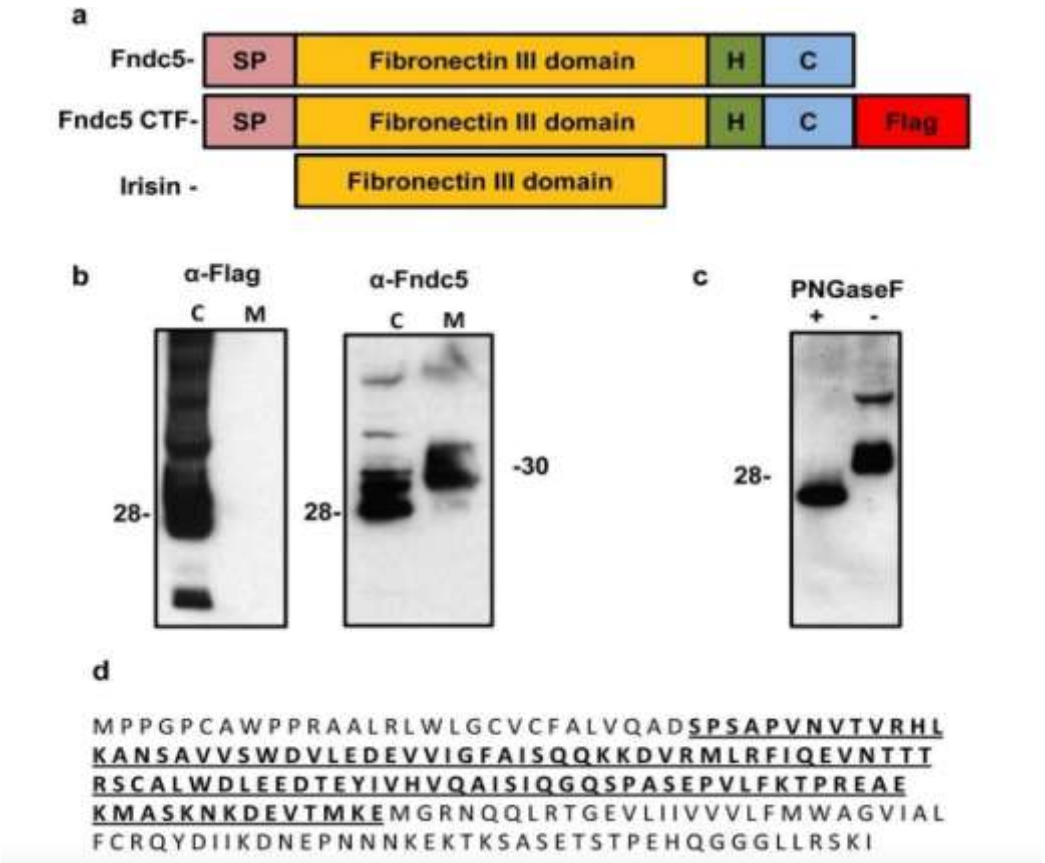

Figure 1: Proteolytic cleavage of Fndc5 (Bostrom *et al.*, 2012)

Irisin is found in both human and mice plasma and is seen to be highly conserved with 100% homology. Even low concentrations of Irisin in plasma induce conversion white adipose tissue into brown adipose tissue **(Bostrom *et al.*, 2012)**. This phenomenon has been shown to improve obesity and glucose

metabolism *in vivo* **(Seale *et al.*, 2011).** Irisin expression in mice on a high fat diet increased glucose tolerance and decreased fasting insulin levels. Only moderate levels of Irisin can increase energy expenditure, reduce body weight and improve diet induced insulin resistance.

Irisin is associated with a varied number of cancers especially those associated with adipose tissue. This is due to the fact that Adipokines play an important role in cancer progression by promoting inflammation and carcinogen formation. Therefore serum Adipokine levels can be a diagnostic marker for cancer detection and prognosis **(Fryczkowski *et al.*, 2018; Hu *et al.*, 2019)**.

IRISIN AND REGULATION OF VARIOUS CANCERS

Recent studies have been key in establishing the Irisin in cancer formation as cancer increases the energy production by elevation the basal metabolic rate of an individual**.**

BREAST CANCER

Exercise has been shown to cause a significant reduction of breast cancer risk in women as much as 30-40% in a dose dependent manner. Moreover the survival rate among women with breast cancer increases with the increase in exercise (**Chen *et al*., 2011)**. Cytokines like IL-6, and tumor necrosis factor alpha have been previously shown to alter breast cancer aggressive **(Madeleine *et al.*, 2011).** These inflammatory cytokines are counteracted by Irisin which acts as a therepuetic agent for breast cancer without affecting non-malignant cells and induce apoptotic cell death only in malignant cells. This happen by the suppression of NFkB activity **(Gannon *et al*., 2015)**. Non-modified Irisin is more effective in suppression of malignant cell number, viability, and migration. Irisin may also be effective in reducing the dosage of antineoplastic agents increasing

tolerance and prognosis by increasing sensitivity and effectiveness of these agents **(Gannon *et al.*, 2015)**.

Irisin has a different pattern of secretion depending on location of adipose tissue. A number of mechanisms have been suggested to link obesity and breast cancer like altered production of adipokines Regulation of adipokines during carcinogenesis could provide a link between obesity and cancer. Irisin being an adipokine is speculatedto have similar regulatory effects on carcinogenesis. Irisin levels were detected to significantly lower in patients with breast cancer when compared to controls. Even one unit increase in Irisin levels lead to 90% reduction in breast cancer risk among women. Irisin levels were also demonstrated to have a positive relation with cancer stage. Increased levels of both adiponectin and Irisin in patients conclusively proved the similar action of both during carcinogenesis **(Coughlin and Smith, 2015)**. **.**

LUNG CANCER

Non-small cell lung cancer accounts for 80% of the lung cancer cases and still has the worst prognosis **(Glatzel-Plucinska *et al*., 2018)**. Despite of the development of targeted therapies for NSCLC it still remains the leading cause of lung cancer death **(Dela Cruz *et al.*, 2011)**. Due to poor prognosis and increased deaths a viable prognostic technique needs to be developed for earl detection of NSCLC. Irisin has been demonstrated through the means of many studies to be and appropriate fix **(Nowinska *et al.*, 2019)**. In an in vitro study containing lung cancer cells, Irisin inhibited the cell proliferation and migration and epithelial-mesenchymal transition via the PI3K/AKT/Snail Pathway. Snail protein is responsible for EMT whose expression was decreased by Irisin **(Shao *et al.*, 2017)**.

Irisin was found to be secreted not only by lung cancer cells but also in the stromal cells whereas the occurrence of Irisin in normal lung epithelial cells were not found. This expression in stromal cells may have a more significant prognostic value. High Irisin expression in fibroblast cells associated with worse patient prognosis could act as an independent prognostic biomarker. Expression of Irisin in stroma is characteristic only of lung cancer. An important component of tumor stroma are cancer associated fibroblasts. Irisin levels decreased in the cancer cells while they increased in stromal cells with the progression of cancer. Irisin expression has been detected in stromal cells which can be characterized as CAFs **(Nowinska *et al.*, 2019).**

It has been shown through several studies the regulation of EMT by Irisin during various types of cancer. Irisin inhibits the migration and invasion of lung cancer **(Shao *et al*., 2017)**. Secretion of Irisin in stromal cells is positively related to the expression of the Ki-67 antigen and also the levels of Irisin is lower with the higher grade of malignancies and the advanced stage of cancer. Hence Irisin levels in stromal cells may lead to division and proliferation whereas its secretion in cancer cells is associated with inhibition of cancer progression **(Nowinska et al., 2019)**.

Irisin promotes glucose uptake by activating AMPKalpha2-mediated p38 MAPK in muscle cells **(Lee *et al*., 2015).** This may be related to the high levels of Irisin during lung cancer due to high demand of glucose by the proliferating cancer cells. Even though cancer cells produce Irisin, it cannot be incepted from the microenvironment **(Nowinska *et al.*, 2019)**.

PANCREATIC CANCER

Pancreatic Cancer is a high risk disease whose prognosis still remains difficult due to its rapid progression, late stage diagnosis, and early metastasis

(Tan *et al.*, 2014). The existing therapeutics are not very effective in dealing with the disease and only 20% of the patients are eligible for surgical excision of the tumor. According to recent stats, the 5-year relative survival is below 8% **(Siegel *et al.*, 2016).**

Irisin's inhibitory effect is considered to be concentration dependent. Moreover Irisin seemed to have arrested cell cycle in the G0/G1 phase of cell division **(Liu *et al*., 2018; Zhang *et al.*, 2019)**. EMT (Epithelial Mesenchymal Transition) plays a crucial role in the development of pancreatic cancer and in its progression and metastasis **(Iwatsuki *et al*., 2010; Hamada *et al.*, 2012; Karamitopoulou, 2013)**. Irisin could be seen to effectively inhibit EMT in pancreatic cancer cells. This effect of Irisin is believed to be due to its suppression of the AMPK-mTOR pathway **(Liu *et al.*, 2018)**.

AMPK plays an important role in energy homeostasis as it is an energy sensor **(Faubert *et al.*, 2015)**. AMPK has been demonstrated to control various pathways of cancer progression and proliferation including the inhibition of mtor-p70S6K/4E-BP1 pathway which is key in controlling organ size and cell growth **(Cheng *et al*., 2016; Hardie *et al.*, 2016)**. Pancreatic cancer cells were also found to contain Irisin specific membrane receptors **(Liu *et al.*, 2018).**

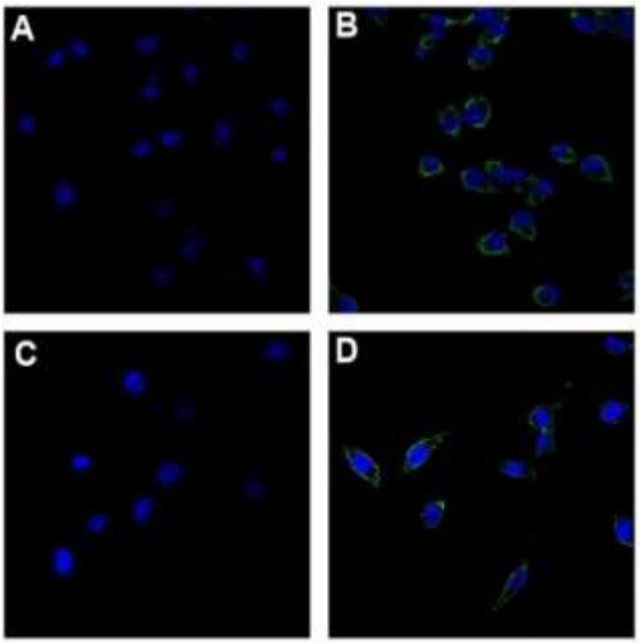

Figure.2: Immuno-fluorescence of Irisin receptors on Pancreatic Cells (Liu *et al*., 2018)

Another study conducted reported that Irisin was able to elevate proapoptotic Bax protein and reduce the levels of anti-apoptotic Bcl-2 protein family inducing apoptosis in pancreatic cancer cell line via mitochondrial apoptosis pathway **(Zhang *et al*., 2019).**

The P13K/AKT signaling pathway is involved in cell proliferation, motility and survival and is key in tumorigenesis but also treatment of cancer. The elevation of P13K and AKT in pancreatic cancer cells leads to poor prognosis. Irisin inhibits this pathway hence stopping the migration and invasion of pancreatic cells **(Zhang *et al*., 2019)**. Irisin also increases the number and function of endothelial progenitor cells via this signaling pathway **(Zhu *et al.*, 2018)**.

Doxorubicin (DOX) is an antitumor drug whose effectiveness increases upon the inhibition of the PI3K/AKT signaling pathway **(Eisa *et al*., 2015; Geng *et al.*, 2015)**. Hence, Irisin was shown to be key in increasing the chemo sensitivity of pancreatic cancer cells to DOX and in turn increase DOX mediated apoptosis of pancreatic cancer cells. This means that a low dose of DOX

combined with Irisin could bring about the same therapeutic effects providing a solution for the resistance of cancer cells towards DOX **(Liu *et al*., 2019)**.

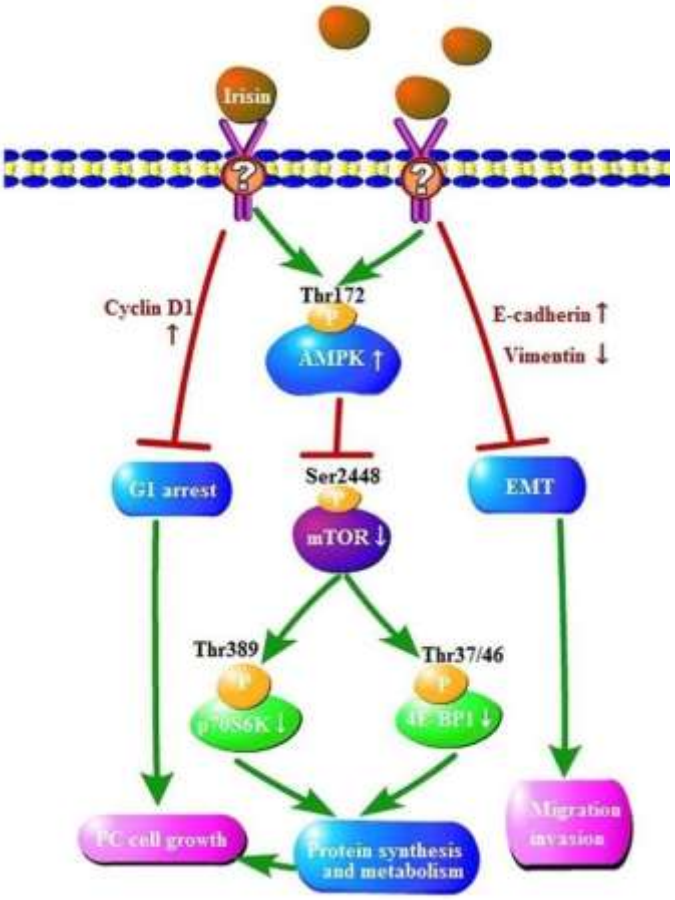

Figure.3: Model of Irisin effects on Pancreatic cells (Liu *et al.*, 2018)

PROSTATE CANCER

Prostate cancer is the second most frequently diagnosed cancer in the world with a frequency of more than 1.1 million cases per year. Prostate cancer accounts for almost 15% of the total detected cases of cancer worldwide **(Tan *et al.*, 2014)**. Prostate cancer is the second most common cause of death in men **(Heidenreich *et al.*, 2014)**. Testosterone is considered to have direct link with the occurrence of prostate cancer and it can be seen that without the effect of hormones the disease won't exist.

A study conducted by **Aslan *et al*, (2020),** demonstrated that serum Irisin levels are considerably low in patients with prostate cancer as compared to

healthy controls. The decreased Irisin levels were suggested to be due to increased malignancy and that high Irisin levels may suppress prostate cancer. They also concluded that malignancies related to hormone imbalance could be associated with decreased serum Irisin levels.

Another study was conducted by **Tekin *et al.,*(2005)** to demonstrate the cytotoxicity of Irisin on different prostate cancer cell lines using an MTT assay observing that Irisin significantly decreased the viability of these cell lines in concentration dependent manner which emerge via an androgen receptor independent mechanism.

CONCLUSION

Irisin is an exercise induced adipokine, a crucial molecule in converting white fat cells into brown fat cells. Act as a key molecule in regulating glucose homeostasis of the body and hence regulating obesity. The link between obesity and cancer has been known for a long time and studies have shown the reduction of risk to cancer merely through exercising as seen in breast cancer studies. The upregulation or downregulation of Irisin levels during carcinogenesis is of great therapeutic value. Although significant research has been carried out in this area, it has been mainly through the means of cell lines and the studies have mostly been *in vitro*. In cancers that occur due to imbalance in hormones like prostate or breast cancers, Irisin levels seem to downregulated while they are seen to be upregulated in cancers like lung cancer and pancreatic cancer. There is an urgent need for reliable biomarkers for prostate cancer for pre-treatment decision making. Irisin levels have been highlighted to act as a novel biomarker for various types of cancer like pancreatic cancer, non-small cell lung cancer wherein definite prognosis still doesn’t exist although the mechanism of action still needs elucidation.

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MUCORMYCOSIS AND ITS ASSOCIATION WITH COVID19

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ABSTRACT

The term Mucormycosis refers to a life-threatening infection in the immunocompromised patients which is caused by fungi which belongs to the Mucorales genera which consists of Rhizopus, Absidia, Morterella and mucor. These fungi are widespread in the nature these grow on the decaying matter and organic material. Although the fungi and spores of Mucorales show minimal pathogenicity in normal beings, it shows very aggressive and fatal effects on immunosuppressed and diabetes patients. Because this is an airborne illness, it usually begins in the upper or lower airways, and is associated with the clinical development of sinusitis, rhino cerebral mucormycosis and pulmonary infection. The spreading of the infection to skin, brain and other sites is less common, yet direct extension to the contagious site is common if proper treatment is not received. Risk factors for the development of mucormycosis diabetes ketoacidosis, neutropenia, protein-calorie malnutrition, and iron overload. Covid associated mucormycosis was a serious issue in the second wave of covid causing serious fatal infection in the covid recovered patients, colloquially known as black fungus, it commonly causes necrosis head, neck, nose, paranasal sinusitis, orbits, facial bones with possible intercranial spread. This infection has a connection with the covid delta variant. This has created a pandemic around the globe with 40 countries including the USA, UK and other countries.

Keywords:- Black fungus, mucormycosis, COVID-19

INTRODUCTION

Mucormycosis is an invasive infection with a high mortality rate even after active management (**Chakrabarti and Singh, 2014)**. This infection is caused by a group of filaments molds belonging to the order Mucorales **(Reid *et al*., 2020**). Mucoromycetes is a fungus with at least 20 pathogenic species divided into 12 genera (**Rao *et al*., 2021**). These Mucorales are decomposers and are found in the decaying matter, rotten fruits, vegetables other than the environmental niche, they reproduce rapidly asexually, it is human pathogenic affecting skin, lungs, brain, gut and other organs (**Divakar, 2021**).

The types of mucormycosis are rhino cerebral ocular mucormycosis (ROCM), cutaneous mucormycosis, pulmonary mucormycosis, gastrointestinal mucormycosis, and disseminated mucormycosis. This infection is commonly seen in immunocompetent patients and people with uncontrolled diabetes mellitus (DM) (**Prakash and Chakrabarti, 2019**). DM is the most common reason for mucormycosis in India and Asian countries, where haematological malignancies and organ transplants are the reason in the US and European countries (**Prakash & Chakrabarti, 2019**).

In low and middle-income countries including India, mucormycosis is associated with high mortality (45%-90%) (**Patel *et al*., 2020**). Unfortunately, despite disfiguring surgical debridement and adjunct antifungal therapy, the overall mortality rate for mucormycosis remains >50% (Ibrahim *et al.*, 2012). During the second wave of COVID-19 countries like India was worst hit and secondary fungal infection such as mucormycosis became an epidemic in the already

existing pandemic. ROCM was the most common form of CAM observed in India, even after many management methods it still has a very high mortality rate.

The aim of this thesis is to give a better knowledge of mucormycosis and CAM and also to address the need to have more research in developing better diagnostic tools and management methods to reduce the mortality rate.

MUCORMYCOSIS

Mucormycosis is a fungal infection caused by a group of filamentous moulds within the order Mucorales and Entomophthorales. Molds from the order Entomophthorales are different the Mucorales. Entomophthorales produce chronic subcutaneous infections in immunocompetent patients, usually in the tropical and subtropical climates. Entomophthorales are not Angio invasive and rarely disseminate. The order Mucorales comprise of numerous genera (e.g.: - *Rhizopus, Mucor, Lichtheimia* [formerly absidia], *Apophysomyces spp, Cunninghamella, Rhizomucor* and *Saksenaea*) and others. *Rhizopus* is the most common genus associated with mucormycosis, followed by *Lichtheimia and Mucor*. Other genera such as *Rhizomucor, Cunninghamella* and *Saksenaea* are less common. *Cunninghamella berthollethiae* appears to be the most dangerous Mucorales strain in humans, with a high fatality rate (**Reid *et al*., 2020**).

In India, *Rhizopus arrhizus* is the most common related mucoral to cause human mucormycosis, followed by *Rhizopus microspores, Apophysomyces* and *R. homothallicus* (Divakar, 2021). *Rhizopus homothallicus and Apophysomyces elegans* species are emerging in India. Even uncommon species such as *Mucor irregularis* and *Thamnostylum lucknowense* are also reported **(Chakrabarti and Singh, 2014**). These genera of Mucorales are one of the best decomposers of organic substances and are found in decaying organic substances such as rooted

fruits and vegetables, plant litter and animal litter, also they are found in the environmental niches including the soil (**Reid *et al*., 2020**). They reproduce asexually through spores that develop endogenously within in a vesicle called the sporangium. Mucormycosis infection spreads by inhaling the sporangiospores or by eating contaminated or by exposure of the spores to open wounds. The spores are 3 to 11 µm in diameter and are easily aerosolized. This infection is not transmitted from one person to other (**Divakar, 2021**).

The brain, sinuses, lungs, skin, and other organs are commonly affected. Uncontrolled DM is the most common reason for this infection in India and Asian countries, whereas haematological malignancies and organ transplants are the common cause in the US and the European countries. This is a Angio invasive infection with high mortality rates.

INCIDENCE AND EPIDEMOLOGY

The incidence of mucormycosis is rising globally, but a drastic increase seen in China and India in patients with uncontrolled DM. However, a recent review of 851 cases over the period January 2000 to January 2017, provides a different view showing the disease burden more in the European countries compared to the Asian countries. Whereas this cannot be completely agreed as most of the cases are not properly reported in the Asian countries. According to the leading international fungal education (LIFE) has estimated the burden of the infection. According to them estimation, the annual prevalence of mucormycosis maybe 10,000 cases excluding India. After the inclusion of India, the global burden rose to 910,000 cases globally. The estimated incidence of the continents are given per million, in Europe from 0.2 in Denmark to 95 cases in Portugal, USA 3.0 cases, Canada 1.2 cases and Australia 0.6 cases (**Prakash and Chakrabarti, 2019**).

However, mucormycosis still remains an uncommon disease, even in the high-risk patients and represents 8.3% to 13% of all fungal infections in the autopsy of patients. The post-mortem reports tell us that the mucormycosis infection is 10 to 50-fold less frequent than aspergillosis or candidiasis with a frequency of 1 to 5 cases per 10,000 autopsies.

Mucormycosis epidemiology in India differs from that of European countries and the United States. In India, uncontrolled diabetes mellitus outnumbers other risk factors such as haematological malignancy and organ transplantation, both of which are substantial risk factors in affluent countries. The current study validates this, as 56.8% of the patients had diabetes mellitus, and the majority of them presented with ROCM (65.7 percent). Uncontrolled diabetes as risk factor was significantly higher in north India though the prevalence of diabetes mellitus is more in the south Indian population compared to north India **(Prakash *et al*., 2019**).

PATHOGENESIS

Pathogenesis of mucormycosis begins with the inhalation of the sporangiospores, or inoculation of the spores through open wounds or trauma. In healthy individuals, mononuclear and polymorphonuclear phagocytes (PMNs) eliminate fungal spores and hyphae by oxidative and nonoxidative killing mechanisms **(Bhogireddy *et al*., 2021**). Phagocytic activity deficiencies enhance the organism's survival or growth. (e.g., deficiencies in phagocyte function or neutropenia). Hyperglycaemia and acidosis, in particular, impede chemotaxis and phagocytic killing. Rhizopus also produces the enzyme ketone reductase, which allows it to flourish in acidic and glucose-rich situations like ketoacidosis. Mucorales have a natural resistance to being killed by human phagocytes, which could explain their virulence.

In the aetiology of mucormycosis, iron metabolism is crucial. Patients with iron overload (including those receiving deferoxamine chelation therapy) are predisposed to mucormycosis. Deferoxamine stimulates Mucorales growth in vitro by functioning as a siderophore. In addition, greater blood iron availability in people with acidosis, which is partly attributable to transferrin's decreased affinity for free iron at pH below 7.4, may increase vulnerability to mucormycosis. Mucormycosis has a penchant for entering blood arteries, causing thrombosis and tissue necrosis as a result. Angioinvasion may be aided by the interaction of fungal spores with endothelial cells. Further, interaction with host endothelial cell receptors may promote endothelial cell damage and fungal spread (Reid *et al*., 2020).

SIGNS AND SYMPTOMS

Persistent dryness of the nose, local pain on the cheekbone, fever, shortness of breath, toothache, and tooth loss are the first indications of mucormycosis, which can be further extended to eye pain, blurred or double vision with pain. In later stages, the nervous system can also be involved and patients can experience neurological symptoms like headache, confusion, and altered mental status **(Kumar, 2021**). The Angio invasive nature of mucormycosis is characterised by thrombosing vasculitis and its role in host invasion have been attributes to increased expression of platelet derived growth factor signalling (**Rao *et al*., 2021**).

RISK FACTORS

DIABETES MELLITUS (DM)

Patients with uncontrolled hyperglycaemia, mainly those with ketoacidosis are most susceptible (**Petrikkos *et al*., 2012**). Poorly controlled DM is associated with defects in innate immunity particularly phagocytosis, chemotaxis, and killing by monocytes or macrophages. Diabetes with ketoacidosis are more prone to develop the rhino cerebral mucormycosis, where pulmonary and disseminated mucormycosis is unassociated with diabetes-mucormycosis (**Reid *et al*., 2020**).

The percent of DM being a risk factor is 17% to 88% globally. Whereas in India two multi-centre studies say that over 57%of the patients have DM and 10% have diabetic ketoacidosis. Another study showed that the prevalence of mucormycosis in diabetes patients is 1.6 per 1000 patients. The diabetes risk factor was seen higher in the north regions compared to the south regions (**Prakash & Chakrabarti, 2019**).

HEMATOLOGIC MALIGNANCIES (HM)

In hematologic malignancies the chemotherapy related defects in the innate host defences, phagocytotic dysfunction, neutropenia or mucociliary dysfunction predispose to the infection. Mucormycosis is rare but it is life-threatening to patients with hematologic disorder and neutropenia.

The percent of hematologic malignancies being a risk factor is 38% to 62% in Europe and the U.S (**Prakash and Chakrabarti, 2019)**. Among the patients with HM, those suffering from acute myelogenous leukaemia (AML) are at the

highest risk, with a incidence of 1% to 8%, the incidence is less in other chronic and acute HM's (**Petrikkos *et al*., 2012**).

ORGAN TRANSPLANTATION

Mucormycosis is a rare complication found among the patients in solid organ transplants (SOT) but has a high mortality rate (Reid *et al*., 2020). As all patients undergo chronic immunosuppression, with high doses of corticosteroids which disseminates the infection to skin and soft tissues, this does not affect the sinuses, brain or lungs. The estimated incidence of SOT's ranges from 0.4% to 16% depending on the type of SOT's. The renal transplant recipients have a range of 0.2% to 1.2%, liver transplant recipients have a range of 0% to 1.6%, heart transplant recipients have a range of 0% to 0.6%, and lung transplant recipients have a range of 0% to 1.5% and neutropenia is absent in SOT's patients (**Petrikkos *et al*., 2012**).

Overall, the SOT's recipients have a incidence of 2% to 15% in mucormycosis cases globally. The patients with HSCT have a greater risk of contracting mucormycosis during the neutropenic phase. The HSCT has a range of 0.9% to 2% (Petrikkos *et al*., 2012). A multi-centre cohort studies shows that France has a rate of 0.4%, Italy has <0.1% incidence rate, whereas U.S has 8% incidence of invasive fungal infection (**Prakash and Chakrabarti, 2019**).

AUTOIMMUNE DISORDERS

Opportunistic mucormycosis is commonly seen in people suffering from auto immune disease. Mainly with patients suffering from Wegener granulomatosis, mucormycosis mimics with the relapse of the already underlying conditions and go undiagnosed making the mortality rate very high.

The autoimmune disease has a rate of 12% of mucormycosis cases, whereas a study conducted showed that 2% of individuals with mucormycosis had an autoimmune illness as a co-morbidity (Prakash and Chakrabarti, 2019).

IMMUNOSUPPRESIVE THERAPY

The use of corticosteroids for an extended period of time is a major risk factor for mucormycosis as it causes defects in macrophages and neutrophils, or steroid induced diabetes. In this clinical form can be present but disseminated mucormycosis is found to be common with high mortality rate **(Petrikkos *et al*., 2012).**

HIV OR AIDS

Mostly mucormycosis in HIV patients is due to intravenous drug. Whereas the occurrence of mucormycosis is very rare. This also shows that the occurrence is less in HIV or AIDS patients compared to other immunocompetent patients **(Petrikkos *et al*., 2012).**

IRON OVERLOAD

DFO therapy, an iron chelator used to treat iron and aluminium overload in dialysis recipient is reportedly a risk factor for Angio invasive mucormycosis. Other than DFO therapy, iron overload either trans fusional or caused by dyserythropoiesis, is also a risk factor for mucormycosis. The most common presentation of mucormycosis is disseminated mucormycosis, associated with high mortality rate (**Petrikkos *et al*., 2012**).

A study conducted showed that 78% of the patients with the deferoxamine (DFO) therapy had mucormycosis which made gave the scientists that DFO therapy is a risk factor for mucormycosis with a high mortality rate of 80%,

however the new iron chelator is not pre-disposing any risks for mucormycosis **(Prakash and Chakrabarti, 2019).**

PROLONGED USE OF VORICONAZOLE

The widespread use of aspergillus-active agents, especially voriconazole, mainly in patients with HM and recipients with haemopoietic stem cell transplants are at the higher risk for mucormycosis is linked with increased incidence of mucormycosis (**Petrikkos *et al*., 2012**).

CLINICAL FORMS OF MUCORMCOSIS

RHINO ORBITO CEREBRAL MUCORMYCOSIS

Rhino cerebral mucormycosis (ROCM) is the most common form of infection in patients suffering from uncontrolled DM. It may occur in patients with underlying conditions such as HemeM, HSCT, SOT and other risk factors. The infection develops after inhaling of the fungal sporangiospores into the paranasal sinuses. The infection may then rapidly spread to the adjacent tissues. After germination the invading spores may invade inferiorly the palate, posteriorly invade the sphenoid sinus, laterally into the cavernous sinus and the orbits, or into the cranially to invade the brain. The fungus invades the cranium either through the orbital apex or cribriform plate of the ethmoid bone which ultimately kills the host.

The initial symptoms of ROCM are consonant with those of the periorbital cellulitis, sinusitis, eye or facial pain and followed by facial numbness leading to blurry vision. Multiple cranial nerve palsies, unilateral periorbital facial pain, eyelid oedema, blepharoptosis, proptosis, orbital inflammation, acute ocular

motility abnormalities, acute vision loss, and headache are all signs and symptoms of mucormycosis.

A classic hallmark of this infection is a necrotic black eschar or crust in the nasal cavity, palate, nasal dorsum or face (Chouhan *et al*., 2021). However, the absence of the eschar does not exclude the possibility of the mucormycosis. Fever is varied, and in half of the cases, it may be absent. There is a drastic elevation in the WBC's count as long as the patient has a functioning bone marrow.

A study conducted on 43 individuals showed the following percent for the symptoms nasal discharge (69%), face swelling (69%), headache (88%), decreased vision (21%), face pain (65%), fever (14%), epistaxis (9%) and with cranial nerve palsy (25%). The diagnostic tools used are CT scans, MRI and tomography **(Therakathu *et al*., 2018)**.

PULMONARY MUCORMYCOSIS

Pulmonary mucormycosis is the second most type of infection seen in HM and organ transplant recipients (**Prakash and Chakrabarti, 2019**). Pulmonary mucormycosis is seen mostly in the middle-aged men group, and also it has no occurrence in people without any predisposing conditions or factors (**Lin *et al*., 2017**).

The clinical features of pulmonary mucormycosis cannot be specified and cannot be differentiated from pulmonary aspergillosis **(Petrikkos *et al*., 2012**). The symptoms of pulmonary mucormycosis are patients usually show up high persistent fever, cough, neutropenia, and dyspnea (**Lin *et al*., 2017)**. In rare circumstances, this form of infection can present tracheal lesions or endo bronchial lesions especially in diabetic patients. Endo bronchial mucormycosis

can cause airway blockage resulting in lung collapse and also lead to massive haemoptysis. this can invade to lung adjacent organs such as the chest wall, pericardium and the mediastinum

The diagnosis used are CT and MRI imaging the imaging shows the presence of large nodules or consolidations with a reverse halo sign or large perilesional ground glass halos are common in pulmonary mucormycosis. In patients with severe disease the imaging showed multi focal pneumonia pattern, and this pattern is associated with a high mortality rate.

CUTANEOUS MUCORMYCOSIS

Cutaneous mucormycosis results from the direction inhalation of the fungal sporangiospores into the open wound, burns or the skin. Cutaneous mucormycosis are of two types the primary form in which the infection is caused due to the direct contact of the spores into the skin, whereas the secondary form in which the infection is disseminated from another infected site. Primary infection is usually caused by Rhizopus variabilis in immunocompetent patients. Whereas, secondary infection is caused from rhino cerebral or disseminated mucormycosis which is an acute onset and a high mortality rate (**Castrejón-Pérez *et al*., 2017**).

Cutaneous mucormycosis is classified as localized when it affects the skin and the subcutaneous tissue, and it is termed as deep extension infection when it spreads to the bones, muscles and tendons. The clinical findings show necrotic eschar surrounded by erythema. And it also shows superficial lesions with elevated circinate and squamous borders, targetoid plaques with an outer erythematous rims and presence of blackened necrotic centres, it would look like a bread mould in open wounds.

GASTROINTESTINAL MUCORMYCOSIS

Gastrointestinal mucormycosis is a very rare condition and is seldomly diagnosed in living patients, and this case the diagnosis is delayed and has high mortality rate. The infection is predominantly seen in premature neonates, malnourished children and individuals suffering from HM's, uncontrolled DM, organ transplants and patients with previous corticosteroid therapy. This infection is caused due to the consumption of fermented porridges, fermented milk, dried bread, contaminated herbal and homeopathic medicine.

The infection may occur in any part of the alimentary canal, the common symptoms are fever, abdominal pain, GI bleeding, nausea and perforation **(Monte *et al*., 2020**). It also comes with cecal, ileac or appendiceal mass perforation with frequent massive bleeding. In premature neonates, the GI mucormycosis presents necrotizing enterocolitis. The diagnosis is usually delayed due to unspecific symptoms and a deep examination should be conducted, leading to an early endoscopic biopsy analysis which helps in early detection.

This GI mucormycosis spreads to other organs such as the liver, spleen, and pancreas as well as the blood vessels and the bowel walls. This causes massive GI bleeding, bowel perforation which is the reason of the death of the host.

DISSEMINATED MUCORMYCOSIS

Mucormycosis in one organ can spread hematogenously to other organs this form of infection is termed as disseminated mucormycosis. The most commonly organ associated with dissemination is lungs, this can also occur in the alimentary tract, cutaneous lesions and burns as well. However, brain being the

common site of spread, the lesions can be found in liver, heart, spleen and other organs.

The patients with iron overload, corticosteroids, HSCT and active leukaemia are the groups at risk for disseminated mucormycosis. The symptoms may vary from person to person and also the level of vascular infection. Without early detection, proper treatment this type of mucormycosis is always fatal (**Petrikkos *et al*., 2012**).

DIAGNOSIS

Diagnosis is a very important step as it helps in the detection of a particular infection or disorder. In mucormycosis initiation of early treatment is the key to control invasive Mucorales, this can be achieved only through early diagnosis. An early diagnosis in case of mucormycosis is very beneficial as it may prevent the invasion of the infection to the vascular tissues (**Sharma *et al*., 2021)**.

Development of improved diagnostic methods can be achieved by recognising the factors such as host factors, clinical manifestations, early use of computed tomography (CT), and magnetic resonance imaging (MRI) modalities, evaluation of histological and cytological preparations, implementation of advances in molecular detection and optimal use of clinical microbiological methods (**Walsh *et al*., 2012)**.

DIAGNOSTIC IMAGING

Early detection of pulmonary and sinuses lesions is taken by CT and has been a key advance to give sinus and chest radiographs. In immunocompromised patients who are at a high risk for invasive pulmonary infection, early CT may reveal pulmonary lesions in the absence of findings in the conventional

radiographs. CT have the ability to detect lesions that are characteristically associated with Angio invasive mucormycosis, such lesions include halo signs, reverse halo signs, nodules, cavities wedge-shaped infiltrates and pleural effusions associated with pain. The only disadvantage of imaging is that it's risky to do this procedure for children as it requires ~ 6mSv to get each CT scan done. However imaging may help in early diagnosis but defective diagnosis should be established by biopsy and culture (Walsh *et al*., 2012).

LABORATORY DIAGNOSIS

The signs and symptoms and radiological findings may be nonspecific at times, so defective diagnosis is important for correct identification of the infection and the causative agent. This can be done in numerous ways, one way is by the direct examination of sputum, paranasal sinus secretions, bronchoalveolar lavage(BAL) fluid is used by met mount or calcofluor technique for the identification ofthe organism. Secondly by direct microscopy by doing a biopsy, this gives a precise diagnosis of the infection by identifying the hyphae in the collected samples. And lastly by the use of staining methods such as periodic acid Schiff stain, Gomori methenamine silver stain and also cultures and antigen detection(**Garg *et al*., 2021**).

MOLECULAR DIAGNOSIS

Although direct examination is the standard method for detection, recovery of the organisms using the microbiological techniques become difficult at times, as the elements may not be abundant and the infection may not be seen. Due to this a lot of infection is confirmed in the post mortem examination.

With the increase in the number of cases in the recent years, molecular approaches for the detection are important, as it helps in early and fast

detection leading to direct therapy. Studies have shown that molecular identification is accurate and fast it is done using the internal transcribed spacer (ITS) region as a first line sequencing target for identification of the organisms in pure culture. Another laboratory has developed two quantitative PCR (qPCR) assays, targeting the 28S ribosomal RNA gene. The first qPCR assay distinguishes Rhizopus, mucor and Rhizomucor species through meta curve analysis. The second qPCR detected Cunninghamella species using different primer probe set technique. Mucormycosis can be diagnosed molecularly, which allows for more research into the standardisation of a wider range of primers and properly designed clinical trials, as well as improving early diagnosis and detection **(Walsh *et al*., 2012).**

MEDICAL MANAGEMENT OF MUCORMYCOSIS

Early diagnosis and proper treatment are a very important part of mucormycosis as it helps in better outcome and it may increase the survival as well **(Kontoyiannis & Lewis, 2011).**

ANTIFUNGAL THERAPY

The choice of which antifungal drug should be used is decided by the firm diagnosis.

Amphotericin B is the first-line treatment for the illness, and it can improve patient outcomes dramatically. Amphotericin B treatment is required until clinical improvement is evident, which usually takes a few weeks. Instead of amphotericin B deoxycholate, which is a cheaper and more toxic alternative, a lipid formulation of IV amphotericin B is commonly utilised. Metabolite repletion following amphotericin therapy should also be checked (**Yasmin *et al*., 2021**).

A point of conflict and disagreement is the initial dose and the lipid amphotericin B formulation to employ. In order to manage the infection, most clinicians employ a lipid formulation of amphotericin B with a typical initial dose of 5 mg/kg that is sometimes escalated to 7.5 to 10 mg/kg per day. We believe that amphotericin B lipid complex is an effective option, albeit we have found that doses greater than 5 mg/kg per day are less well tolerated than amphotericin B liposomal formulation. High-dose liposomal amphotericin B has a higher risk of nephrotoxicity and hasn't been proven to be more effective than a daily dose of 3 mg/kg.

According to animal models of pulmonary mucormycosis, higher amphotericin B tissue concentrations may be required early in the infection for efficient mucormycosis treatment. Because of the frequent and early start of renal toxicity in haematology patients with opportunistic mycoses, we do not utilise amphotericin B deoxycholate (**Kontoyiannis and Lewis, 2011)**.

Individuals with compromised renal function should be started on triazoles, such as Posaconazole and Isavuconazole, which suppress ergosterol production in the fungal cell membrane and are hence nephrotoxic. These broad-spectrum azoles are available in both oral and parenteral formulations and are effective against mucormycosis agents.. These are also used as Salvage therapy and as a step-down therapy in patients who can tolerate amphotericin B (**Yasmin *et al*., 2021**).

Patients prescribed Posaconazole should be provided education and counselling by the physician, nurse, or pharmacist and be seen by the nutritionist will evaluate you in order to establish a specific nutritional plan that will aid in medicine absorption and compliance. Because of their ability to produce prolonged tissue concentrations, we occasionally use intermittent doses of lipid amphotericin B (5 mg/kg 3 times per week, or even 2 times per

week) as an alternative to Posaconazole therapy after at least 3 to 4 weeks of initial therapy. These intermittent dosing strategies also provide a more facile transition to outpatient, Posaconazole based oral therapy **(Kontoyiannis and Lewis, 2011)**.

SURGERY

Poor drug bioavailability to the infection site can be caused by angioinvasion and blood vessel thrombosis. Patients should be prepared and prioritized for surgery, even with the slightest suspicion of mucormycosis. Because of the infection's aggressive nature, surgical debridement of isolated cutaneous or sinus lesions is critical and must be done as soon as possible. Repeated removal of necrotic tissue or aggressive surgical measures, such as enucleation of the eye may be required to prevent dissemination.

An MRI/CT guided endoscopic sinus approach should be performed to remove the infected tissue. Orbital exenteration and vigorous debridement of the paranasal sinuses should be used to control rapid invasion of the orbits (less than 72 hours). IV amphotericin B should be continued, followed by step-down therapy. Triazoles should be used to treat refractory instances (**Yasmin *et al*., 2021**). Surgery in conjunction with systemic antifungal therapy has been shown to significantly improve survival rates in pulmonary mucormycosis.

ADJUNCTIVE THERAPY

Hyperbaric oxygen therapy is a beneficial adjunct therapy for mucormycosis, particularly diabetic patients with rhino cerebral or extensive cutaneous disease (**Kontoyiannis & Lewis, 2011)**. By correcting acidosis, the higher oxygen pressure provided is known to promote neutrophil function and increase AMB activity. Finally, increasing oxygen pressure slows wound healing and inhibits

fungal development by limiting spore germination. As a result, HBO treatment for mucormycosis has been recommended as an addition to surgical and antifungal therapy. However, because this treatment has not been thoroughly tested to determine its efficacy, it cannot be suggested on a regular basis. Immune augmentation strategies are also considered in patients with refractory mucormycosis, including administration of cytokines.

Unfortunately, despite disfiguring surgical debridement and adjunct antifungal therapy, the overall mortality rate for mucormycosis remains >50% **(Ibrahim *et al*., 2012)**

CAM (COVID ASSOCIATED MUCORMYCOSIS)

The World Health Organization (WHO) proclaimed Coronavirus Disease 2019 (COVID-19) a global pandemic in March 2020, attributable to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The pandemic continues to be an ongoing public health concern with more than 162 million cases recorded, and more than 3 million deaths globally (**Selarka *et al*., 2021**).

The deadliest epidemic of the millennium, Novel Coronavirus Sickness (COVID-19, SARS-CoV-2), has thrown the world's health system to its knees, with millions of people falling victim to this fatal disease. Countries all around the world, whether developing or developed, were forced to impose a lockdown and a measure of social separation to prevent the spread of infection. WHO). B.1.617.2, one of three B.1.617 strains discovered in India, is a Variant ofConcern (VOC) with a higher public risk.

Moreover, due to an increase in the number of cases due to the new variety, the country experienced a high demand for medical oxygen and post-COVID cardiac problems during the second wave. To make matters worse, during the second

wave of the COVID-19 pandemic in May 2021, India saw another epidemic, this time of mucormycosis (black fungus). More than 31,000 cases have already been reported so far across the country and more than 2100 people have died as a result of mucormycosis (**Gambhir *et al*., 2021**).

The average time between COVID-19 diagnosis and the start of mucormycosis symptoms is 15.6±9.6 days. A delay of even 6 days in seeking treatment doubles the 30-day mortality from 35% to 66% .

CAUSES OF CAM

ABUSE OF STERIODS

The most common cause of black fungus infection. Even though steroids are effective in treating many types of inflammation, including rheumatoid arthritis and lung disorders like asthma and chronic obstructive pulmonary disease, their long-term use or overdose suppresses the immune system and make the patient more prone to other infections like mucormycosis or black fungus infection in India (**Bhogireddy *et al*., 2021**).

IMPROPER USAGE OF OXGEN CYCLINDER

Another possible cause of the increase in post-COVID mucormycosis is unsanitary oxygen supply or low-quality tubing systems in hospital ICUs, oxy-gen cylinders with filthy masks or humidifiers utilising contaminated/tap water, and prolonged use of the same mask for more than two patients.

UNCONTROLLED DIABETES

In post-COVID-19 patients, diabetes may increase the incidence of black fungus infection, with more than 8 out of 10 black fungus cases found in diabetic

patients. Diabetics have a reduced immunological response, which is exacerbated by hypoglycaemic medications. Extensive care should be taken in Covid-19 patients with the history of diabetes mellitus.

TOCILIZUMAB THERAPY

Tocilizumab is a recombinant anti-IL-6 receptor monoclonal antibody that has been humanised. The patient's immune response is weakened by long-term use of this medication to manage inflammation caused by the severe acute respiratory syndrome-associated corona virus (SARS-CoV). In this way tocilizumab increases the risk of mucormycosis in post-COVID-19 patients (Bhogireddy *et al*., 2021).

Another factor could be an overabundance of uncontrolled traditional precautions. Repeated steaming, for example, may disrupt the beneficial microbiome and virome of the nasal tract. Nasal microbial imbalance (dysbiosis) may reduce local immunity, allowing fungal infection to thrive. During Covid-19, an increasing number of people took Zn disproportionately through vitamins and other dietary supplements in an attempt to prevent/ameliorate viral illness. It is evident that the Zn deprivation inhibits fungal growth in the body.

TABLE 1: **Clinical manifestations of CAM (Yasmin *et al*., 2021)**

Type	Pathogenesis	Clinical Manifestation	Risk Factors
Rhino-cerebral mucormycosis	Spores invade sinuses, cribriform plates, and	Infects the sinuses and spreads to the brain. Destroys maxillary-facial structures and causes ptosis,	Common in patients with uncontrolled diabetes and kidney transplant.

	through the cavernous sinus.	proptosis, and permanent vision loss.	
Pulmonary mucormycosis	Spread of fungal infection through the bloodstream.	Destroys bronchial airways, causes dyspnoea, tracheal invasions of the lungs, and a reverse halo sign on CT scan.	Patients with cancer, post-transplant immunosuppressive therapy
Gastrointestinal mucormycosis	Inhaling spores that invade the GI tract.	Fever, bowel, and per rectal bleed.	Consistent use of broad-spectrum antibiotics, malnutrition, and neutropenia.
Cutaneous mucormycosis	Direct inoculation of skin through site of trauma or thermal burns.	Black discolouration and lesions on the skin.	Skin trauma such as surgery or burns. It does not involve an impaired immunological response.
Disseminated mucormycosis	Occurs when the infection spreads through the bloodstream to another part of the body	Commonly affects the brain, but also other organs such as the spleen, heart, and skin.	Iron overload, neutropenia, suppressed immune system

MEDICAL MANAGEMENT OF CAM

Mucormycosis is treated through early diagnosis and treatment, as well as surgical debridement of infected tissue, antifungal therapy, and management of the underlying condition. And other management methods as mentioned above in management of mucormycosis. Other management techniques include **(Gambhir et al., 2021).**

- **Proper cleaning and sterilization of the humidifiers and ventilators**
- **Use of disposable or disinfected personal protection equipment**
- **Following high standard of personal hygiene**
- **Keeping a high standard of oral hygiene**
- **Disposal of used tooth brush after recovering from covid**
- **Steroids to be used wisely as advised by doctors**

CASES OF CAM

In total, 28 articles from the PubMed (24/28) and Google Scholar (4/28) databases were found to report the original case(s). A total of 101 cases of mucormycosis were found in patients with confirmed (RT-PCR diagnosis) COVID-19 (including confirmed [95/101] and suspected [6/101]). 82 instances (81.2%) of mucormycosis in COVID-19 patients were reported from India, followed by 9 cases (8.9%) from the United States and 3 cases (3.1%) from Iran. There are just 19 of them (18.8 %)cases as of now were reported from other parts of the world (**Singh *et al*., 2021**).

An upsurge of mucormycosis is being reported throughout the world over the past two decades, however, the rise in developing countries including India has been phenomenal (Chakrabarti & Singh, 2014).

Table 2:Cases of mucormycosis and CAM in India (Kumar *et al*., 2021)

	Study Period	Study duration	Place of study	Total cases
Pre-COVID-19 period	1990–2004; 2006–2007	15 years 6 months	Chandigarh (North India)	382
	2005–2015	10 years	Tamil Nādu (South India)	184
	2010-2014	5 years	Chandigarh (North India)	82
	January 2013–May 2015	2 years 5 months	Gujarat (West of India)	27
	2013–2015	3 years	North and South India	388
	January 2016–September 2017	1 year and 9 months	Across India	465
	2015–2019	4 years	Tamil Nādu (South India)	38
	September 1–December 31, 2019	4 months	Across India	112
COVID-19 period	September 1–December 31, 2020 January 1, 2020 - May 26, 2021	4 months 17 months	Across India Across India	295 2826

During the second wave of covid India was hit the worst with increase in secondary fungal infection mucormycosis locally known as black fungus. The first case was reported in Gujarat, whereas Uttar Pradesh reported the first death caused by mucormycosis. With Rajasthan recording the highest deaths. Gujarat, Maharashtra, Karnataka, Andhra Pradesh, and ten other Indian states reported the majority of the instances (**Bhogireddy *et al*., 2021**). In India more than 45,432 cases and 4252 deaths are reported due to CAM (**Kumar *et al*., 2021)**.

CONCLUSION

Mucormycosis is a fungal infection caused by a group of filamentous moulds within the order Mucorales and Entomophthorales. Molds from the order Entomophthorales are different the Mucorales. Entomophthorales produce chronic subcutaneous infections in immunocompetent patients, usually in the tropical and subtropical climates. Entomophthorales are not Angio invasive and rarely disseminate. The order Mucorales comprise of numerous genera (e.g.: - *Rhizopus, Mucor, Lichtheimia* [formerly absidia], *Apophysomyces spp, Cunninghamella, Rhizomucor* and *Saksenaea*) and others. *Rhizopus* is the most common genus associated with mucormycosis, followed by *Lichtheimia and Mucor*.

The incidence of mucormycosis is rising globally, but a drastic increase seen in China and India in patients with uncontrolled DM. DM is the most common reason for mucormycosis in India and Asian countries, where haematological malignancies and organ transplants are the reason in the US and European countries.

Pathogenesis of mucormycosis begins with the inhalation of the sporangiospores, or inoculation of the spores through open wounds or trauma. Mucormycosis symptoms include persistent dryness of the nose, local pain on the cheekbone, fever, shortness of breath, toothache, and tooth loss, as well as eye pain, blurred or double vision, and soreness.

The risk factors of mucormycosis are uncontrolled DM, hemeM, SOT's and HSCT, autoimmune disease, immunosuppressive therapies, HIV, trauma, etc. The types of mucormycosis are rhino cerebral ocular mucormycosis (RCOM), cutaneous mucormycosis, pulmonary mucormycosis, gastrointestinal

mucormycosis, and disseminated mucormycosis. The diagnosis for mucormycosis is imaging, laboratory techniques, molecular diagnosis. The management methods are antifungal therapies, salvage therapy and surgery.

CAM was the worst epidemic in a already pandemic situation creating havoc around world, being a secondary fungal infection it comes with high mortality rate. The risk factors of CAM are uncontrolled DM, unhygienic ventilators, misuse of steroids. The precautions taken to control is to have a proper oral hygiene, clean the ventilators, use fresh water in humidifiers.

Concluded here that mucormycosis is a rare but a fatal disease with high mortality rate, the most common form of mucormycosis is ROCM, the most common form of CAM was pulmonary followed by ROCM the maximum no of cases of mucormycosis is observed in the European countries followed by India and China. The maximum no of CAM was observed in India during the second wave of covid. It can also be that CAM is more fatal than normal occurring mucormycosis. It also shows that uncontrolled DM is the major and the most common factor for both mucormycosis and CAM.

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LIFE HISTORY AND DAMAGE SYMPTOMS OF *PLUSIS* SP. ON *ADATHODA VASICA*

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ABSTRACT

The biology and damage symptoms of *Plusis* sp. has been studied. They have completed their life cycle in 30.4 ± 5.3 days respectively with five larval instars. The mean developmental duration of third instar larva, fourth instar larva, fifth instar larva, prepupa and pupa were 3.9 ± 0.6, 3.00 ± 0.6, 3.6 ± 0.4, 1± 0.4 days and 4.50± 1.6 respectively. The third instar and last instar larva measured 17.23+0.13 and 32.62+0.29 in length respectively. The incidence of *D. caesalis* was recorded throughout the year during 2013–2016 indicated an overlapping of generations. However, two distinct peaks in the population level were recorded during June– July and October–November.

Key words: Life cycle, Medicinal plants, damage symptoms

INTRODUCTION

In Indian system a large number of medicinal plants have been used for many centuries for treating various diseases (Wright and Philipson,1990) These plants have a wide variety of chemical constituents and some of them have the ability to inhibit the growth of micro-organisms (Iqram,1984). It is estimated that in this modern age 80% of home care and traditional native system of medicinal and major part of these therapies use plant extracts or their active principles

(Ramesh et al, 1985). The use of plants for treating various diseases predates human history and forms the origin of much of the modern medicine. Long before the advent of modern medicine, herbs were the main stream remedies for nearly all ailments. People commonly diagnosed their own illness, prepared and prescribed their own herbal medicines or bought them from the local apothecaries (Tyler, 2000).

According to WHO, herbal medicine is defined as plant derived material or preparation which contain raw or processed ingredient from one or more plants with therapeutic values (Dev, 1999). The increasing use of medicinal plant and extracts in industrial society has been traced in the present years for the development of several drugs and chemotherapeutics from these plants but occurrence of pests on these medicinal plants is a major drawback.

Adhatoda vasica Nees (Acanthaceae) commonly known as vasaka distributed throughout India up to an altitude of 1300 m) (figure 1a). It is a small evergreen sub herbaceous bush. This bush is 4-8 feet height and the leaves are 10 to 15cm in length The leaves, flowers, fruit and roots are extensively used for treating cold cough, whooping cough, chronic bronchitis and asthma, as sedative, expectorant and antispasmodic (Pandita, 1983; Gangwar and Ghosh, 2014).

Adathoda sp. is infested by many insect pests such as lepidopteran moth (*Plusia sp*.), grasshopper, spider and scale insect. The previous studies conducted by other researchers reported on this plant was infested by grasshopper in the months of July to September. Larva of lepidopteron moth infestation was maximum during the months of November to January in the month of October–November scale insects' infestation was recorded. Another

pest, weaver spider was noticed on this plant in the month of May and June (E Emimal Victoria, 2010). The other pests reported on Adhatoda is jewel buds damage by sucking cell sap and convert the plant matter into liquid by injecting proteolytic enzymes through saliva and then the required material. Thus, leaves turn dead and cause reduction in alkaloid content production (Arun kumar tripathi, 2004).

Plusia sp. is a moth belonging to family noctuoideae. *Plusia festucae* was reported on Carex, *Sparganium erectum, Iris pseudacorus and Alisma*. Pupates in a whitish cocoon on the underside of a blade of grass, doubled over for the purpose. Eventhough there are many reports of insects feeding on *A. vasica*, there was no serious studies on the life history and life cycle of this pest on *A. vasica*. Antifeedent and Toxic activity, Photosynthetic activities of *A. vasica* is available. Hence the current study aims at a life history and damage symptoms of Plusia sp. on *A. vasica*.

MATERIALS AND METHODS:

Study Area

The area chosen for this study was the Jyoti Nivas college Autonomous of Bangalore city, Karnataka. Study area is situated between 12.9331" N and 77.6169" E. The pest was collected from leaves of *Adhatoda* plant.

Sampling Methods

A few individuals of larval stages which were found damaging the leaves of the host plant with the help of hand picking method. Photographs were taken. The insect pests were identified based on the morphological and structural characteristics. The pests were brought to lab with leaves. The pests brought were in the third, fourth and fifth instar Phases. There were eggs seen on the

young tender leaves of the plant which was observed under the dissection microscope.

Laboratory Culture of the Pest

The study was carried out under the mean temperature 29.0 ± 2.6 °C and mean relative humidity 43.5 ± 5.9%. The pest was culture in the laboratory on *A. vasica* leaves. Adults of *Plusia* sp. emerged in the laboratory were allowed to mate in a nylon mating cage (15 × 15 × 15 cm) lined with black cloth and provided with 10% honey solution in cotton swab as food. The eggs laid on the black cloth were kept separately along with fresh jackfruit leaves and buds in Petri dishes (15 cm diameter) to determine the duration and number of different larval instars.

Morphology and life cycle

The rearing containers were examined daily for exuviae and head capsules to confirm the moulting of larvae. The egg, larval, prepupal and pupal period and the number of instars in their life cycle were recorded. The egg, each larval instar, the head capsule of each instar, prepupa, pupa, and adult were measured with a calibrated micrometre fitted within the eyepiece of a stereo zoom microscope. External morphology of the egg, larval stages, prepupa, pupa and adult was recorded.

It was observed that the larvae of third and fourth instar was molting to change into fifth instar. It will shed its exoskeleton and it was found in the tray. Differences between instars was seen in body proportional, color, pattern and the number of segments. After molting the larvae continued its life cycle until they pupate.

RESULTS AND DISCUSSION

The larvae started pupating after 7-8 days. Pupa is the resting stage in which the caterpillar undergoes a major rebuilding of body tissues to emerge as a mature adult. They pupate on the walls of the tray and the leaves of *Adathoda vasica.* They stopped feeding and gets shrunken in size. The larva started secreting silk thread like filaments, whitish silky cocoon with in which it is converted into pupa. After 6-7 days the adult moth came out of the cocoon. Themoths were kept in transparent container, which was covered with nets. Cottondipped in honey was kept in the walls of the container and plants of *Adhatoda* were also kept for egg laying. The moth was seen feeding on honey. The life stages during its life cycle the egg, larva, pupa and adult.

Egg

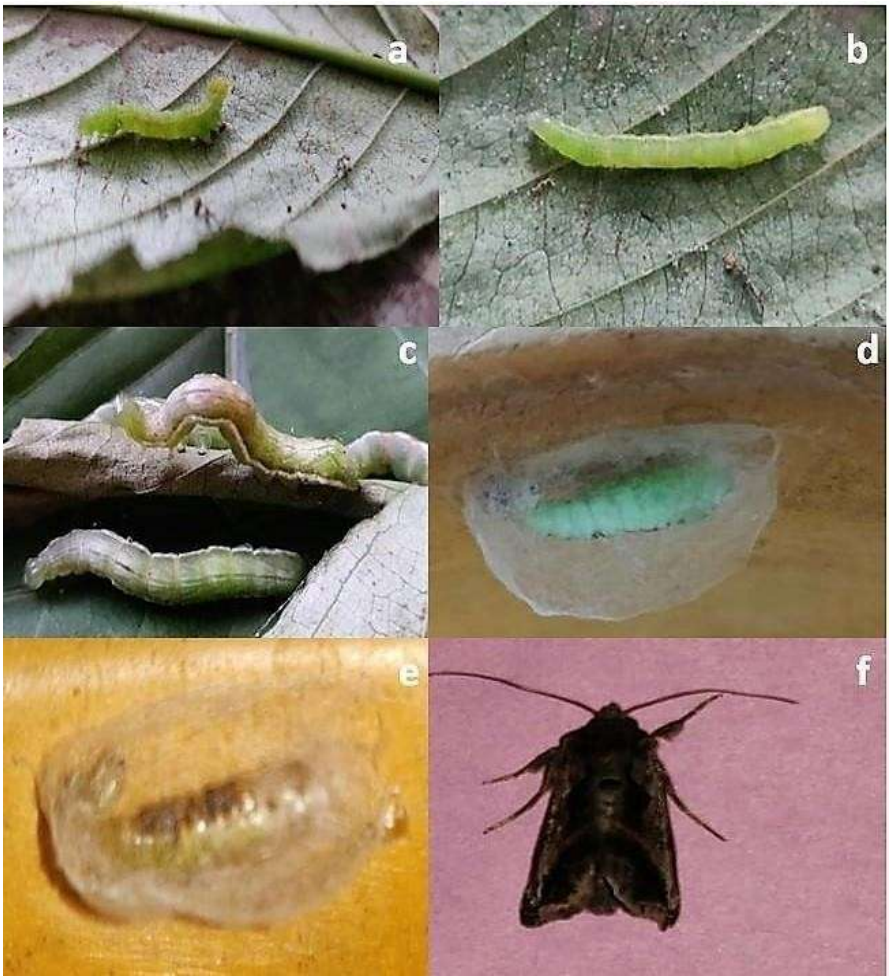
The eggs of *Plusia* sp. were more or less spherical in shape, with a flat side affixed to foliage. It was found deposited singly on either the upper side or lower surface of the leaf although clusters of six to seven eggs are common. These eggs were yellowish white to greenish in colour, and further sculptured by numerous fine striations on the dorsal surface and bear longitudinal ridges. These eggs hatched in about two, three and five days at 32, 27, and 20 Degree Celsius respectively.

Young Larvae

The Young larvae of *Plusia* sp. was initially dusky white, but turned pale green as they commence feeding on foliage. They were somewhat hairy initially, but the number of hairs decreased gradually as the larvae matured. The matured larva was predominantly green, but was usually marked with a distinct white stripe on each side. The thoracic legs and head capsule were pale green. Dorsally the larva bearded several narrow, faint white clustered into broad white bands. The

matured larva was entirely green. The body is narrower at anterior end and broader towards the posterior end. *Plusia* sp. can be easily distinguished by the presence of small nipple like structures located ventrally and abdominal segments 3 and 4.

Figure 1 Different life stages of *Plusia* sp.



a) Second instar larva, (b) Third instar larva, (c) Fourth and Fifth instar larva, (d) Prepupa, (e) Pupa, (f) Adult

Third Instar

During the 3rd instar, on an average body length was 17.23mm , width was 3.58mm and head capsule width was 0.89mm. Head and cervical shield was

pale green in colour. Thoracic and abdominal segments were apple green with white stripes. True legs were pale green colour basally and brownish apically **[figure 1]**.

Table 1 Measurements of the larva, prepupa and pupa of *Plusia* sp.

Larval stages	No. of individuals	Body length (mm)+SE	Body width (mm)+SE
Third Instar	13	17.23+0.13	3.58+0.27
Fourth Instar	9	24.49+0.25	4.47+0.29
Fifth Instar	7	32.62+0.29	6.18+0.23
Pre-pupa	5	19.78+0.34	3.94+0.21
Pupa	5	19.78+0.15	3.94+0.11

During the 4th instar it was observed that on an average the body length was 24.493mm , body width was 4.477mm and the head capsule's width was 1.685mm . Head and cervical shield was pale green in colour. Ocellus was black, head was smaller than the body, skin was smooth. Thoracic and abdominal segments were leafy green above and dark green in colour below. The body was covered by small whitish tubercles with black centre dots from which arise short hairs. True legs were light brown in colour. Prolegs were dark green in colour and crochet was biordinal and light reddish brown in colour.

Pre-Pupal Stage

The pupal stage in plusia sps. was preceded by a pre-pupal stage. The advanced fifth instar larvae, when ready to pupate moved towards the upper sides of the container or between the adjoint leaves that served as the substrate. At this stage the body length was 19.786mm on an average. The body width was

3.944mm on an average and width of head capsule was 1.894 mm on an average. The larvae stopped feeding and became shrunken in size and this represented the prepupal stage, which lasted for 5-6 days.

Table 2: Developmental period of different stages of Diaphania caesalis under laboratory conditions

Life stages	Body colour	Duration of stages
Third Instar	Pale green	3-4 days
Fourth Instar	Light green	3-4 days
Fifth Instar	Leafy green	3-4 days
Pre-pupa	Green with brown patches	1-2 days
Pupa	Brown	3-5 days

Pupal Stage

During pupation the body length, width and width of the head capsule remained the same as prepupal stage. The pupa of moth is called cocoon. There was the formation of dark brown covering around the larva. This cocoon was covered with thin, white, silky filaments around it. These filaments were partially folding the leaves on which the pupa was formed. The anterior part of the cocoon was very loosely spun so has to enable hindrance free emergence of the adult. The freshly formed pupae were soft, tender and much smaller than the fully grown larvae.

Adult

The adult form emerged out of the cocoon laterally i.e,. through one side of the cocoon. There was slit like formation on the cocoon and the moth was seen coming out of the cocoon. The adult moth had two pairs of wings: a pair of forewings and a pair of hind wings. The forewings were attached at the region of mesothorax (second thoracic segment). It was grey brown in colour with light brown lines on it. The hind wings were attached at the region of metathorax (third thoracic segment). It was light brown at the base and the distal portions were dark brown

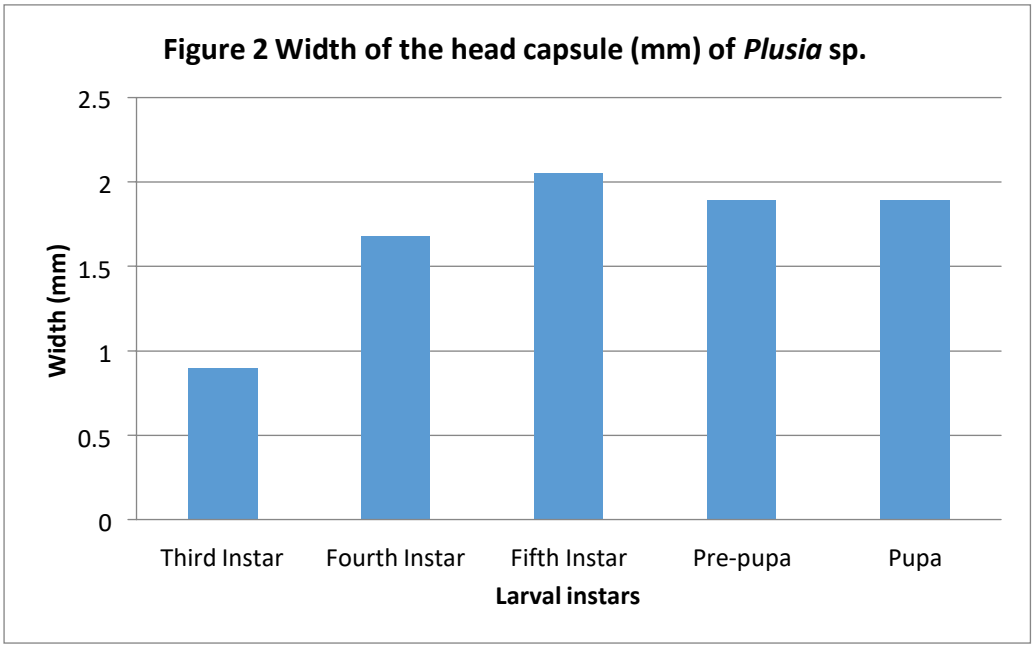


Figure 2 Width of the head capsule (mm) of *Plusia* sp.

Damage Symptoms

The larvae was found to be a leaf feeder, consuming leaf of the ***Adhatoda*** plant throughout its larval stage preparing for their pupal stage and metamorphosis

to adulthood. They were leaf feeders which consume almost the entire leaf leaving behind only the thick veins. The locomotion was by INCHING movement in these larva. Thoracic legs and the posterior most prolegs aided as anchors. When the posterior anchor is moved forward, the middle of the body is lifted away from the surface and the body of the larvae resembles an omega(Ω) following which it releases the front grip and extends the body forward forming a cantilever that requires the body to be relatively stiff (figure 4).

Figure 3 Movement of Plusia sp.

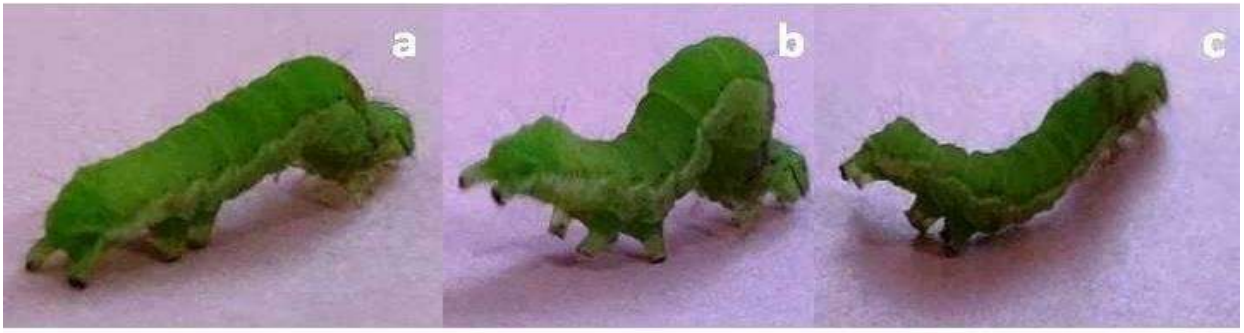
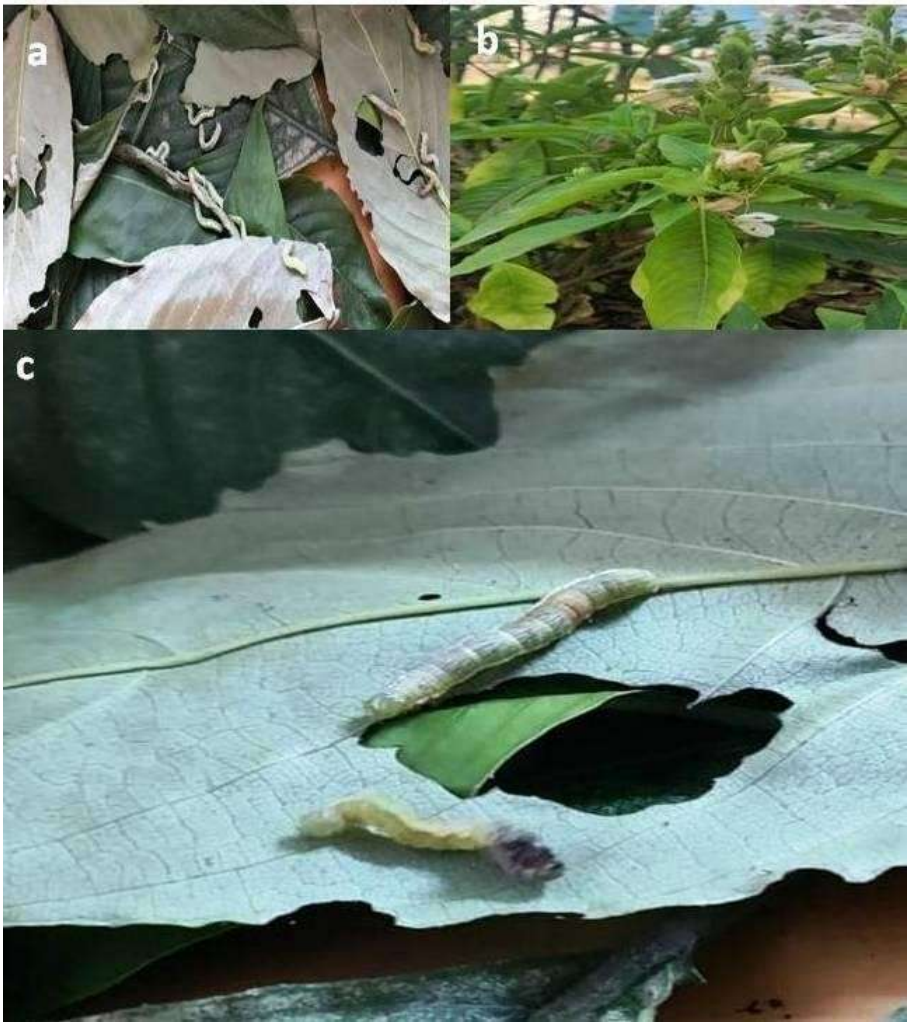


Figure 4 Damage symptoms of Plusia Sp. on Adathoda vasica



In *Plusia* the metamorphosis is holometabolous like other Lepidopterous insects and development phases through the eggs, larva, prepupa, pupa and adult stage. The eggs and larvae were formed on the host plants. Leaves and the larvae feed actively on the foliage. The pupa is an inactive stage between the larvae and adult and is formed inside the silky cocoon between leaf folds. The adult were formed at the completion of the pupal stage and is most active as compared to other stages of life. It differs drastically from the larvae in respect to its food as well as habitat.

The eggs are yellowish white in colour with fine striations on dorsal surface and they usually lay eggs singly on the underside of the leaves **(**Chow, Jennifer K.; Akhtar, Yasmin; Isman, Murray B(2005) The eggs were mostly found on the leaves that are both larger and higher on the plants. It is not clear why egg are preferentially laid on these leaves (Wilson et al. 1982). Larvae were usually dusky white but become pale green as they start feeding on the foliage. The larval eclosion is signalled by the appearance of blackish larval head capsule. Prothoracic shield and setae pass through the egg chorion. When ready to emerge the first instar larva bites a hole in the egg shell at its apex and pulls out its head and thorax. The freshly emerged larvae consume its own shell or those of others partly or completely. This type of behaviour corresponds with that of freshly emerged larvae of *trichoplusia* ni [shorey et al.,1962], *P. brassicae* [David and Gardiner,1962] and *Othireis materna* [Srivastava and Bogwat,1969] but is in contrast with that of *P.argentifera* first instar larvae [Cunnigham,1971].

The larvae were observed to congregate on the sides of the container that received brighter light. The newly emerged *Plusia* larvae are pale white coloured but turn light green soon after feeding on epidermis and mesophyll on the ventral sides of the foliage. With the advancement of the age the food consumption by the later instar larvae increased and feed voraciously.

CONCLUSION :

For the present study, *plusia sp* pest was collected from the medicinal plant *Adhatoda* from Jyoti Nivas college. They belong to order Lepidoptera which is They caused heavy damage to the plants and made it unpalatable and ineffective in clearing simple respiratory ailments .During the life cycle of this pest from 3rd instar to 5th instar several morphological took place such as Colour

of the body, length ,width of the body and width of head capsule as reported in results .

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DIGITAL EYE STRAIN: PREVALENCE AND ASSOCIATED FACTORS OF COMPUTER VISION SYNDROME (CVS) AMONG INDIVIDUALS OF DIFFERENT AGE GROUPS

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ABSTRACT:

The aim of this study was to determine the prevalence of Computer Vision Syndrome (CVS) and commonly associated symptoms among the different age groups . A cross-sectional study was conducted among 200 individuals and the most remarkable result of the study was that 32% of the respondents used to spend 3-5 hours and 35.5% spent 6-8 hours on a daily basis in front of the computer screens or mobile phones. 71% of the respondents used to spend continuous hours on the digital screen without any interruption. Participants complained about CVS symptoms including dry eyes, redness of eyes, watering of eyes and eye strain. During the pandemic the prevalence and use of digital devices increased and there is a need to educate people about limiting the use of these gadgets and preventive measures for eye care from the digital eye strain.

INTRODUCTION

Digital eye strain is the temporary discomfort that follows two or more hours of digital device use and has been the concern ever since there has been an increase in the use of digital devices (Sanodiya I *et al.,* 2019). Simultaneous usage of electronic devices or when switching frequently from one device to another like televisions, desktop and laptop computers, smartphones can cause digital

eye strain. Using the devices improperly by holding them at the wrong angle or too far from the eyes can also cause strain.

Several people experience eye discomfort and vision issues with digital devices if used for extended periods. Digital eye strain has been reported in a number of studies indicating that a large proportion of the population are at risk. Since the last decade smartphones are used extensively by people of all age groups and are believed to be responsible for the sudden rise in Computer vision syndrome and other related symptoms (Sheppard, A. L. & Wolffsohn, J. S. 2018).

The extent of discomfort seems to be related to the extent of digital screen use. Digital related eye strain symptoms like blurring of vision, dryness, aching or watering can be experienced by people of all ages and this kind of eye strain is not different from the symptoms experienced while reading, or doing work like sewing for a long stretch of time. (Salal Khan *et al.,* 2021).

In the twenty-first century, computers have become almost as ubiquitous as the humble pen and paper of the past in many people's daily life. There are approximately six computers per thousand population with an installation of 18 million personal computers (PCs) and their number is increasing. (Sharma AK *et al.,* 2006).

Usage of digital devices for various purposes and for many hours a day is now very common among individuals of all age groups . Students of all ages have gradually moved to computer-based learning, and it is believed that it is seen as an attractive option to the conventional teaching in classrooms. This paradigm shift has deteriorating effects on the student's vision relating it to digital eye strain. This study aims to investigate the prevalence of digital eye strain among people of different age groups who use digital devices and to understand the associated risk factors.

MATERIALS AND METHODS

A cross sectional study was conducted for a duration of 2-3 months in 2021-2022 among 200 people randomly selected.. The aim of this study was to perform a survey to detect the prevalence and severity of the CVS among a sample of 200 people. An online questionnaire link was circulated . The questionnaire form included fourteen questions designed by the researchers and related to the personal data and habits of the individual. Respondents were informed about the research topic and content by researchers. The survey was performed

among all age groups of different professions after a brief explanation about CVS survey.

These fourteen questions included age, the frequent computer or smartphone digital screen use, the hours the individual spend every day on the screen, whether the individual screen-hours are continuous or interrupted, the presence of any symptoms related to CVS, whether the individual is using glasses or contact lenses, the number of screen hours the student spends on the screen, whether the individual believes that CVS affects his life style and eye health or not and finally whether the individual is willing in the future to decrease his screen hours.

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RESULTS AND DISCUSSION:

SL. No.	FACTORS ASSOCIATED WITH DIGITAL EYE STRAIN (DES)	FREQUENCY N (%)
1.	The frequent gadgets you use for your work or personal purposes.	YES NO
	laptop	(123) 61.5% (77) 38.5%
	Notepad/ tablet	(38) 19% (162) 81%
	Ordinary computer	(46) 23% (154) 77%
	Android phones	(184) 92% (16) 8%
	Iphone	(27) 13.5% (173) 86.5%
2.	How many hours do you spend on gadgets?	
	< 2 hours	(19) 9.5%
	3-5 hours	(64) 32%
	6-8 hours	(71) 35%
	10-12 hours	(27) 13%
	> 12 hours	(19) 9.5%
3.	Do you wear power spectacles?	
	Yes	(118) 41%
	No	(82) 59%

4.	If yes, is your vision getting worse during the pandemic?	
	Yes	(91) 45.5%
	No	(109) 54.5%
5.	When did you start using power spectacles?	
	Before pandemic	(70) 35%
	During pandemic	(12) 6%
	I don't wear	(118) 59%
6.	The hours you spend on gadgets?	
	Continuous	(142) 71%
	Interrupted	(58) 29%
7.	After using my smartphones for prolonged hours, I complaint of_____.	
	Shoulder pain	(36) 18%
	Eye strain	(101) 50.5%
	Blurred vision	(36) 18%
	Darkening of vision	(9) 4.5%
	Others	(18) 9%
8.	Do you wear any eye protection while working on digital screen?	
	Anti-reflective spectacles	(52) 26%
	Lens	(6) 3%
	Power spectacles	(61) 30.5%
	Others	(15) 7.5%
	Nothing	(66) 33%
9.	Do you prefer the Reading Mode (Feature) while using your smartphones?	
	Yes	(127) 63.5%
	No	(73) 36.5%
10.	The average distance between you and your digital screen.	
	< 8 inches	(56) 28%

	8-18 inches	(113) 56.5%
	20-30 inches	(26) 13%
	30 inches	(5) 2.5%
11.	Do you experience any of the following symptoms?	YES NO
	Headache	(127) 63.5% (73) 36.5%
	Blurred vision	(79) 39.5% (121) 60.5%
	Redness of eyes	(57) 28.5% (143) 71.5%
	Watering of eyes	(98) 49% (102) 51%
	Others	(104) 52% (96) 48%
12.	Do any of your family members wear power spectacles?	179 responses
	Yes	(129) 65.8%
	No	(67) 34.2%
	PREVENTIVE MEASURES ASSOCIATED WITH DES	**N (%)**
13.	Do you feel that the digital screen is affecting your health and lifestyle in the worst way?	
	Yes	(91) 46.5%
	No	(20) 3.5%
	Maybe	(89) 44.5%
14.	Do you believe that decreasing the hours on these gadgets will help in any way?	
	Yes	(134) 67%
	No	(7) 3.5%
	Maybe	(59) 29.5%

Table 1: Questions associated with Digital Eye Strain.

In this study, an attempt was made to do a survey on CVS in different age groups. However, the data collected showed a larger percentage of people in the age groups of 16-20 years, followed by the 21-35 years age group (Fig 1).

The age group included 2% people of 10-15 years old, 60% people of 16-20 years old, 36.5% people of 21-35 years old, 1% people of 36-50 years old,0.5% people of 50+ years old.

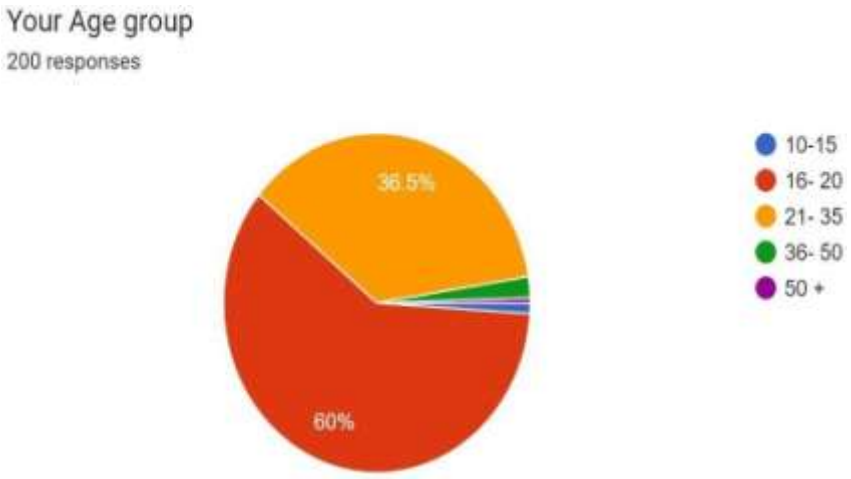

Figure 1: Graph showing respondents age group.

The second question was about the hours they spend on Gadgets. The results were 9.5% people used to spend less than 2 hours, 32% people used to spend 3-5 hours, 35.5% people used to spend 6-8 hours, 13.5% used to spend 10-12 hours and 9.5% used to spend more than 12 hours on Gadgets. According to the study conducted by Gammoh in 2021 among a university student population in Jordan majority of the students used digital devices for more than 6 hours per day. In our study 32% of the respondents spent 3-5 hours while 35% spent 6-8 hours. (Fig 2).

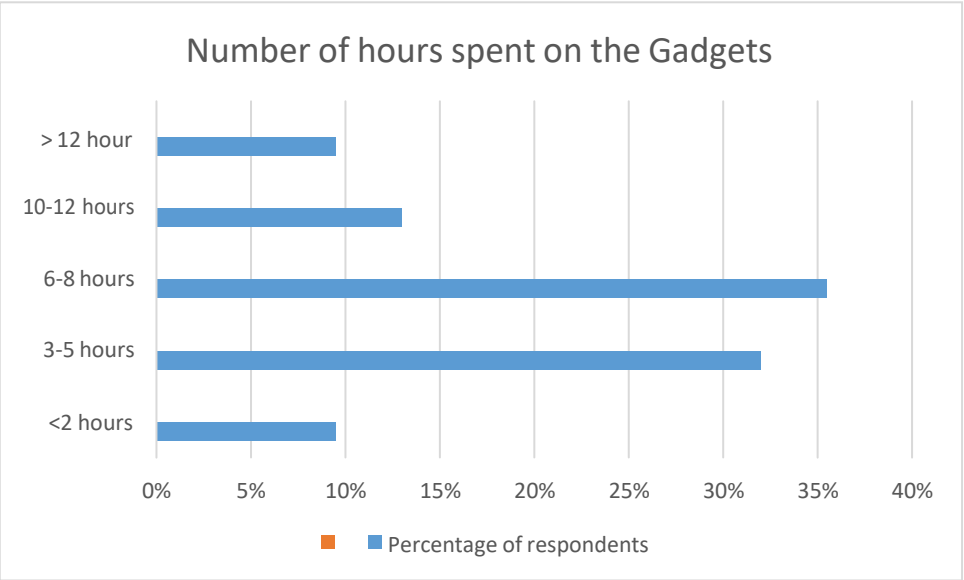

Figure 2: Graph showing hours respondents spent on gadgets.

In our study 35.5% of the respondents spend around 3- 5 hours per day on digital gadgets. These results corroborates the study conducted by Richa Gupta *et al* 2021 among 654 school children where the average per day digital device exposure was 5.2 ± 2.2 hours .

In a study recorded by Iqbal M *et al* 2018, 86% of the medical students sample used to spend 3 hours or more on a daily basis and complained of one or more of the same CVS symptoms recorded by our study.

The third and fourth questions were about wearing spectacles and 59% of people used spectacles while 41% did not wear spectacles. 45.5% of them believed and experienced their vision got worse during the pandemic.

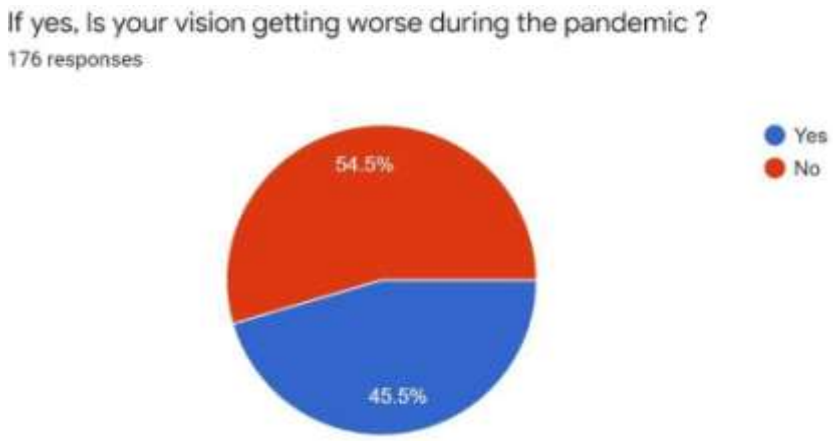

Figure 3: Graph showing the percentage of respondents

The sixth and seventh questions was about whether the hours they spend on Gadgets were continuous or interrupted and the discomfort they faced after usage. The results were 29% of people were spending interrupted hours on gadgets while 71% people were spending continuous hours on gadgets. However Iqbal *et al.,* 2018 reported that 34% of the students were spending continuous hours while 66% were spending interrupted hours on their screens

According to a study by KY Loh and SC Redd (2008) symptoms experienced in CVS are caused by three mechanisms : i) Ocular mechanism: causes symptoms like neck stiffness, headache, back ache, and shoulder pain.(ii) extraocular mechanism which causes blurry vision, double vision, presbyopia, myopia, slowness of focus change.(iii) Accommodative mechanism which causes symptoms such as dryness and redness of eyes, gritty sensation and burning after extended period of computer usage. In our study although the hours spent on the gadgets were interrupted, they faced many eye related issues. 50.5% of people complained of Eye strain,18% of people complained of Blurred vision,18% of people complained of Shoulder pain, 4.5% of people complained of darkening of vision, 2% of people complained of headache and 7% had similar issues.

In a survey done by Ghufran A. *et al.,* 2020 among 651 UG medical students in Saudi Arabia reported high prevalence of CVS being observed in which 95% reported at least one symptoms of CVS during studying computer, with reference to demographical data in the study, female gender was observed to have high risk of CVS than male gender. Person having myopia or hyperopia was

not associated with CVS but astigmatism showed significant association with CVS.

The eighth question was about if they wear any Eye protection while using digital screen and the result showed 26% people were using Anti-reflective lens, 3% of people were using lens, 30% of people were using power glasses, and 32.5% wore nothing while using the gadgets, which means their eyes were directly exposed to the harmful rays.

The ninth question was about the preference for the reading mode while reading on Smartphone and the results of this question was 63.5% of people use reading mode and 36.5% of them did not use any reading mode feature.

The tenth question was about the average distance between Digital screen and them. 28% of the people were using the distance less than < 8 inches, 56.5% of the people were using the distance between 8-18 inches, 13% of the people were using the distance around 20-30 inches and 2.5 % of the people were using greater than 30 inches. Venkatesh *et al* 2016 reported that the viewing distance from the display screen of the computer was less than 25 inches for 34.75% participants.

65.85% of their family members used power spectacles and 34.2% of their family members did not use spectacles. Among those using spectacles 49.7% of the respondents were in the age group of 50 years old and above while 25.5% were 20-35 years old.

The thirteenth question was about their opinion whether they believe that digital screens is affecting the health and lifestyle of people or not. 46.5% of the people felt that the digital screen affected their health and lifestyle, 44.5% of the people felt that the digital screen may affect their health and lifestyle and 3% of people confirmed that the digital screen had no effects on their health and life style.

The fourteenth question was about the belief of people whether decreasing the hours of using Gadgets will help them in anyway and 67% of people believe that decreasing the hours of using gadgets will help them and 29.5% of the people felt it may be of some help and 3.5% of people did not believe decreasing the hours will help them.(Figure :4)

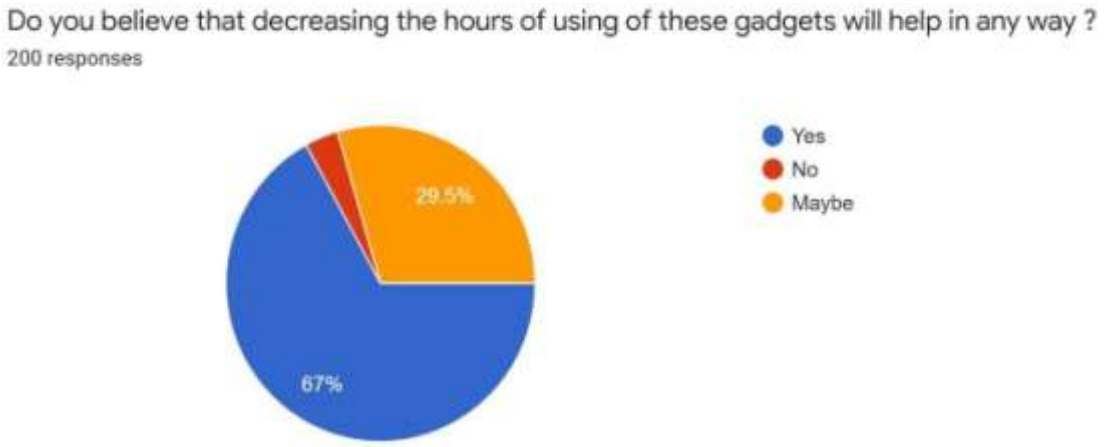

Figure 4 : Percentage of respondents

CONCLUSION:

Due the corona virus disease [COVID19] pandemic, the use of multi digital gadgets was seen for different purposes like office work, college online classes, online interaction sessions, webinars, examinations and assignments. This technology not only broke the limit of boundaries of walls of a room but also took the use of technology to another level. But the impact of these gadgets is neglected

Based on the survey performed in this study, 72% of the participants complained about the CVS symptoms including dry eyes, redness of eyes, watering of eyes, eye strain etc. This study recorded that the most common symptoms were headache and eye strain which acts as the root cause of many physical and psychological problems. There is a need to educate people about limiting the use of these gadgets and time to come up with solutions for the same.

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A CLINICAL INVESTIGATION –

INDUCING OBESITY IN *DANIO RERIO* WITH THE ADMINISTRATION OF HIGH PROTEIN DIET

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ABSTRACT

Along with improved technology and healthcare, there has been a spike in the number of people suffering from obesity, consequently hyperglycaemia and diabetes as well. Being a set of syndromes, a disorder, diabetes and its root causes in specific have been difficult to trace. One major risk factor that contributes to the occurrence of diabetes has been found to be obesity. Obesity is becoming a worldwide crisis, with an increase in both adults and children. Obesity and related metabolic diseases are now, therefore, being studied using zebrafish as a model organism. This research is specifically directed towards obesity linked with a high protein diet as many people are unaware of the effects of a high protein diet on their bodies. The model used for this research is the adult zebrafish. It is a popular vertebrate model, with several benefits linked to its development, life cycle, and translational abilities. They have significant structural and functional parallels to humans and have been used to model a variety of human disorders, including an obesity genetic model. Zebrafish have been used to investigate many obesity-related disorders in humans and have proven to be a successful model for mammalian obesity. The goal of thisresearch was to create a zebrafish model of diet-induced obesity (DIO). One Control and four protein-rich dietary groups of zebrafish were constructed. One set of zebrafish was overfed chickpeas, while the other was overfed broccoli with high protein content. Each dietary group comprised a subset of further twogroups. For 10 weeks, zebrafish were fed these dietary regimes. The secondaryaim of the research was to observe the implications of this DIO on

neurobehavior, toxicity, and other factors by means of various tests. It was observed that the fishes provided with a high protein diet showed higher BMI and aggression and slower locomotion. The presence of micronuclei was found in fishes that were fed a high protein diet.

Key words: Obesity, Diet-induced obesity (DIO), *Danio rerio*, High Protein Diet, Chickpeas, Broccoli, Neurobehavior, Micronuclei.

INTRODUCTION

The World Health Organization (WHO) estimated that 650 million individuals globally were obese in 2016. Obesity is one of the main risk factors that has been linked to the development of diabetes. The prevalence of obesity is rising among both adults and children worldwide. Nowadays, people looking to lose weight and gain muscle mass turns to a low carbohydrate and high protein diet. Most people are unaware of the dangers of a high-protein diet in excess. A high protein diet does help in reducing eight in considerable amounts. But this lost in weight is temporary if the protein content is excess in amounts.

Protein accounts for 20% or more of the total daily calories of a high protein diet. The biochemistry of the excess protein is that our bodies break it down to its simpler constituents, amino acids, which when present in excess may be converted to sugar (glucose, a monosaccharide, then pyruvate), which is then stored as fat, depending on how easily other sources of energy are available. These changes in diet can therefore lead to disorders such as obesity, hyperglycaemia, and consequently diabetes. Hence, this research is primarily focused on obesity associated with a high protein diet.

The model used for this research is the adult zebrafish (*Danio rerio*). In recent years, the zebrafish (*Danio rerio*) has become a well-known vertebrate modelfor biomedical study (Oka *et al.,* 2010) and they can be maintained in tap water, conditioned by letting it set (Westerfield, M., 1994). High fecundity rate, small size, short generation time, optical transparency during early embryogenesis, the productionof a large number of eggs in each mating, and the fact that all stages of development are visible due to external fertilization, have long appealed to researchers in a range ofdomains, including animal behaviour, fish physiology, and aquatic toxicology (Willemsen *et al*., 2011).

The primary objective of this research is to induce obesity by administration of a high protein diet. Further tests under the Secondary objectives of this research were performed:

1. **Breathlessness due to obesity**- This test serves as an early warning system for obese fish.
2. **BMI (Body Mass Index)** - This test shows us the increase in the weight and length of the fish. Using BMI ranges we could differentiate the fishes in various criteria.
3. **Qualitative Analysis of Proteins** - The protein consumed by the fishes, could be excreted by the fishes if their organs were affected by a high protein diet.
4. **Locomotive test** - This test reveals how the fishes move and how their movements change when they are obese.
5. **Neurobehavioral test**- These tests show a link between obesity and the working of the Central Nervous System (CNS).
    a. **Mirror biting test**- This is a test for aggression. When the fish see themselves in a mirror, the ones that are bolder and more aggressive will show a contrasting difference in results as compared to the shy and less aggressive fish. This shows us a direct correlation between obesity and aggression.
    b. **Novel dive tank test** - This test shows the measure of anxiety. It is examined by the time each fish spends at the top of the tank in contrast to the time spent at the bottom. Fish with less anxiety is found at the top of the tank, while those with more anxiety are found at the bottom.

MATERIALS AND METHODOLOGY

BOWLS	DIET
CONTROL (C)	Standard diet (0.04 g of protein)
TEST- 1 (T1)	Standard diet + broccoli (1.5 g broccoli ~ 0.06 g of protein)
TEST- 2 (T2)	Standard diet + broccoli (2 g broccoli ~ 0.08 g of protein)
TEST - 3 (T3)	Standard diet + chickpea (0.3 g chickpea ~ 0.06 g of protein)
TEST - 4 (T4)	Standard diet + chickpea (0.4 g chickpea ~ 0.08 g of protein)

Fig 1

There are five groups in total. One control and 4 protein rich dietary groups of zebrafish were constructed. One set of zebrafish was overfed with chickpea and other fed with broccoli. Each dietary group comprised a subset of further 2 groups with different dosage of proteins. (Fig 1)

METHODOLOGY OF MEASURING BMI OF ZEBRAFISH

The fish are measured from their head to the tail end of their body. This was done by placing the fish in a glass container and placing a graph sheet under the container. (Fig 2)

- Further, pictures of the setup along with the fish were taken and measurements were taken.
- Digital weighing balance was used to measure the dry weight of the fish.
- A transparent measuring tube was filled up to a known volume with water.
- This setup was weighed, and the weight was noted (W).
- Each fish to be weighed was put into this setup and weighed (w).
- The weight of the fish was found to be (W-w).

- BMI of the fish was calculated by dividing the body weight by the square of the body length measured.

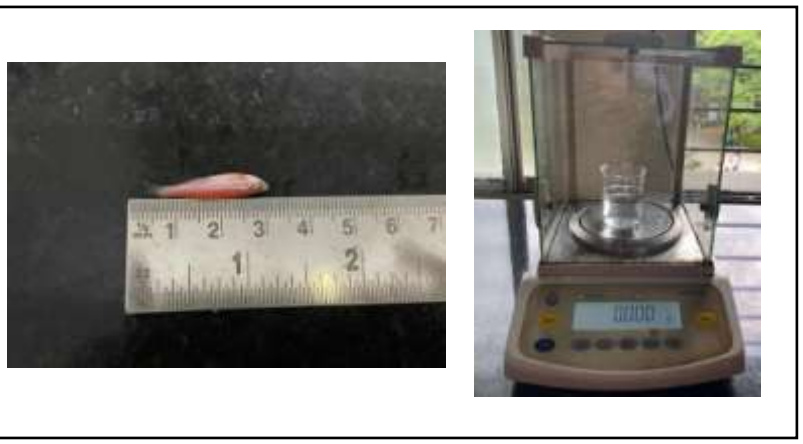


Fig 2

PROCEDURE OF QUALITATIVE ANALYSIS OF PROTEIN

1. The biuret reagent is prepared by using 0.0225 grams of CuSO4 and 0.09 grams of Sodium Potassium tartrate which is dissolved in 7.5 ml of distilled water, which is then dissolved in 1.5 ml of 2M NaOH, and the solution is made up to 10ml.
2. 5 ml of the water sample was taken from Control, Test 1, Test 2, Test 3, and Test 4 in different test tubes.
3. For the sample, a few drops of biuret's reagent were used and mixed.
4. The test tubes were observed, and the results were analysed.

PROCEDURE OF LOCOMOTIVE TEST:

MATERIALS USED

1. **SETUP**

The glass tank is rectangular in shape of the following measurements-

- Length of the tank - 30 cm
- Breadth of the tank -14 cm
- Height of the tank - 20 cm

The inner side of the tank was layered with black aquarium paper on 3 sides. (Fig 3)

2. **WATER**

5 litres of standardized water, which had a height of 14 cm in the tank.

PROCEDURE OF TEST

1. A transparent 100ml measuring beaker was filled up to 50 ml with water which we used as a pre-treatment container.
2. One fish was taken from the bowl and was kept in the pre-treatment beaker for 5 minutes in acclimatization conditions.
3. It was, then, poured into the rectangular tank.
4. The movements were observed with respect to the clockwise direction for 5 minutes.
5. The behaviours of all the fish were noted down.
6. Steps 1-4 were repeated for the other fishes of the C, T1, T2, T3, and T4.

NEUROBEHAVIOURAL TEST

I. MIRROR BITING TEST

Materials used

1. **SETUP**

- A rectangular glass tank of the following measurements was taken-
    - Length of the tank - 30 cm
    - Breadth of the tank -14 cm
    - Height of the tank - 20 cm

- The novel tank apparatus was as follows-

The inner side of the tank was layered with black aquarium paper on 3 sides. (Fig 3)

- On the uncovered side, the mirror was placed against the outer wall, the reflective side facing inside.
- The tank was divided into 2 parts using a thermocol brick, measuring 2 cm in width and 15 cm in length, placed vertically.

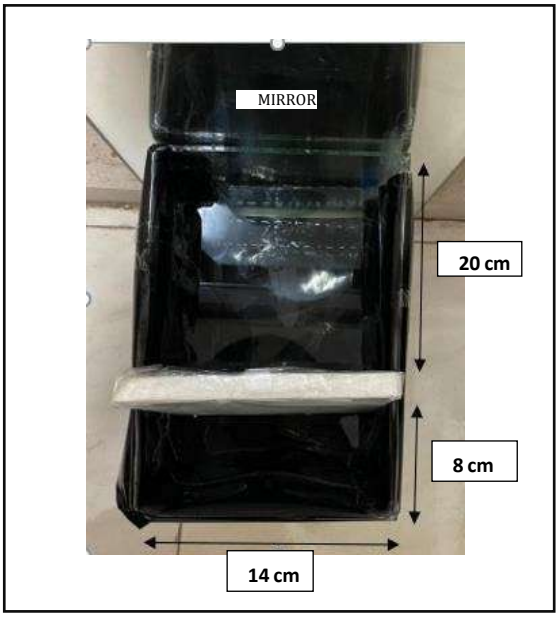


Fig 3

- The measurements of the side with the mirror were as follows-
  Length - 20 cm
  Breadth (Mirror side and thermocol side) - 14 cm
- The measurements of the acclimatization side were as follows-
  Length - 8 cm
  Breadth (Wall and thermocol side) - 14 cm

- A light line on the bottom layer of the tank was made, with a marker, 0.5 cm from the mirror, to represent the zone of "contacting the mirror".
- Another line 2.5 cm from the first line (3 cm from the wall of the tank with the mirror) was drawn, to represent the zone of "approach to the mirror".

2. **WATER**
   5 Litres of water were filled into the tank, which was 14 cm in height.

PROCEDURE OF TEST

1. The fish was placed on the acclimatization side of the tank.
2. It was left undisturbed for 5 minutes.
3. After this, the thermocol was lifted and the fish was allowed to move into the mirror containing the side of the tank.
4. The following values were noted-

- *Mirror biting frequency*: Number of times the fish bite the mirror.

- *Approaches to the mirror*: The number of crossing the line denoting the mirror approach zone, but without mirror contact.
- *Mirror contacts*: The number crossing the line denoting the mirror contact zone.

II. NOVEL TANK DIVING

Materials used

1. **SETUP**

- A rectangular glass tank, as used in the "MIRROR BITING TEST".
- The novel tank apparatus was as follows-

The inner side of the tank was layered with black aquarium paper on 3 sides as shown in the figure.

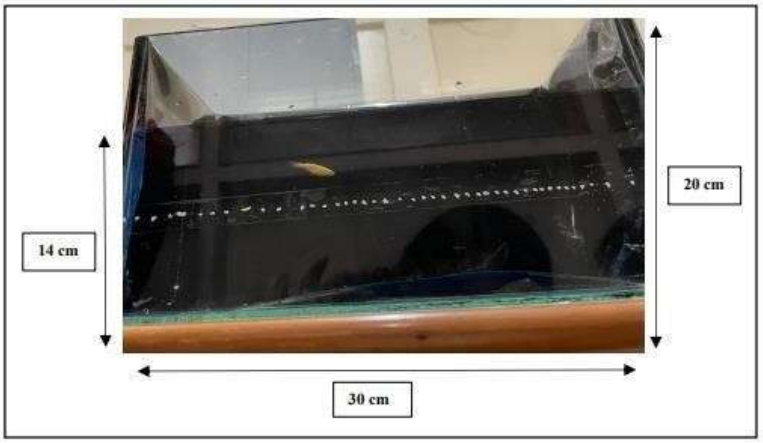


Fig 4

2. **WATER**

- The tank was then filled with water up to 14 cm deep is 5 litres.
- The tank was divided into 2 parts using a marker into two equal parts measuring 6.5 cm.

PROCEDURE OF TEST

1. A transparent 100ml measuring beaker was filled up to 50 ml with water which we used as a pre-treatment container.
2. One fish was taken from the bowl and was kept in the pre-treatment beaker for 5 minutes in acclimatization conditions.
3. It was then poured into the rectangular tank.

4. The fish was let to dive inside the rectangular tank and the following values were noted-
- *Time spent around the top part of the tank (in 300 seconds)*
- *Time spent around the bottom part of the tank (in 300 seconds)*
5. Steps 1-4 were repeated for the other 2 fishes of the C, T1, T2, T3, and T4.

OBSERVATION AND RESULT

1. BREATHLESSNESS DUE TO OBESITY

Zebrafish spend most of their life span at the bottom of the tank. As previously indicated, obesity causes breathing problems in all obese fishes. With the pace of time, they become obese, and this may lead the fish to become morelaboured in their breathing. The test is carried out to see if high-protein diet foods such as broccoli and chickpeas are causing the fish to become obese. Because this test alerts us to the possibility of obesity. When the fish become obese, they rise to the surface in search of oxygen, and some of them die as a result. (Fig 5)

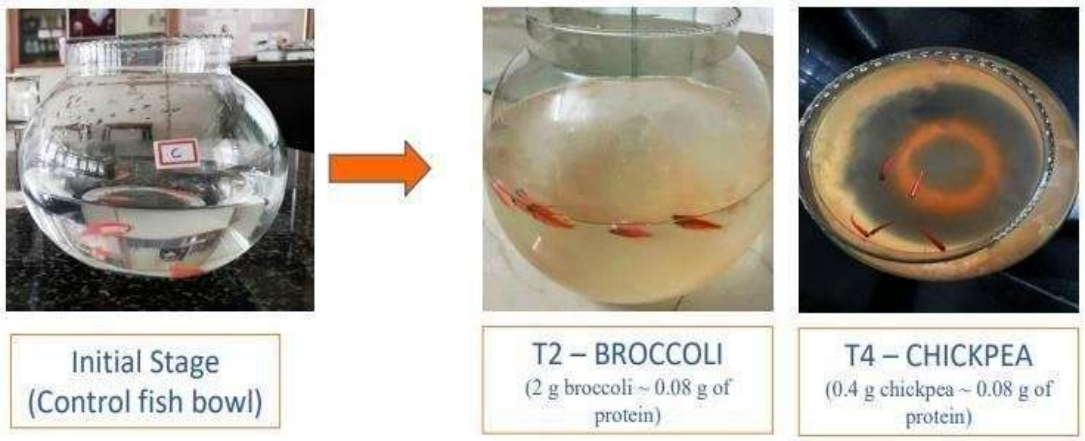
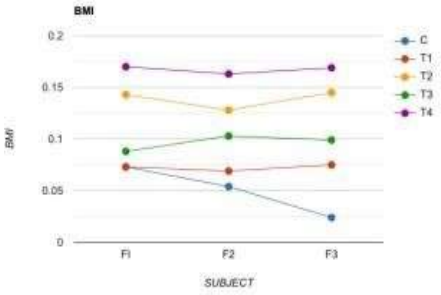


Fig 5

2. BODY MASS INDEX (BMI)

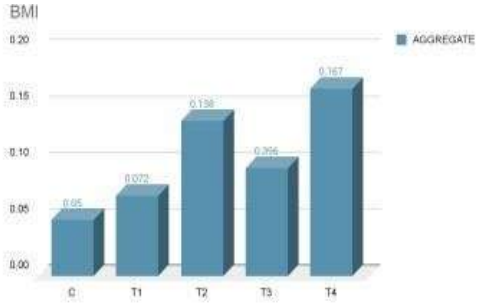
BMI stands for Body Mass Index. It is a measure of body fat. It aids in assessing the occurrence of certain diseases that come with high body fat. The aim of this study is to create a simple obesity model of zebrafish and to arrive at an average

length, weight, and body mass index (BMI) of zebrafish. Here, we compare the changes in BMI in contrast to fish fed with regular food versus fish fed with a high-protein diet like broccoli and chickpeas. Based on the observation and result we can clearly see there is a significant change in the BMI between the control and the test (T1, T2, T3, T4) bowls.



T4 shows the highest BMI followed by T2>T3> T1 and C.

C – 0.04 g of protein
T1 -1.5 g broccoli ~ 0.06 g of protein
T2 - 2 g broccoli ~ 0.08 g of protein
T3 - 0.3 g chickpea ~ 0.06 g of protein
T4 - 0.4 g chickpea ~ 0.08 g of protein



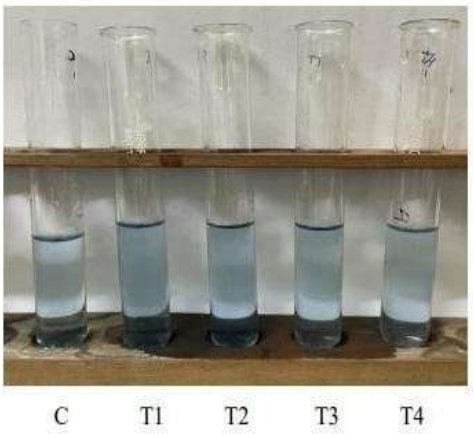
The increase (in percentage) as compared to Control:

1. T1- 13.76%
2. T2-26.38%
3. T3-18.35%
4. T4-31.93%

As a result, we can see that T4 has the highest BMI, followed by T2, T3, T1, and C the least.

3. QUALITATIVE ANALYSIS OF PROTEIN

Proteins are one of the major constituents of the body and it plays a significant role in the biological systems hence there becomes a need for them to be absorbed. A high protein diet (Broccoli, Chickpea) was given to the fishes, and to check if they are excreting proteins out making it necessary as the proteins are not generally excreted out as such making the qualitative analysis important.



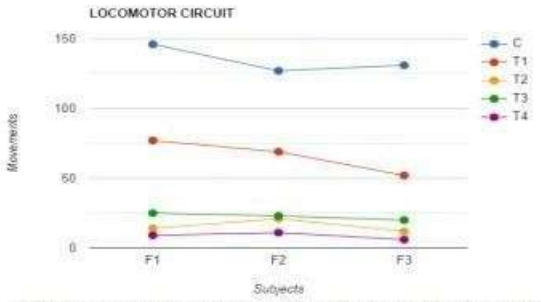
Negative results were observed in qualitative analysis of protein in fish bowl water.

Excretion of proteins indicates that the proteins they are consuming are not getting absorbed in the body and as per our experiment the fishes have to consume proteins for getting obese and according to the results, we can say that **the proteins were being absorbed by the zebrafish.**

Fig 6

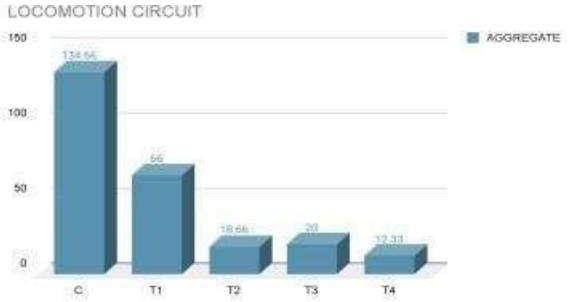
4. LOCOMOTIVE TEST

The main goal of this experiment is to compare and contrast the locomotive behaviours of fish fed with regular food versus fish fed with a high-protein diet. This test is used to assess their movements and behavioural changes, with the total distance travelled, the number of clockwise rotations, erratic movements (typically evaluated by the angle of rotation), and immobility being the primary parameters common to all activities.



T4 shows the slowest motion followed by T2>T3> T1 and C.

C – 0.04 g of protein
T1 -1.5 g broccoli ~ 0.06 g of protein
T2 - 2 g broccoli ~ 0.08 g of protein
T3 - 0.3 g chickpea ~ 0.06 g of protein
T4 - 0.4 g chickpea ~ 0.08 g of protein



The increase (in percentage) as compared to Control (53.6%) -

1. T1- 26.3%
2. T2- 7.3%
3. T3- 8%
4. T4- 5%

As a result, we can see that **T4 has the slowest motion**, followed by **T2, T3, T1, and C**, which **have the fastest movements.**

5. NEUROBEHAVIORAL TESTS

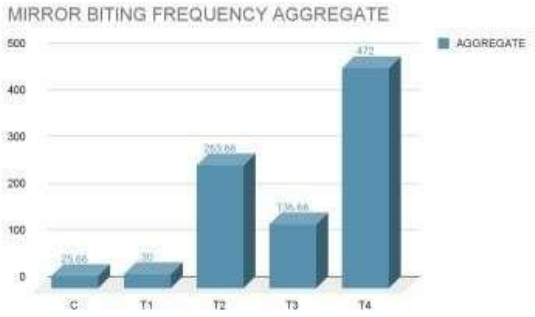
I. Mirror biting test

The main aspect of this test is to determine and compare the aggressive behaviour between the fishes fed with normal food and the fishes fed with a high protein diet. Here, we will consider the Mirror biting frequency to compare the aggressive behaviour of the fishes from the different bowls.

I. MIRROR BITING TEST

T4 shows the highest frequency of mirror biting followed by T2>T3>T1 and C.

C – 0.04 g of protein
T1 -1.5 g broccoli ~ 0.06 g of protein
T2 - 2 g broccoli ~ 0.08 g of protein
T3 - 0.3 g chickpea ~ 0.06 g of protein
T4 - 0.4 g chickpea ~ 0.08 g of protein



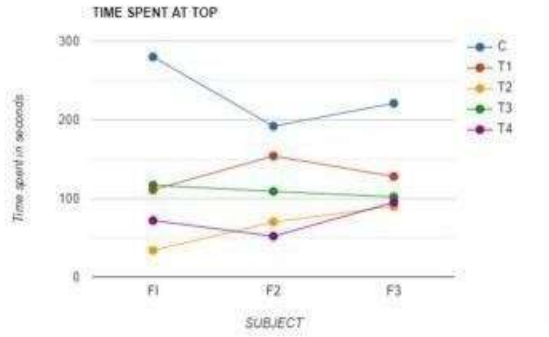
The increase (in percentage) as compared to Control-

1. T1- 0.46%
2. T2- 25.611%
3. T3- 11.901%
4. T4- 48.166%

Therefore, we can see that **T4 shows the highest level of aggression** followed by **T2, T3, T1, and C the least.**

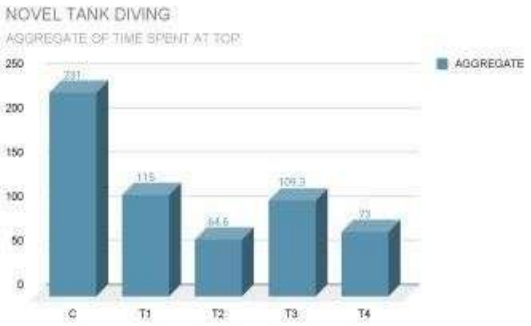
II. Novel tank diving

The major goal of this experiment is to examine and contrast anxiety levels in fish fed regular food and fish on a high-protein diet. Here, we will consider how much time the fish spends at the top and bottom parts of the tank and compare it with the rest. Spending more time at the top signifies less anxiety, whereas spending more time at the bottom shows higher anxiety levels.



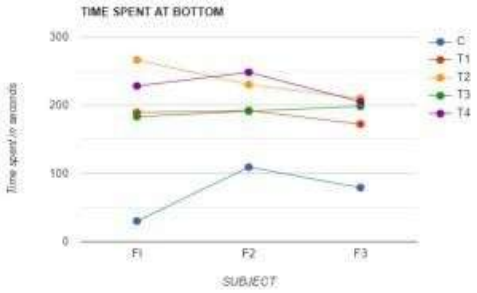
C shows the highest time spent at top followed by T1>T3> T2 and T4.

C – 0.04 g of protein
T1 -1.5 g broccoli ~ 0.06 g of protein
T2 - 2 g broccoli ~ 0.08 g of protein
T3 - 0.3 g chickpea ~ 0.06 g of protein
T4 - 0.4 g chickpea ~ 0.08 g of protein



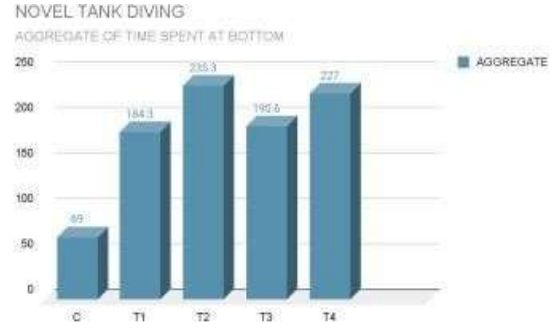
The increase (in percentage) as compared to control for "time spent at top"-

1. T1- 19.40 %
2. T2- 10.90%
3. T3- 18.44%
4. T4- 12.31 %



T4 shows the highest time spent at the bottom followed by T2>T3> T1 and C.

C – 0.04 g of protein
T1 -1.5 g broccoli ~ 0.06 g of protein
T2 - 2 g broccoli ~ 0.08 g of protein
T3 - 0.3 g chickpea ~ 0.06 g of protein
T4 - 0.4 g chickpea ~ 0.08 g of protein



The increase (in percentage) as compared to control for "time spent at bottom"

1. T1- 20.34%
2. T2-25.97%
3. T3-20.97%
4. T4-25.07%

Therefore, we can see that **T4 shows the highest level of anxiety followed by T2, T3, T1, and C the least.**

CONCLUSION AND DISCUSSION

High-protein diets are becoming popular in recent days as a beneficial method for weight loss as they provide the dual benefits of increased satiety and decreased fat mass.However, there are some consequences that may affect the health too. Animal models that closely resemble the state of being obese in humans and that enable the characterization of the metabolic pathways involved in obesity development, such as zebra fish, are of significant interest (Oka *et al.,* 2010).

The primary objective of this research was to induce obesity in our fish by administration of a high protein diet which was successfully achieved. The secondary objectives and their results were as follows-

Breathlessness due to obesity - The fishes when fed a high protein diet, they came up to the surface of the bowl. This was a sign of breathlessness which means that the fishwere getting obese.

BMI (Body Mass Index) - The fish fed a high protein diet showed the highest BMI as compared to the control fishes. A measure of how much weight is gained per calorie consumed, or feed conversion efficiency, is said to be influenced by protein sources through changes in energy uptake, utilisation, metabolism, and/or food consumption behaviours (Smith *et al.,* 2013).

Qualitative Analysis of Protein - The food containing high protein was given and it wasfound that the fishes did not excrete any proteins, indicating that the fishes were consuming the proteins on a daily basis in the span of 2 months.

Locomotive test - The locomotion of the obese fishes progressively slowed down and the aggression level increased with respect to the fishes in the control bowl.

Neurobehavioral tests - These tests show a link between obesity and the working of theCentral Nervous System (CNS).

- Mirror biting test - The fishes fed a high protein diet showed an increase in aggression, while the control fishes were comparatively less aggressive towards their own reflection that they assumed to be a predator. Even zebra fish fed with a High Fat Diet show their boldness by butting or biting it (Picolo *et al.,* 2021).

- Novel tank diving test - The fish fed a high protein diet showed an increase in anxiety while the control fishes were comparatively less anxious.

The fishes present in the control showed normal growth and were calm in nature.

The fish fed with a high protein diet, with broccoli and chickpea, showed a progressively increasing trend in their mass, length, aggression, and a decreasing trendin their locomotion.

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STUDY OF EXTERNAL MORPHOLOGY OF DIFFERENT POLLEN GRAINS

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ABSTRACT

External morphology of Pollen Grains from ten different flowers is recorded. It is done by simply mounting the pollen grains from a flower onto a microscopic glass slide and observing it under 10x magnification of a compound microscope. Their shape, size, aperture, polarity, sporoderm is recorded. The diversity of external morphology in pollen grains can be observed. Their diversity could be the result of different requirements required by different flowers for their pollination.

Key words- Pollen grains, polarity, aperture, sporoderm

INTRODUCTION

Angiosperms, one of the largest and diverse group of the Kingdom Plantae, are flowering plants that bear flowers and fruits. The word “Angiosperm” is derived from Greek words ‘*angeion*’ and ‘*sperma*’, and refers to those plants that produce their seeds enclosed within a fruit.

Reproduction in living organisms is an essential part of their life. In angiosperms, flowers bear the reproductive organs- the Stamen and the Carpel. The stamen

comprises of an anther and a filament. The carpel comprises of stigma, style and ovary. The anther bears pollen grains which contain the male gamete and the ovary bears ovules which contain the female gamete. The gametes fertilize to form a seed which is enclosed by the fruit.

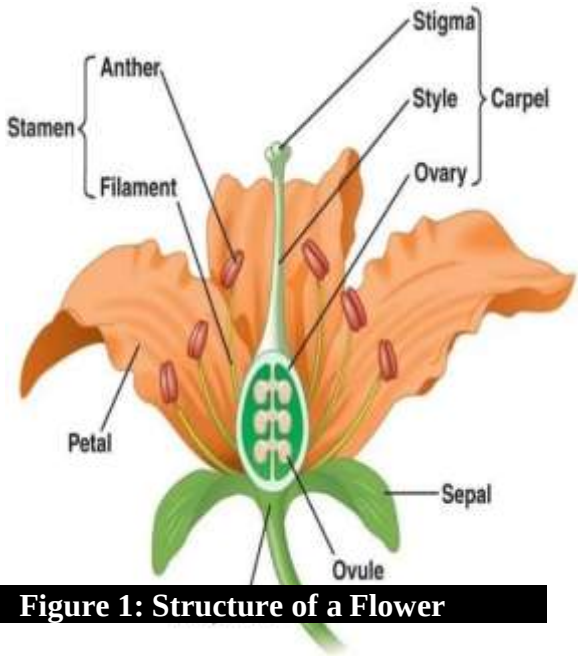


Figure 1: Structure of a Flower

In this paper, the external morphology of pollen grains from different flowers will be observed. Pollen grains are microscopic structures that range incredibly in their size, shape and form.

POLLEN GRAINS

Pollen can be defined as a fine or a coarse powder, which consists of microgemetophyte and produces the male gametes or the sperm cells. Palynology is the branch of Biology, which mainly deals with the study of pollens and their properties.

The pollen grain's interior region comprises of cytoplasm and tube cell (which transforms into a pollen tube and generative cell (which releases the sperm nuclei).

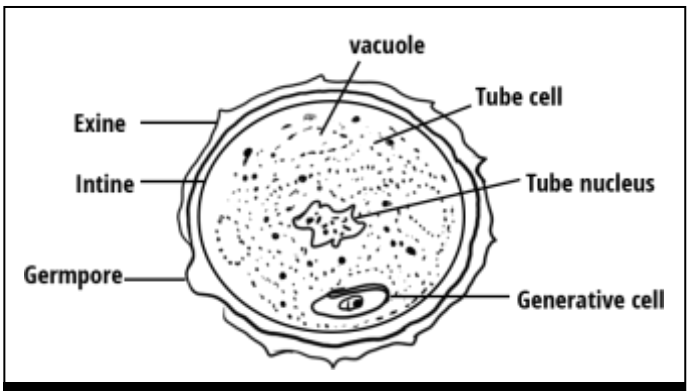


Figure 2: Structure of a Mature Pollen Grain

Pollen grains are structures on a tiny scale that range in both their size and their form. In general, the size of individual pollen grains varies depending on the species and can range anywhere from 3-200 micrometres or microns. The shape of the pollen grain is commonly found in round, ovule, triangular, disc or in a bean-shape with a smooth to spiky texture. The natural colour of pollen grains are white, which may also vary depending on the plant species. Some are yellow or orange or cream or light green in colour.

Some characteristics by which pollen grains can be differentiated are-

(a) **Shape-** The ratio between the polar axis (P) and the equatorial diameter (E) of a pollen gives us the shape of the pollen. Pollen shapes have been classified as follows-

SHAPE	P/E
Perprolate	> 2
Prolate	2-1.33
Subprolate	1.33-1.14

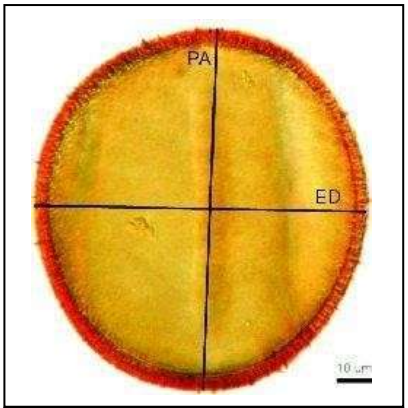


Figure 3: Polar Axis and Equatorial Diameter

Prolate-spheroidal	1.14- 1.00
Spheroidal	1
Oblate-spheroidal	1.00 - 0.88
Suboblate	0.88 - 0.75
Oblate	0.75 - 0.50
Peroblate	< 0.50

Table 1: Showing different shapes

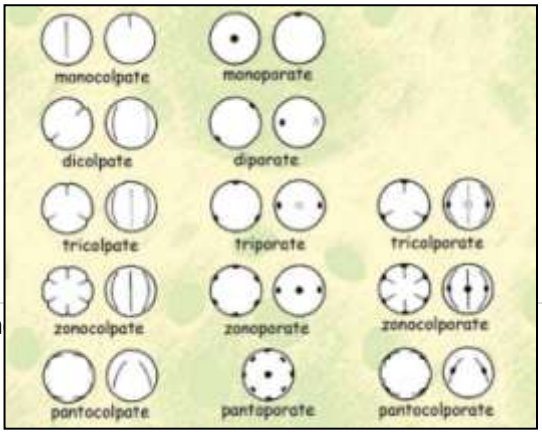
(b) Size- These vary greatly, but are generally divided into six categories, although this number could be increased.

Equatorial Diameter	Feature
<10 µ	Very Small Pollens
10 - 25 µ	Small Pollens
26 - 50 µ	Medium Pollens
51 - 100 µ	Large Pollens
101 - 200 µ	Very Large Pollens
> 200 µ	Giant Pollens

Table 2: Showing different sizes

(c) Apertures- They are areas on the wall of a pollen grain, where the wall is thinner and/or softer. These are the places where the pollen tube is able to break through the (elsewhere very tough) pollen wall. Two different types of apertures can be distinguished: pores (circular apertures) and colpi (Boat-shaped/long apertures).

Pollen grains with pores are porate and those with colpi are colpate. If both pore and colpus are combined in the same aperture, the pollen grain is colporate.



(d) **<u>Polarity</u>-** It is defined as the condition of having distinct poles in a pollen grain. The polarity is best detected at the tetrad stage of the pollen grain. There are two poles in a pollen grain - proximal and distal. The proximal face is the part of a pollen grain and spore that faces toward the centre of tetrad. The distal face is the part of a pollen grain and spore that faces away from the centre of tetrad and opposite the proximal part. If the poles are equal, the pollen is defined as isopolar and if they are unequal as anisopolar. Spherical pollens have no polarity (apolar).

(e) **<u>Sporoderm</u>**- It is the outermost protective wall around the pollen grain or microspore. It is made of many layers with the two main layers being exine and intine. Structure (layers forming the sporoderm as a whole) and sculpture (ornamental elements modelling the sporoderm surface) in this wall can differentiate a pollen.

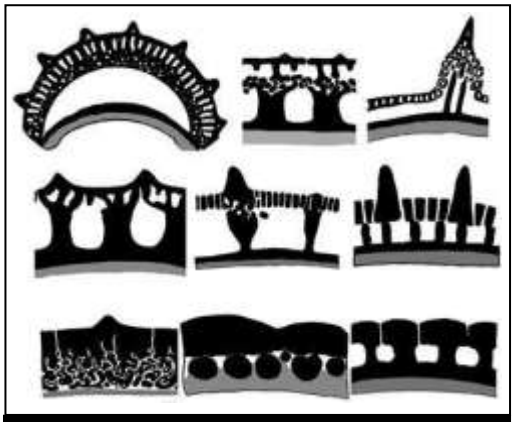


Figure 5: Various Sporoderm Ultrastructures

I. EXPERIMENT

AIM

To study the external morphology of pollen grains from different flowers.

MATERIALS REQUIRED-

Flowers, brush, needle, a pair of tweezers, glass slides/cavity slides, cover slips, water, dropper.

PROCEDURE

(i) A drop of water was added to the centre of a clean glass slide/cavity slide.

(ii) A flower was taken and anther was located. If the anther was found to be healthy it was examined for pollen grains on its surface. If it was present, it was brushed to the slide. If it was not present, the anther was placed on the water droplet using a pair of tweezers and teased with a needle to release the pollen grains.

(iii) A cover slip was placed on the drop of water carefully to eliminate any air bubbles. The excess water was removed using a filter paper.

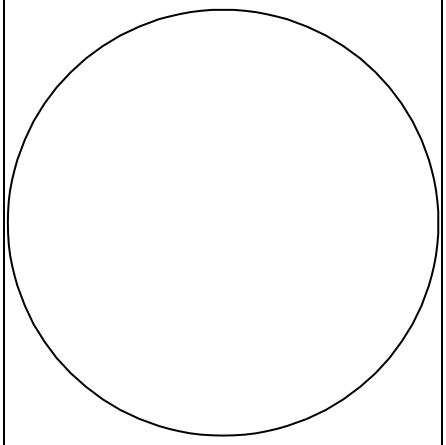
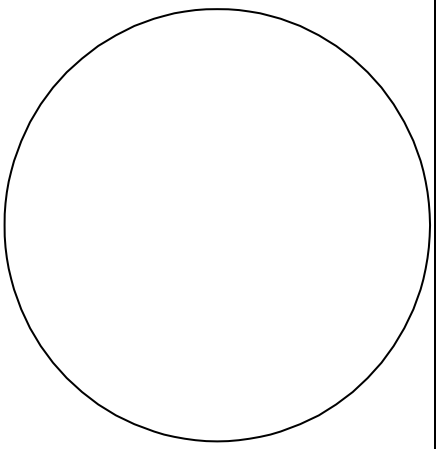
(iv) The slide was observed under 10x magnification and the observations were recorded.

(v) The process was repeated for the remaining flowers.

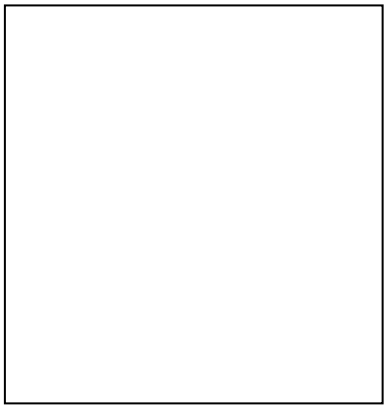
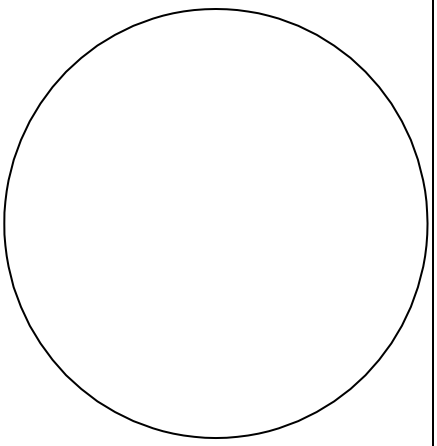
OBSERVATIONS

Table 3: Observations Recorded

S.no.	Flower	Pollen Grains (10x)	Characteristics
1.	Scientific Name- *Nerium oleander* Common Name- Pink Oleander		• Medium-sized (26-50 µm) • Spheroidal shape • Circular outline • Porate aperture • Isopolar polarity • Clumps of pollen was present
2.	Scientific Name- *Podranea ricasoliana* Common Name- Pink Trumpet Vine		• Medium-sized (26-50 µm) • Spheroidal shape • Circular outline • Isopolar polarity
3.	Scientific Name- *Jatropha panduripholia* Common Name- Peregrina		• Large-sized (51-100 µm) • Spheroidal shape • Circular outline • Isopolar polarity

4.	Scientific Name- *Spathodea campanulata* Common Name- African Tulip		• Medium-sized (26 - 50 µ) • Spheroidal shape • Circular outline • Isopolar polarity
5.	Scientific Name- *Hibiscus rosa-sinensis* Common Name- Shoeblack Plant		• Very Large-sized (>100 µm) • Spheroidal shape • Circular outline • Exine overspread with echini of variable height and width • Pantoporate aperture
6.	Scientific Name- *Ipomea horsfalliae* Common Name- Cardinal Creeper		• Large-sized (51-100 µm) • Spheroidal shape • Circular outline • Minaret-shaped spinae

7.	Scientific Name- *Plumbago auriculata* Common Name- Cape Leadwort		• Large-sized (51-100 µm) • Oblate (P/E ratio: 0.75 - 0.50) • Circular outline • Tricolpate aperture • Isopolar polarity
8.	Scientific Name- *Impatiens balsamina* Common Name- Garden Balsam		• Medium-sized (26 - 50 µ) • Oblate (P/E ratio: 0.75 - 0.50) • Elliptical outline • Isopolar polarity
9.	Scientific Name- *Asystasia gangetica* Common Name- Ganges primrose		• Large-sized (51-100 µm) • Prolate (P/E ratio: 2 - 1.33) • Tricolporate aperture • Isopolar polarity

10.	Scientific Name- *Ravenia spectabilis* (Pink Flower) Common Name- Lemonia 		• Medium-sized (26 - 50 µ) • Prolate-spheroidal (P/E ratio: 1.14 - 1.00)

CONCLUSION

Pollen grains with a variety of external morphology were observed through this experiment.

The pollen grains vary in their external morphology for ease in pollination, as reproduction is an important phase in a living organism's life cycle. Since different flowers require different requirements, the diversity in the external morphology of pollen grains is vast.

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