

EXPLORING THE EFFECT OF DIFFERENT VISIBLE WAVELENGTH LIGHT IN ENHANCING THE REGENERATIVE POTENTIAL IN EARTHWORM, *Eudrilus eugeniae*

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

SRISHTI RAJA (39230045)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

APRIL - 2023



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **SRISHTI RAJA (39230045)** who carried out the project entitled "**EXPLORING THE EFFECT OF DIFFERENT VISIBLE WAVELENGTH LIGHT IN ENHANCING THE REGENERATION POTENTIAL IN EARTHWORM, *Eudrilus eugeniae***" under our supervision from September 2022 to April 2023.

Internal Guide
DR. L INBATHAMIZH
PhD

External Guide
DR. S JACKSON DURAIRAJ
PhD

Head of the Department
DR. V RAMESH KUMAR, PhD

Submitted for Viva voce Examination held on 11/05/2023

Internal Examiner

External Examiner

DECLARATION

I, **SRISHTI RAJA (39230046)** hereby declare that the Project Report entitled "**EXPLORING THE EFFECT OF DIFFERENT VISIBLE WAVELENGTH LIGHT IN ENHANCING THE REGENERATION POTENTIAL IN EARTHWORM, *Eudrilus eugeniae***" done by me under the guidance of **DR. L INBATHAMIZH, PhD, DEPARTMENT OF BIOTECHNOLOGY (Internal)** and **DR. S JACKSON DURAIRAJ, PhD, INTERNATIONAL RESEARCH CENTRE (External)** at **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY (DEEMED TO BE UNIVERSITY) ACCREDITED WITH A GRADE "A" BY NAAC, JEPPIAAR NAGAR, RAJIV GANDHI SALAI CHENNAI- 600119** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in **BIOTECHNOLOGY**.

DATE: 11/05/2023

PLACE: CHENNAI

Srishti

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **Board of Management of SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. V Ramesh Kumar**, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I express my thankfulness to my Internal Guide **Dr. L Inbathamizh, Assistant Professor, Department of Biotechnology**, for her constant support and encouragement provided during my project.

I would like to express my sincere and deep sense of gratitude to my External Guide **Dr. S Jackson Durairaj, Scientist 'D', Centre for Molecular and Nanomedical Sciences, International Research Centre**, and Research Scholars **Mr. Kamarajan** and **Mr. S C Karthikeyan, International Research Centre** for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of my review work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the **Department of Biotechnology** who were helpful in many ways for the completion of the project.

ABSTRACT

Regeneration is a highly complex process that requires the coordination of numerous molecular events, and identifying the key ruler that governs is keen to investigate. Light has an influence on the biological system. Some wavelengths of light have a positive effect while some have a negative effect. For this purpose, earthworms are studied for understanding the influence of different wavelengths of light. This study deals with *Eudrilus eugeniae* which is used as a model organism to study the effect of visible light (400 – 700 nm). Amputation studies, light exposure. Western blotting, SDS-PAGE and histology are techniques that are used for understanding the different wavelengths of light on *E. eugeniae*. While SDS-PAGE and western blotting procedures are used to distinguish between the expressions of different antibodies, histology is utilized to analyze tissues and organs at the microscopic level. To understand the normal regeneration of earthworm, the earthworm was initially amputated at the 10th segment. The bud formation was seen to have started on the 4th day post wound healing. Out of the colours. in visible wavelength, sunlight was used as the control while the other colours were used for finding out their effect on the earthworms. The observation was that yellow light (570–590 nm) had a positive effect while violet light (380–450 nm) had a negative effect. According to obtained histology results, worms exposed to yellow light had a significant longitudinal layer while those exposed to violet light had a reduced longitudinal layer. This longitudinal layer is essential for observing regenerative patterns. The expression of H2AX and p53, which are stressful markers, is maximum in violet because the wavelength of violet lies near the UV region. Furthermore, as H2AX and WNT3a antibodies function in opposition to one another, both must be produced concurrently for proper regeneration. Apoptosis, stem cells and immune responses are vital factors for successful regeneration. Earthworms are used as model organisms since they are effective in displaying normal regeneration. On treatment with light, we can conclude that *Eudrilus eugeniae* reacts differently to different wavelengths. Yellow light has a positive effect on the earthworm and violet has a negative effect.

TABLE OF CONTENTS

S.NO.	TITLE	PAGE NO.
	ABSTRACT	iv
	LIST OF ABBREVIATIONS	vii
	LIST OF FIGURES	viii
	LIST OF TABLES	x
1	OVERVIEW	1
	1.1 Introduction	1
	1.2 Regeneration Studies	3
	1.3 Role Of Stem Cells in Regeneration	4
	1.4 Phototherapy	4
	1.5 Role of Riboflavin in Regeneration	6
	1.6 <i>H2AFX Gene</i>	6
	1.7 Bacteriorhodopsin	8
2	LITERATURE SURVEY	9
3	AIM AND SCOPE	15
4	MATERIALS AND METHODS	16
	4.1 Earthworm culture and maintenance	16
	4.2 Exposure to visible light categorized according to their wavelengths	16
	4.3 Protein isolation	16
	4.4 SDS-PAGE	16
	4.5 Western Blotting	21
	4.6 Histological studies of Regeneration of bud	22
	4.7 Statistical analysis of <i>in-vivo</i> bud regeneration of <i>E.eugeniae</i>	23
5	RESULTS AND DISCUSSION	25
	5.1 <i>In-vivo</i> regeneration of blastema	25
	5.2 Morphological characterization of <i>in-vivo</i> regeneration blastema	27

	5.3 Statistical analysis for survivability of <i>E.eugeniae</i>	31
	5.4 Western blotting analysis	35
	5.5 Histopathological studies of <i>E.eugeniae</i>	37
6	SUMMARY AND CONCLUSION	42
7	REFERENCES	43
8	ANNEXURE	48

LIST OF ABBREVIATIONS

AEC	Apical Epithelial Cap
ATP	Adenosine Triphosphate
BRdU	Bromodeoxyuridine
CIT	Cancer Immunotherapy
CNS	Central Nervous System
CSU	Chronic Spontaneous Urticaria
CTCL	Cutaneous T-cell Lymphoma
DAMPs	Damage-Associated Molecular Patterns
DDR	DNA Damage Response
DSB	Double Strand Break
ECM	Extracellular Matrix
ECP	Extracorporeal Photochemotherapy
GVHD	Graft versus Host Disease
ICD	Immunogenic Cell Death
IPA	Isopropyl Alcohol
NER	Nucleotide Excision Repair
NIR	Near Infrared
PAHs	Polycyclic Aromatic Hydrocarbons
PDT	Photodynamic Therapy
ROS	Reactive Oxygen Species
ssDNA	Single Strand DNA
TME	Tumour Microenvironment
TRAIL	TNF-related Apoptosis-inducing Ligand
β-ME	β-Mercaptoethanol

LIST OF FIGURES

FIGURE NO.	FIGURE TITLE	PAGE NO.
Figure 1.1	Adult earthworm <i>Eudrilus eugeniae</i>	3
Figure 1.2	Structure of <i>H2AFX</i> gene	8
Figure 5.1	The primary setup for light exposure given for <i>Eudrilus eugeniae</i>	26
Figure 5.2	Depiction of the first 10 segments amputated for observing the wound healing and regenerative capacities of <i>E.eugeniae</i> under different wavelengths of light	26
Figure 5.3	Control bud growth	27
Figure 5.4	Violet bud growth	28
Figure 5.5	Blue bud growth	28
Figure 5.6	Green bud growth	29
Figure 5.7	Yellow bud growth	29
Figure 5.8	Orange bud growth	30
Figure 5.9	Red bud growth	31
Figure 5.10	(a) A cumulative graph depicting the survival ability of earthworms in 30 mins exposure	32
	(b) A cumulative graph depicting the survival ability of earthworms in 2 hours exposure	33
Figure 5.11	Statistical graphs showing the survival of <i>Eudrilus eugeniae</i> under different time period	34
Figure 5.12	(a) SDS-PAGE results	36
	(b) Western Blotting results	36

Figure 5.13	Histology sectioning of the 7th day bud of <i>E.eugeniae</i>	40, 41
	(a) Histology sectioning of worm under normal conditions	40
	(b)Histology sectioning of worm under violet light	40
	(c) Histology sectioning of worm under yellow light	41

LIST OF TABLES

TABLE NO.	TABLE TITLE	PAGE NO.
Table 4.1	Concentration of separating gel	17
Table 4.2	Stacking gel concentration	18
Table 4.3	Reagent preparation for Lowry's method	19
Table 5.1	Survival data for 30 mins exposure	32
Table 5.2	Survival data for 2 hours exposure	33

1. OVERVIEW

1.1 INTRODUCTION

Regeneration is how cells, tissues, organs, and even complete body parts in plants and animals are naturally replaced or brought back to full functionality. In other words, regeneration is the process of tissue development, renewal, and restoration that renders genomes, cells, organisms, and ecosystems resistant to natural disturbances or occurrences. Regeneration is being studied by researchers for its possible applications in medicine, including the treatment of various illnesses and injuries. By studying regeneration, scientists also hope to gain more insight into the ageing process in humans. This rapidly developing field is known as regenerative medicine. At its most basic level, regeneration is regulated by molecular mechanisms of gene regulation and involves the cellular processes of cell proliferation, morphogenesis, and cell differentiation. Regeneration in biology, on the other hand, primarily refers to the morphogenic processes that characterize the phenotypic plasticity of traits, allowing multicellular organisms to repair and retain the integrity of their physiological and morphological states. Regeneration is fundamentally controlled by asexual cellular activities above the genetic level.

The soil organisms known as "farmer's friends" are called earthworms. More than 6000 species of earthworms (Crassieitellata) have been identified (Anderson et al., 2017), except in Antarctica. Nightcrawlers belong to a sizable, widely distributed population with a profound evolutionary history. Most earthworm species can be found along trees, on riverbanks, in decaying logs, and in leaf litter. Earthworms have a very significant economic influence and operate as engineers of the terrestrial ecosystem. Farmers receive significant benefits from earthworm compost.

Some bioluminescent worms release light as a defensive and protective strategy to keep predators at bay. An excellent example of a bioluminescent worm that would provide a scene that would be unpleasant to predators for preventative purposes and those which do not appreciate bright lighting but prefer to hunt in the dark is *Microscolex phosphorous*. (Friend, 1919) Saint Danies and their colleagues discussed the biochemical reaction of an earthworm to benzo(a)pyrene across different concentration levels, and they focused on the potential response selected as a marker for sub-lethal assays in the lab and monitoring of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). (Saint-Denis et al., 1999)

1.1.1 Earthworm *Eudrilus eugeniae*

Eudrilus eugeniae is a member of the Clitellata class and the Eudrilidae family of annelids. The term "African nightcrawler" is used to describe the worm. The rear segments of the African Nightcrawler are uniformly tapered to a tip and have a uniform purple-grey gloss. The worm makes an excellent model for biological research involving cells and molecules. The worm can be used to study the intricate cell and molecular biological processes of regeneration because of its remarkable capacity for regeneration.

E.eugeniae is an affordable model system for cell and molecular biological studies of regeneration and stem cell biology. To survive and regenerate, the clitellum-dependent earthworm requires undamaged clitellum segments. It is an excellent source of protein and has great pharmaceutical potential. This species of earthworm is endemic to tropical West Africa, but it is now common in warm places because of vermicomposting.

1.1.2 Characteristics of *E.eugeniae*

E.eugeniae was used as a regenerative biology experiment model. The pros include:

- a) The cost of raising fish, mice, and other animals is comparably expensive, however, maintaining earthworms in a lab is affordable and simple.
- b) It grows more quickly, maturing in as little as 5 weeks.
- c) It reaches 12 mg of body weight each day.
- d) It reproduces relatively quickly.
- e) It can withstand temperature differences anywhere between 15-30°C.

An adult worm has between 80 and 100 segments. The vital organs of *E.eugeniae* are the mouth, simple brain, two pairs of hearts, testis, seminal vesicles, rudimentary ovary, oviduct, and auxiliary ovarian glands which are present in the first 13 segments of the earthworm. The 13th to 18th segments contain the clitellum, a thick, cylindrical structure that resembles a collar and is crucial to reproduction. A pair of prostate glands, the gut, and the anus are among the essential organs located in the posterior portion. The coelomic fluid, which also contains several cell types known as coelomic cells, contains all the internal organs.



Fig 1.1 Adult earthworm *Eudrilus eugeniae*

1.2 REGENERATION STUDIES

Studies on regeneration first began in the 19th century. The research on regeneration is significant to Dinsmore, Mittwoch, and Batson. In his book "The Cell in Development and Inheritance," E. B. Wilson wrote about the suspension sponge cells that were used in the first sponge regeneration investigations. Jothi A. Kode (Kode et al., 2009) created an allogeneic strategy to identify and grow mesenchymal stem cells, which is essential in cell replacement treatment and may be a useful tool in regenerative medicine. Planarian worms are often the major subject of scientific research; however, they have a very limited capacity for regeneration. Fish and salamanders, which are lower vertebrates, are highly capable of axon regeneration as well as the development of neurons after a head injury (Sikes & Newmark, 2013), according to an analysis made by James about the regeneration of the Central Nervous System (CNS) in a variety of vertebrates, including lower vertebrates. (Tanaka & Ferretti, 2009)

Biopolymers are crucial to the process of regeneration in the medical industry. Nalinkant and Damani demonstrate that bioresorbable polymers may aid in the process of regeneration by enhancing the mechanism of tissue regeneration by employing the least amounts of solvents, medicines, and biopolymers. To speed up the regeneration process, autogenous peritoneum was precisely applied to the wound, and the peritoneum includes stem cells that could be surgically harvested from the individual. (Matapurkar & Rehan, 1997) The SFF approach is mostly utilised for device manufacturing, but it also improves tissue regeneration and cell culture. (Vacanti, J.P.,

Cima, L.G. and Cima, 1995) Mark Keating talked about cellular dedifferentiation, *in-vitro* and *in-vivo* regeneration of mammalian tissue approaches and compositions. This aided to improve the regeneration process by providing knowledge about various types of polypeptides, receptors, and growth factors in the tissue. (Poss et al., 2003) According to studies, the *Eudrilus eugeniae* earthworm has a remarkable capacity for regeneration and can replace destroyed body parts in as little as 24 days. (Johnson Retnaraj Samuel et al., 2012) Riboflavin is also crucial for the regeneration process, which was started by the earthworm's gut microbial flora. Riboflavin is produced by notable *Bacillus endophytic* in earthworm *E. eugeniae* for effective earthworm regeneration.(Sudalaimani et al., 2017)

1.3 ROLE OF STEM CELLS IN REGENERATION

Because they can differentiate into a variety of different cell types and replicate themselves millions of times, stem cells serve a crucial role in regeneration that more specialized cells like nerve cells cannot. Stem cells primarily function to repair and maintain the region where they are present. It is possible to direct stem cells to differentiate into cells that can be employed in individuals to regenerate and restore tissues that have been harmed or affected by illness. Stem cells can be developed into new tissue for use in transplant and regenerative medicine. Researchers continue to expand their understanding of stem cells and their uses in transplant and regenerative medicine.

1.4 PHOTOTHERAPY

Phototherapy is a medical treatment that uses natural or artificial light to improve a patient's health. Several disorders can be effectively treated using phototherapy. It is mostly used to treat a variety of common skin conditions, but it can also be used to cure neonatal jaundice. It entails the controlled application of non-ionizing radiation to the skin in a variety of dermatoses, which includes both ultraviolet and visible light radiation from the electromagnetic spectrum. Phototherapy is the most popular form of treatment for various skin conditions, including eczema, parapsoriasis, pityriasis lichenoides chronica, and psoriasis.

1.4.1 Clinical Significance

A number of dermatoses can be treated safely, easily, and effectively with phototherapy, which is also less expensive and has fewer side effects. Due to the significant prevalence of dermatological illnesses, phototherapy is more effective than topical or systemic therapies in treating individuals with vitiligo, psoriasis, atopic dermatitis, pityriasis Versicolor, chronic urticaria, and pityriasis Versicolor. Because phototherapy is a safe alternative to systemic therapy, patients are safeguarded from its side effects.

1.4.2 Different Colours of Phototherapy Light

1.4.2.1 Blue Light Therapy

The blue LED lights resemble a cell phone's light output. When a high-intensity light source is used with blue light therapy to activate photosynthesizing (or light-sensitive) medicines, the treatment becomes characterized as photodynamic therapy. It is seen as an alternate kind of treatment because the light used is a natural violet or blue light. The most typical uses of blue light treatment are to cure sun damage and pre-cancerous or malignant skin growths. Removing sebaceous glands can be used to enhance texture and alleviate depression. Blue lights, which emit a blue hue, is used frequently used to treat jaundice.

1.4.2.2 Red Light Therapy

LEDs with a reddish hue are referred to as red LED lights. Red light encompasses laser lights. Low-level red light wavelengths are used therapeutically in red light therapy to cure a variety of ailments, including persistent wounds, scars, and wrinkles on the skin. It is known as photodynamic therapy when this therapy is combined with photosensitizing drugs. The light in this sort of therapy only serves as a stimulant for the drug. Red light is thought to function by strengthening the mitochondria through a biochemical effect in cells. A cell can produce more ATP by enhancing the mitochondria's performance by utilizing red light therapy. Cells may perform more effectively, regenerate, and repair damage with increased energy.

1.4.2.3 Yellow light therapy

Amber light therapy is another name for yellow light therapy. The yellow light that enters the skin is absorbed by keratinocytes, melanocytes, Merkel, and Langerhans cells, all of which are essential for maintaining the epidermal layer of the skin. According to studies, cytochrome c oxidase, a crucial mechanism for changing metabolic pathways, is capable of absorbing yellow light. Red blood cell synthesis is

stimulated by yellow light therapy, and red blood cells are essential for skin healing and skin cell renewal.

1.5 ROLE OF RIBOFLAVIN IN REGENERATION

The worm needs riboflavin to regenerate, and riboflavin supplementation enhances its regeneration. Riboflavin has also been discovered to help speed up planarian regeneration. The liver in vertebrates contains a higher concentration of riboflavin than any other organ. It has been established that earthworms and the liver of vertebrates both have increased levels of riboflavin. It is interesting that the liver and earthworms both possess strong regeneration abilities. Riboflavin is a crucial component of the intricate regeneration process, according to studies.

1.6 *H2AFX* GENE

The *H2AFX* gene produces the histone protein H2AX, which belongs to the H2A family. When double-strand breaks arise, the phosphorylated form γ H2AX (S139) takes on a significant role. Histone octamers, which are made up of the main histones H2A, H2B, H3, and H4, are wrapped around DNA in humans and other eukaryotes to form chromatin. H2AX aids in the synthesis of nucleosomes, chromatin remodelling, and DNA repair. It can also be utilized *in-vitro* to determine DSBs in dsDNA. Due to the loss of genetic continuity, DSBs are among the most severe types of DNA damage. They result from several factors, such as the activity of DNA-damaging chemicals as well as typical processes like meiosis and antibody class change. The histone variation H2AX is an essential participant in the DDR for handling DSBs.

One of the histones H2A proteins encoded in the genomes of eukaryotic organisms is H2AX, the human genome holding 21 genes of H2A forms. H2A.1 and H2A.2, two biochemically distinct isoforms of the canonical human H2A, are present. Despite no variation within each isoform, there appears to be a basis for their separation that depends on residue 51, respectively which encodes either leucine or methionine. There are four other H2A variations with unique roles encoded in humans and other higher eukaryotes that includes H2AX, H2A.Z, macroH2A1, macroH2A2, H2ABbd and H2A.F/Z.

1.6.1 γ H2AX for double-strand breaks

Although the assay may also reveal other less significant phenomena, an assay for γ H2AX often reflects the existence of DSBs in DNA. On the one hand, based on

absolute yields and distributions caused per unit dosage, overwhelming data shows a significant, quantitative link between the creation of γ H2AX foci and the induction of DNA DSBs after exposure to ionizing radiation. On the other hand, cellular responses to numerous stressors other than ionizing radiation have been reported to include not only the development of discrete γ H2AX foci but also the production of γ H2AX signals across the nuclear membrane. At DNA double-strand breaks, the γ H2AX signal is always stronger than in chromatin which has not been damaged.

According to some theories, H2AX in undamaged chromatin may be produced by active kinases directly phosphorylating H2AX, most likely because of the protein diffusing from DNA damage sites. It is crucial to understand that γ H2AX is a downstream proxy that can help depict DNA damage repair when employing it as a marker for DSBs. When analyzing data from such assays, it is important to keep in mind that it does not represent DSBs as a whole.

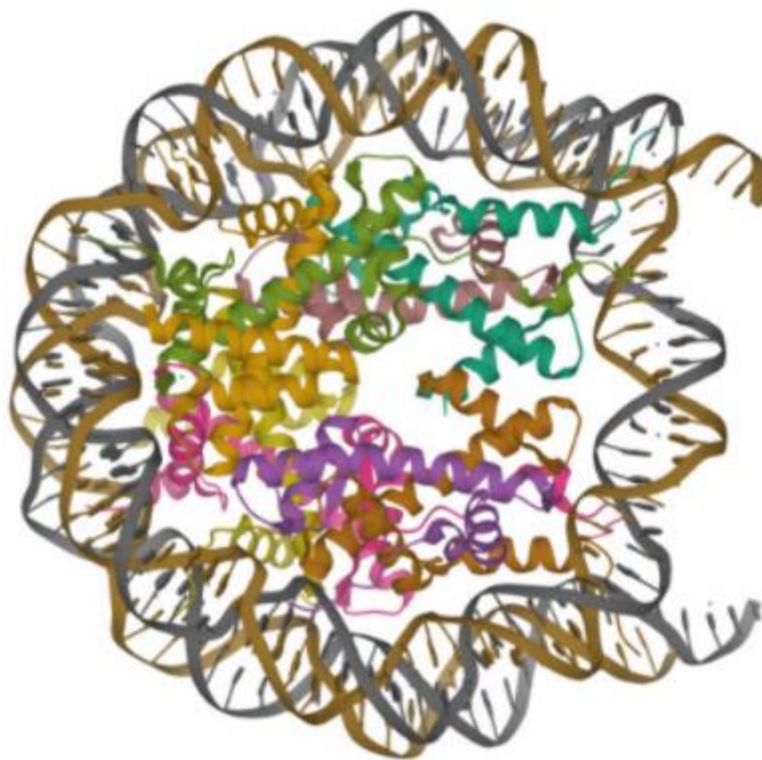


Fig 1.2 Structure of H2AFX gene (Entry ID – 6K1I)

1.7 BACTERIORHODOPSIN

A reddish-purple pigment called bacteriorhodopsin changes salt lakes from brick red to deep purple. The only light accessible to the cyanobacteria, which developed after the archaea, is a reddish to purple tint that pervades the layer of water housing the archaea. Bacteriorhodopsin is a purple pigment that is similar to rhodopsin, better known as visual purple, the light-sensitive pigment in the eyes. Due of rhodopsin's high sensitivity to light, it is possible to see in dim light. Rhodopsin is activated by light and releases energy generated in the retinal cell in the form of an electrical impulse that travels via the retinal nerve.(Durand, 1963)

The protein is found in the cell membranes of some bacteria, particularly in a group of organisms known as halophiles. It functions as a light-driven proton pump, which means that it can use light energy to transport protons across the cell membrane and generate a proton gradient that can be used to produce ATP (adenosine triphosphate), the energy currency of the cell. Bacteriorhodopsin contains a pigment called retinal, which is covalently bound to the protein and acts as a light-absorbing chromophore. When retinal absorbs light, it undergoes a conformational change that triggers a series of events that ultimately result in the transport of protons across the membrane.

Bacteriorhodopsin is of interest to scientists because it represents a simple example of a light-driven ion pump that can be studied in detail. It has also been used in various biotechnological applications, such as in the development of optogenetic tools for controlling neural activity in living organisms.

2. LITERATURE SURVEY

Gopi Daisy et al. (2016) conducted studies on the regeneration of the central nervous system (CNS) of *Eudrilus eugeniae*. They concluded that this species of earthworm has a well-developed nervous system compared to other invertebrates like the hydra. Self-assemblage may help these earthworms survive even in flooded areas. These worms get buried about 12 inches deep into the soil. The earthworm has an incredible ability to renew and mend itself in a matter of weeks or months without altering its internal processes and so maintaining its economic relevance.

Studies conducted by **Pithawala & Jain (2012)** on the expression of alpha-fetoprotein suggested that this animal model is perfect for research on stem cells and regeneration because of its remarkable capacity for regeneration and simple maintenance requirements. In *E.eugeniae*, the regenerative blastema develops from an expanding mass of proliferating undifferentiated progenitor cells. Clitellar segments were shown to play a crucial role in the earthworm *E.eugeniae*, according to studies.

BRÜCKE et al.(2019) conducted studies correlating phototherapy and immunotherapy in treating metastatic cancer. They concluded that it is more effective to treat metastatic cancer by combining phototherapy and immunotherapy because they boost the immune system's reaction. They designed a covalently cross-linked polyethylene glycol that is pH sensitive to function as a tumour ECM targeting ROS nanoscavenger. Such a nanoscavenger is able to remove ROS from TME, alleviate immunosuppressive ICD brought on by particular chemotherapy, and extend T cell survival for customized CIT. Eliminating the ROS in the TME in a breast cancer model induced antitumor immunity and enhanced T lymphocyte infiltration, producing a highly effective anticancer impact.

Paul et al. (2021) carried out studies on the transcriptome of anterior regeneration of *E.eugeniae*. He summarized that the annelids are renowned among invertebrates for having a significant potential for regeneration. Among the oligochaete worms of the annelid phylum, segmental regeneration was best seen in *Eisenia fetida*, *Perionyx excavates*, and *Eudrilus eugeniae*. The extraordinary capacity of the epigeic

oligochaete earthworm *E. eugeniae* to regenerate both anterior and posterior segments has earned it global recognition.

Aztekin et al. (2022) suggested from the studies conducted that when an animal loses an appendage, some lineages exhibit a morphologically identifiable dedifferentiation phenotype, and at the same time, a simple wound epidermis covers the amputation plane. This basic wound epithelium develops into the apical-epithelial-cap, a specialized wound epithelium (AEC). The AEC then serves as the primary signalling hub during regeneration, facilitating the growth of lineage-specific stem and progenitor populations, also known as blastema.

Studies performed by **Rajiv et al. (2014)** suggested that the transport of inorganic and organic toxins through the community's inhabitants on polluted soils is modulated by the earthworm, either directly or indirectly. Earthworms are frequently used in ecotoxicological investigations on soil toxins as an ecosystem indicator species. Earthworms are delicate, easily accessible, and manageable, and historical data from their use in toxicity assessments exist.

Tian et al. (2019) analyzed the albumin-chaperoned cyanide dye which yielded superbright NIR-II fluorophore. He suggested that compared to the current NIR-I systems, NIR-II fluorescence-based biomedical imaging has improved contrast resolution and penetration depth. The loss of imaging signal and the inescapable production of background noise during fluorescence imaging are caused by laser-biological tissue interactions brought on by interacting excitation light, interface reflection, scattering, absorption, and autofluorescence. The use of NIR-II fluorescence imaging with lower scattering coefficients across almost all tissue types at longer wavelengths has many benefits. Extending clinical image-guided surgery into the NIR-II/short-wave infrared region is incredibly promising.

Selvan Christyraj et al., (2020) conducted studies for understanding the role of clitellum in regeneration of *E.eugeniae*. They concluded that earthworms, which share this trait with other invertebrates, have the capacity for regeneration of entire body segments. One group of species of earthworms depend on the clitellum for their regeneration, but the other group of worms do not. These two unique regeneration

occurrences are observed in earthworms. Wound healing is the first step in the regeneration process because it helps to stop additional tissue damage and microbial invasion. After a wound has healed completely, annelids use two separate mechanisms—epimorphosis and morphallaxis—to continue the regeneration process.

Blakemore Vermecology, (2015) conducted studies on the taxonomic profile of *E.eugeniae*. It was concluded that this species of the earthworm is an excellent vermicomposter. It can survive in any condition by adapting itself to the current environment. It has been observed that they are found not only in areas of high organic matter, but also in gardens and fields. So, the expansion of this species may require more closer monitoring.

Ma et al. (2019) suggests that regardless of the wavelength of incident lasers, photothermal treatments in *in-vitro* settings elicited immunogenic cell death (ICD) that was accompanied by the production of damage-associated molecular patterns (DAMPs). In *in-vivo* conditions, DAMPs were released and distributed more uniformly under NIR(II) light in the deeper regions of the tumours. In the cancer vaccination assay, 5/8 of the mice remained tumour-free thanks to NIR(II) photothermal therapy's simultaneous activation of innate and adaptive immune responses following the induction of ICD. Additionally, both main and distant tumours were permanently under control by the NIR(II) photothermal therapy in conjunction with checkpoint blockade therapy.

Hönigsmann et al. (2016) analyzed about oral photochemotherapy. It was observed that PDT is effective in treating superficial neoplastic skin lesions, including actinic keratoses, Bowen disease, and superficial BCCs. PDT may be used to treat a subset of patients with inflammatory dermatoses. However, it is important to be aware of the limits of both systemic and topical PDT. ALA/MAL-PDT is also useful for treating rosacea and acne, albeit the best regimens have not yet been established.

Hönigsmann, (2013) conducted studies on phototherapy. Phototherapy is a solution for many skin diseases. Extracorporeal photochemotherapy, often known as photopheresis, is a new type of phototherapy that was developed to treat erythrodermic cutaneous T-cell lymphoma (CTCL). Later, a number of uncontrolled

clinical trials were conducted to demonstrate its efficacy. Patients with erythrodermic CTCL were advised to begin treatment with ECP. With great response rates, ECP is particularly crucial in the management of chronic graft versus host disease (GVHD) following allogeneic bone marrow transplantation.

The studies undertaken by **Barros *et al.* (2021)** concluded that for improved disease control, phototherapy is utilized in conjunction with topical or systemic drugs. Phototherapy has been used successfully in a number of dermatoses, including atopic dermatitis, vitiligo, cutaneous T-cell lymphoma, and cutaneous sclerosis, among others, even though psoriasis is the most common indication.

Chen *et al.* (2015) studied the structure and function of histone H2AX. The histone variation H2AX, which is a crucial part of the chromatin packing of eukaryotic genomes, is a crucial component of the DNA Damage Response (DDR) for dealing with DSBs. The human genome has 21 genes for various histone H2A forms, with H2AX being one of a group of histone H2A proteins encoded in eukaryotic genomes. The ability of the H2AX variation to accept phosphorylation on a serine close to the C-terminus through the activity of PIKKs like ATM, ATR, and DNA-PK on the consensus motif SQ[E/D] Φ (where Φ is a hydrophobic residue) is what distinguishes it from other forms of the protein.

Hong *et al.* (2020) studied about phototherapy which can be used as an adjuvant for chronic spontaneous urticaria. It was reported that while comparing the antihistamine monotherapy control group to the NB-UVB + antihistamine combination therapy group, there was a statistically significant decrease in the urticarial activity score. This shows that NB-UVB might work well as a supplemental treatment for CSU that is refractory.

Bae *et al.* (2017) analyzed the effect of phototherapy on the disease vitiligo. The study illustrated how different types of phototherapy, treatment durations, and body sites affected the treatment response of vitiligo. Because this study only included studies of phototherapy alone, treatment outcomes in the clinical situation may be better than those found in our analysis. In addition to phototherapy, other adjuvant therapies, such as topical calcipotriol, topical calcineurin inhibitors, and systemic antioxidants, may be utilized to improve the therapeutic response. However, in developing the treatment

plan, professionals and patients could use our findings as a useful reference. Because phototherapy typically takes a long time, it is important to reassure and motivate patients to get the best possible results.

Sedelnikova & Pilch, (2011) studied about the effect of histone H2AX on DNA damage and repair. It was confirmed that the capacity to measure DNA DSB counts at organism-survival-safe levels opens a variety of unique possibilities for human health. A person's sensitivity to ionizing radiation and other substances that induce DSBs can be evaluated, which may identify those whose sensitivity is higher than average but remain subclinical. It may be expected that these people would have higher steady-state levels of cellular DSBs.

Solier et al. (2009) studied on the death receptor-induced activation of Chk2- and histone H2AX-associated DNA damage response pathways. It is observed that Chk2 can increase the apoptotic response induced by TRAIL through a feedback loop independent of p53 after being activated by DNA-PK and ATM. Due to upstream caspases 8 and 9, the Chk2-mediated positive feedback extends outside the nucleus. The acceleration of cell detachment, an increase in cell death as shown by clonogenic experiments, an increase in the production of H2AX, and a quicker activation of caspases were all signs of Chk2's functional impact on TRAIL-induced apoptosis. Because of the potentially serious physiological effects of abortive apoptosis, Chk2 may have a considerable functional impact. Oncogenes can be removed from dying tumour cells or cells that have contracted a virus by causing apoptosis, which has been recognized as a key process.

Rajesh et al. (2021) studies the anti-mitotic compounds found in the pseudo-stem of banana and correlated it with earthworms. It was concluded that at least three chemicals found in BPS have the potential to reduce cell growth or halt mitotic cell division. Purification of these antimitotic chemicals and additional research are needed, nevertheless, to determine whether they have the potential to be molecules of significant therapeutic use. Instead of throwing out BPS, it might be exploited as an inexpensive bio-resource to create future therapeutic compounds that have commercial value. It has been shown that earthworms can serve as straightforward *in-vivo* animal model systems for screening antimitotic BPS chemicals.

Chaudhuri (2022) studied the neurosecretion in oligochaetes. It was concluded that in the lack of nonneural endocrine glands, neurohormone (neurosecretion) is the only source of hormone in oligochaetes. In the absence of any distinct endocrine glands or neurohemal organs, the highly vascularized central nervous system serves as the so-called "neuroendocrine system." An illustration of a first-order neuroendocrine reflex is the physiological function of neurosecretion in oligochaetes. Earthworms' neurosecretory cells have a role in colour variation, osmoregulation, thermal acclimatization, food intake, regeneration, conjugation, and the development of cocoons.

Srivastava et al. (2009) analyzed the role of histone H2AX in DNA damage response and cancers. It was found that there appear to be many more unexpected and significant functions for the variant histone H2AX. In addition to spermatogenesis and sex chromosome inactivation, the role of DNA damage signalling during V(D)J recombination has also been clarified. H2AX becomes a significant adaptor protein, enabling the accumulation and retention of repair components at the site of damage, following DSB signalling, however, because of extensive posttranslational changes. H2AX has the potential to function as a haploinsufficient tumour suppressor gene, which is also becoming more and more clear. H2AX gene copies are lost in random breast tumours. In addition, the H2AFX gene is located in a region of the genome that is rich in CpGs, and given that its haploinsufficiency can encourage carcinogenesis, hypermethylation of the promoter area is a likely scenario.

Johnson Retnaraj Samuel et al. (2012) analyzed on the autofluorescence in BRdU-injected cells of *E.eugeniae*. He confirmed that the circular muscles and longitudinal cell layers have BRdU-positive cells. These cells migrate to the blastema from the adjacent segments of the worm. It was also noted that these cells have autofluorescence properties. The cytoplasm was auto fluorescent, but not the nucleus. The major source of autofluorescence is riboflavin. Riboflavin is one of the regenerative factors that alleviates or hampers regeneration.

3. AIM AND SCOPE

- To analyze the effect of different wavelengths of visible light like violet (380-450nm), blue (450-495nm), green (495-570nm), yellow (570-590nm), orange (590-620nm), red (620-750nm), white (400-700nm) and sunlight (control) on earthworm, *E. eugeniae* and evaluate its regeneration potential.
- To evaluate morphological, developmental, and histopathological changes of different wavelength light upon regeneration events.
- To analyze the DNA damage response using H2AX protein expression following exposure to different wavelengths of light in the earthworm, *E. eugeniae*.
- Specific wavelengths of light that enhance regeneration potential in earthworms are analyzed in correlation with WNT3a, a stem cell marker.
- Specific wavelengths that induce regeneration in *in-vivo* conditions are further analyzed in *in-vitro* maintained non-regenerating amputated worm segments.

4. MATERIALS AND METHODS

4.1 EARTHWORM CULTURE AND MAINTENANCE

Earthworms are collected in the Regeneration and Stem Cell Biology Lab, International Research Center of Sathyabama Institute of Science and Technology. Worms were kept in a plastic container with soil, cow manure, and leaf litter. A diet containing an equal mixture of cow dung and jaggery is offered three times each week. Periodically, the vermicast and earthworm bed were removed and replaced with leaf litter and cow dung.

4.2 EXPOSURE TO VISIBLE LIGHT CATEGORIZED ACCORDING TO THEIR WAVELENGTHS

An equal number of worms is taken for each colour, and they are given exposure for a certain period of 2-4 days. Following exposure, worms are subjected to amputation of segments 1-10 post which exposure is continued until clear bud formation can be observed. After this, the bud region of *E.eugeniae* is cut and a protein sample is prepared.

4.3 PROTEIN ISOLATION

Protein disulphide links are broken with beta-Mercaptoethanol (β -ME, reducing agent). 5% of Beta-Mercaptoethanol was added to 2X sample buffer (stock 1). Samples were transferred into a new 1.5 ml vial at 4 °C with an equal amount of sample buffer. After manually homogenising the samples, they were then placed into a dry bath at 100 °C for 5 minutes, centrifuged for 5 minutes at 10,000 rpm, and the supernatant was transferred to a new 1.5 ml vial and maintained at -20 °C.

4.4 SDS-PAGE

4.4.1 Principle

SDS PAGE is an analytical method that separates proteins according to their molecular weight. The study of how charged molecules travel in an electric field is known as electrophoresis. The intensity of the field, the net charge, the size, and form of the molecules, as well as the ionic strength, viscosity, and temperature of the medium in which the molecules are travelling, all affect how quickly they move. For separating bigger molecules like proteins, polyacrylamide gels are frequently utilised.

Proteins are divided based on the gel matrix's resistance, with smaller proteins often having less resistance and bigger proteins typically having more resistance. The denaturing effect of SDS is very strong, and it disrupts the secondary, tertiary, and quaternary structure of the protein to produce a linear polypeptide chain coated with negatively charged SDS molecules. This linear polypeptide chain is then coated with negatively charged SDS molecules, which is how the molecular weight of the protein was determined. Through the reduction of all disulphide linkages, β -mercaptoethanol aids in the denaturation of proteins (both inter-disulphide and intra-disulphide bonds). Protein separation is aided by the mesh-like matrix that polyacrylamide produces. Proteins were appropriately stacked or organised during PAGE electrophoresis, and once they reached the separating gel, they began to separate depending on their size.

4.4.2 Materials

Eppendorfs; Micro-pipettes; Micro-tips; Centrifuge; Water bath; Vortex and Distilled water.

4.4.3 Chemical Preparation for SDS PAGE

30% Acrylamide and 0.3% Bis acrylamide:

Acrylamide	- 30 g
N, N' Methylene Bis-acrylamide	- 0.3 g
Make up into 100 ml with distilled water.	

4X Separating Buffer:

Tris HCL	- 18.2 g
Distilled water	- 60 ml
pH was adjusted to 8.8 with 10N NaOH.	
Sodium Dodecyl Sulphate (SDS)	- 0.4 g

Makeup into 100 ml with distilled water.

4X Stacking Buffer:

Tris HCl	- 6.05 g
Distilled water	- 40 ml
pH was adjusted to 6.8 with 10N NaOH.	
Sodium Dodecyl Sulphate (SDS)	- 0.4 g
Make up into 100 ml with distilled water.	

2X Sample Buffer:

Tris stacking buffer (pH 6.8)	- 25 ml
Sodium Dodecyl Sulphate (SDS)	- 4 g

20% Glycerol - 20 ml

Make up into 100 ml with distilled water.

During protein sample preparation, 5% β -mercaptoethanol and during loading, 0.001% (w/v) of Bromophenol blue was added.

10% Ammonium Per Sulphate (APS):

APS - 100 mg

Make up into 1 ml with distilled water.

1X Running Buffer:

Tris HCl - 3.94 g

Glycine - 15.014 g

SDS - 1 g

Make up into 1L with distilled water.

Staining Solution:

Coomassie Brilliant Blue R-250 - 200 mg

Methanol - 50 ml

Glacial Acetic acid - 7 ml

Make up into 100 ml with distilled water.

The above mixture was filtered using Whatman No 1 filter paper.

Destaining Solution:

Methanol - 30 ml

Glacial Acetic acid - 7 ml

Make up into 100 ml with distilled water.

Table 4.1 Concentrations of separating gel

Sl. No.	Stock Solution	5%	6%	7%	8%	9%	10%	12%	13%	15%
1	30% Acrylamide + 0.3% Bis-acrylamide (ml)	5.0	6.0	7.0	8.0	9.0	10.0	12.0	13.0	15.0
2	4X Tris Separating Buffer (ml) pH 8.8	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
3	Distilled water (ml)	17.5	16.5	15.5	14.5	13.5	12.5	10.5	9.5	7.5
4	10% APS (μ l)	200	200	200	200	200	200	200	200	200
5	TEMED (μ l)	20	20	20	20	20	20	20	20	20

Table 4.2 Stacking gel concentration

Sl. No.	Stock Solution	5 ml	10 ml	15 ml	20 ml
1	30% Acrylamide + 0.3% Bis-acrylamide (ml)	1.25	2.5	3.75	5.0
2	4X Tris Stacking Buffer (ml) pH 6.8	1.25	2.5	3.75	5.0
3	Distilled water (ml)	3.05	5.0	7.5	10.0
4	10% APS (μ l)	100	100	100	100
5	TEMED (μ l)	10	10	10	10

4.4.4 Protein Sample Preparation

Protein disulphide links are broken with beta-Mercaptoethanol (β -ME, reducing agent). 5% of Beta-Mercaptoethanol was added to 2X sample buffer (stock 1). Samples were transferred into a new 1.5 ml vial at 4 °C with an equal amount of sample buffer. After manually homogenising the samples, they were then placed into a dry bath at 100 °C for 5 minutes, centrifuged for 5 minutes at 10,000 rpm, and the supernatant was transferred to a new 1.5 ml vial and maintained at -20 °C.

4.4.5 Lowry's Method

Before starting the experiment, prepare BSA stock solution along with Reagents A,B and C.

Table 4.3 Reagent preparation for Lowry's method

Reagents	Chemicals required	Quantity
BSA stock solution	Bovine Serum Albumin (BSA)	5 mg
	Distilled water	1 ml
Reagent A	Sodium hydroxide	25 mg
	Sodium bicarbonate (NaHCO_3)	50 mg
	Distilled water	5 ml
Reagent B	copper sulphate (CuSO_4)	25 mg
	Sodium potassium tartrate ($\text{C}_4\text{H}_4\text{O}_6\text{KNa}\cdot 4\text{H}_2\text{O}$)	50 mg
	Distilled water	5 ml
Reagent C	Reagent A	4 ml
	Reagent B	1 ml

Take a 96-well plate when reagent preparation is complete. Depending on the amount of samples, we will need one well for the blank, seven for the standards, and maybe more. Put 100 μ l of distilled water into each well. The 7 standard wells should each have 5, 10, 15, 20, 25, 30, and 35 μ l of distilled water removed from them respectively. 2 μ l of distilled water should be taken out of the sample wells. Except for the blank and sample wells, add BSA solution to every well. Replace the distilled water withdrawn with BSA solution for the standard wells. Add 2 μ l of the sample to the sample wells. Then add 100 μ l Reagent C in all the wells and incubate for 10 minutes. Post incubation, add 10 μ l of Folin-Ciocalteu reagent to all the wells. Make sure that dark conditions are maintained while adding Folin's reagent. Now keep this well plate on the shaker, still in dark conditions for 30 minutes. A graph is plotted with the sample values in correlation with the standard values. These sample values can then further be utilized for SDS-PAGE and Western Blotting.

4.4.6 Preparation of Gel

The glass plates were carefully put together after being cleaned with ethanol. To be utilised as a main baseline on gel and aid in the process of agarose sealing, glass plates were attached in the correct equalise way with each corner covered by spacers. The corners of the spacer's surface and bottom were then sealed with prepared 1% agarose that had been cooked until liquid. Separating gel was carefully produced by hand and put onto the gel. Isopropyl alcohol was applied on top of the gel to seal the air out. Following the removal of the IPA and washing with distilled water, draining, and preparation of the stacking gel and its addition, the comb was immediately fixed onto the stacking gel. After giving the gel some time to set, the comb was gently removed, and the wells were cleaned with water to get rid of the bubbles. Carefully removing the spacer's bottom, the setup was installed in the lower chamber, which housed the 1X running buffer as well as the top chamber.

4.4.7 Sample Loading and Electrophoresis

A 10 g protein sample and the necessary molecular markers were added to the wells. 60V was applied while the device was properly polarised and linked to the power pack. The glass plate assembly was removed from the buffer chamber following electrophoresis. The upper glass plate of the spacer was gently bent to separate it from the gel.

4.4.8 Staining and Destaining of Gel

The stacking gel was gently cut off with a cleaver and removed from the glass plate. By carefully gripping the gel's two corners, the separating gel was extracted and put into the Coomassie Brilliant Blue R 250 container (stainer solution). Gently rocking on a rocker should be used to completely immerse the gel in the staining solution for 30 minutes. Once the protein bands could be seen, it was rinsed with distilled water and moved to the destaining solution. Typically, the first destainer wash takes approximately 30 minutes, and the second wash takes roughly overnight.

4.5 WESTERN BLOTTING

For 15 minutes, gels were immersed in 1X transfer buffer. The transfer buffer was used to moisten the blotting equipment. On either side of the apparatus, sponges, tissue paper, and gel are placed in that order, with the gel being placed lightly on the tissue paper after the sponges. After the carbinol on the nylon membrane has been put into effect, the blotting paper is applied to the gel. The twin tissue sheets and sponges were then carefully applied and punctured. Subsequently, the entire blotting setup is moved inside the chamber, which is filled with 1X transfer buffer. The temperature is kept between 4°C and 8°C via the use of ice packs.

4.5.1 Buffer Preparation

10X Transfer Buffer (Stock)

Glycine	- 72.1 g
Tris-base	- 15.2 g
Distilled water	- 500 ml

The 10X transfer buffer was stored at room temperature, and during blotting, the 1X transfer buffer was prepared from the 10X in the ratio of preparation viz.,

For a total volume of 1 litre,

10X Transfer Buffer	- 100 ml
Methanol	- 200 ml
Distilled water	- 700 ml

4.5.2 Blocking

All proteins that would bind to both targetable and non-targetable proteins were blocked using a blocking buffer. In 20 ml of 1X TBST buffer, 5% of Milk Powder or Fetal Bovine Serum (FBS) was prepared for blocking.

4.5.3 Primary Antibody

In the presence of either 5% milk solution or FBS, the suitable primary antibody was added in the ratio 1:5000 (1 part of antibody to 5000 parts of milk solution or FBS). For instance, H2AX, which is produced from rabbit polyclonal, is selected as a primary antibody. This is poured onto the blot placed in a box which is kept on the rocker and incubated at 4°C overnight. Onto the blot, only H2AX will be bound.

4.5.4 Secondary Antibody

The following day, the setup is kept outside until it reaches room temperature. Post 3 washes with TBST buffer, the secondary antibody is added.

In the 5% milk solution or FBS, the secondary antibody is added in the dilution ratio of 1:10000 (1 part of antibody to 10000 parts of milk solution or FBS). For instance, the secondary antibody H2AX, which is produced in goats, is an Anti-Rabbit Immunoglobulin G (IgG). This is added onto the blot and rocked continuously for 30 - 40 mins after which another 3 washes with TBST buffer are performed. The secondary antibody binds exclusively in the regions where the primary has already bound on the blot.

4.5.5 Visualization of the blot using ChemiDock

After the addition of the secondary antibody and 3 washes with TBST buffer, the blot is transferred to another box with a small amount of TBST buffer to avoid drying and patchy spots on the blot. Before visualizing, the ECL substrate solution is prepared by the addition of solution A and solution B in equal parts (1:1 ratio). The blot is visualized under Chemidock after one minute of incubation in the ECL solution.

The resulting blot images are then stored in .jpg or .epg format. Dark conditions are maintained so as for the development of the blot image.

4.6 HISTOLOGICAL STUDIES OF REGENERATION OF BUD

The *E. eugeniae* *in-vivo* regenerated bud was dissected and rinsed with distilled water. The removed portion was then overnight preserved in 50% formaldehyde at 50°C. The bud segment was then treated with 5 concentrations of isopropyl alcohol (IPA) – 60%, 70%, 80%, 90% and 100% for 1 hour. This is to treat the dehydration of the bud section after being incubated in 50% formaldehyde. This was done to remove the formaldehyde from the tissues. The bud section was once more immersed in 100% IPA at 50°C overnight. The IPA was then removed from the tissues by transferring them to xylene. Finally, tissues were immersed in wax overnight. The intended

temperature range is between 58-60°C. Tissues were carefully moved to the second wax after incubation, and after three hours, tissues were placed on the last wax. For sectioning, tissues were moved into the boxes.

7µm slices were obtained from the roto-microtome and put on albumin-coated clean glass slides containing warm water. Slides were placed in a xylene jar for five minutes to remove wax after parts of the wax had been formed into a liquid condition. The 100% IPA Jar was then filled with slides, where they remained for 3 minutes. Slides were mounted with DPX mountant, and visible results were achieved with hematoxylin and eosin stains.

4.7 STATISTICAL ANALYSIS OF *IN-VIVO* BUD REGENERATION OF *E.eugeniae*

GraphPad Prism 8 is a statistical and scientific graphing software developed by GraphPad Software. GraphPad Prism 8 is a powerful and versatile tool for data analysis and visualization in scientific research. Some of the key features of Prism 8 include:

- Extensive statistical analysis capabilities, including t-tests, ANOVA, nonparametric tests, and survival analysis
- Interactive visualization tools for creating and customizing various types of graphs, such as scatterplots, bar graphs, and heatmaps
- Ability to fit and compare different models, such as linear regression and dose-response curves, and generate parameter estimates and confidence intervals
- Integration with other software and platforms, such as Microsoft Excel, R, and PubMed, for seamless data import and export
- User-friendly interface with comprehensive help and support documentation for both beginners and advanced users.

To plot a survival graph in GraphPad Prism 8, follow these steps:

- Enter your data into the software. Survival data typically includes the time to an event (such as death, disease progression, or recurrence) and whether or not the event occurred.
- Select "Survival Analysis" from the "Graph" menu.
- Choose "Kaplan-Meier" as the analysis type.
- Enter the time and event data into the appropriate fields.

- Specify any additional options you want, such as confidence intervals or censoring options.
- Click "OK" to generate the survival plot.
- The resulting plot will display the proportion of subjects still alive (or free from the event of interest) over time, along with confidence intervals if selected. This type of plot can be used to compare survival between groups, such as different treatment arms in a clinical trial.

5. RESULTS AND DISCUSSION

5.1 *IN-VIVO* REGENERATION OF BLASTEMA

Because of their extraordinary capacity for regenerating lost or injured body parts, earthworms are frequently employed as a model organism in studies towards regeneration biology. Due to the existence of clitellum, which starts blastemal development, a wounded earthworm may replace its destroyed tissue in just a few weeks. The clitellum creates a thick, protective cocoon that wraps the regenerating tissue during regeneration, helping to create a safe and supportive atmosphere for the process that leads to blastema production. Connective tissue, muscle and nerves are just a few of the diverse tissue types that blastemal cells can develop into.

The setup is as shown in Fig 5.1. Worms are kept inside a plastic box with a thick layer of moist tissue paper when light exposure is given. It is made sure that no external light source is found near the setup, in which case appropriate results may not be obtained.

Many studies suggest that earthworms have been found to be most sensitive to light in the blue-green range (500-550 nm) and less sensitive to light in the red range (600-700 nm). Exposure to blue-green light has been shown to stimulate feeding activity and increase growth in some species of earthworms, while exposure to red light has been shown to reduce activity and increase the time spent in burrows. (Rodionova et al., 2017)

Four sets of exposure duration has been given, ranging from 2 hours then slowly reducing it for 30 minutes each time. The longer the duration, higher is the intensity of light. Like most organisms, *E.eugeniae* is sensitive to light, and the duration and intensity of light can affect its behavior and physiology. In general, *E.eugeniae* prefers low light levels and is more active during the night. Exposure to bright light can cause stress and affect its reproductive activity.

In terms of duration, *E.eugeniae* can tolerate a wide range of light exposure, but prolonged exposure to light can be harmful. For example, if exposed to light continuously for several days, they may become disoriented, stop feeding, and even die. Therefore, to maintain optimal conditions in vermicomposting, it is recommended to provide low light levels and avoid prolonged exposure to light. Additionally, the intensity and duration of light can be adjusted to stimulate or suppress their activity and reproductive behavior, depending on the desired outcome.

Some studies have suggested that exposure to constant light or darkness can have negative effects on earthworms' regeneration abilities, while exposure to natural light and dark cycles can improve their regenerative abilities. Additionally, certain wavelengths of light, such as blue light, have been shown to stimulate regeneration in other species of earthworms.

The first cycle of exposure is for 2 hours. Six earthworms are taken for each colour in the visible spectrum and kept in separate plastic boxes with a thick layer of moist tissue paper. This is continued for 3 days for a healthy adult *E.eugeniae*. After 3 days, the first 10 segments (shown in fig 5.2) are amputated and the same duration of exposure is given for another 7-8 days until clear bud formation is seen. This is repeated for all the specified time durations and results are collected for each set of data.



Fig 5.1 The primary setup for light exposure given for *Eudrilus eugeniae*



Fig 5.2 Depiction of the first 10 segments amputated for observing the wound healing and regenerative capacities of *E.eugeniae* under different wavelengths of light

5.2 MORPHOLOGICAL CHARACTERIZATION OF *IN-VIVO* REGENERATION BLASTEMA

Regeneration is possible due to the presence of specialized cells called blastemal cells, which are capable of dividing and differentiating into various cell types needed to rebuild the missing body part. In terms of morphological characteristics, regenerated earthworms are generally similar in appearance to their intact counterparts. The regenerated body part may be slightly smaller or differently shaped, depending on the extent of the injury and the regenerative capacity of the species. However, studies have shown that regenerated earthworms are able to perform all the functions necessary for survival and reproduction, indicating that the regenerated body parts are functionally equivalent to the original ones.

E.eugeniae worms are kept in visible light exposure for 3 days after which the first 10 segments are amputated. After amputation, the light exposure is continued for another 7 days until clear bud formation is seen.

Fig 5.3 to Fig 5.9 depicts the bud formation for the first 7 days.

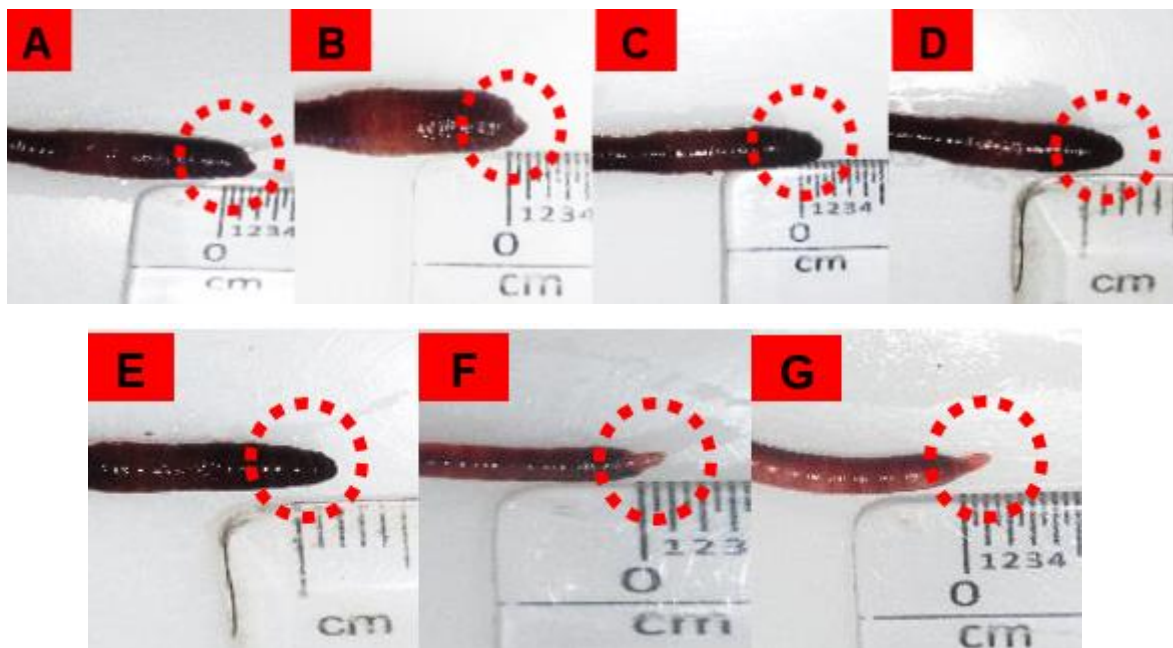


Fig 5.3 CONTROL BUD GROWTH

A. Wound healing starts B. 24 hours after wound healing starts C. 48 hours after wound healing starts, bud formation starts D. Small protrusion found after wound heals

E. Bud growth on day 5 F. Visible bud formation can be seen G. Bud of about 0.20 cm can be observed

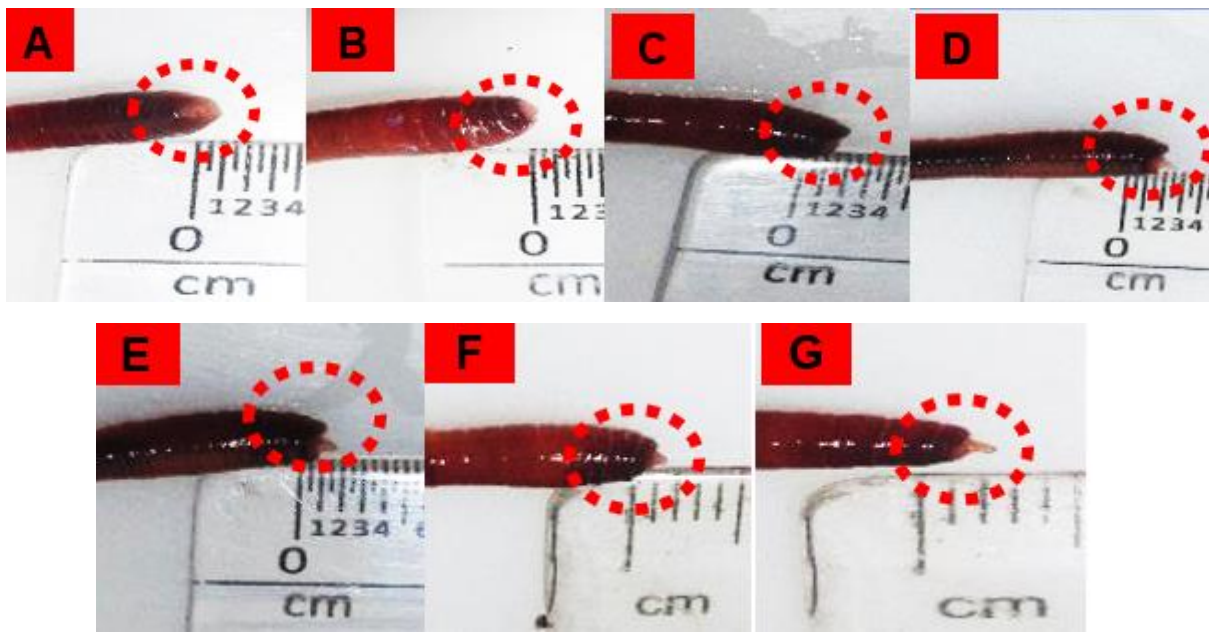


Fig 5.4 VIOLET BUD GROWTH

A. Wound healing starts B. 24 hours after wound healing starts C. 48 hours after wound healing starts, bud formation starts D. Small protrusion found after wound heals

E. Bud growth on day 5 F. Visible bud formation can be seen G. Bud of about 0.15 cm can be observed

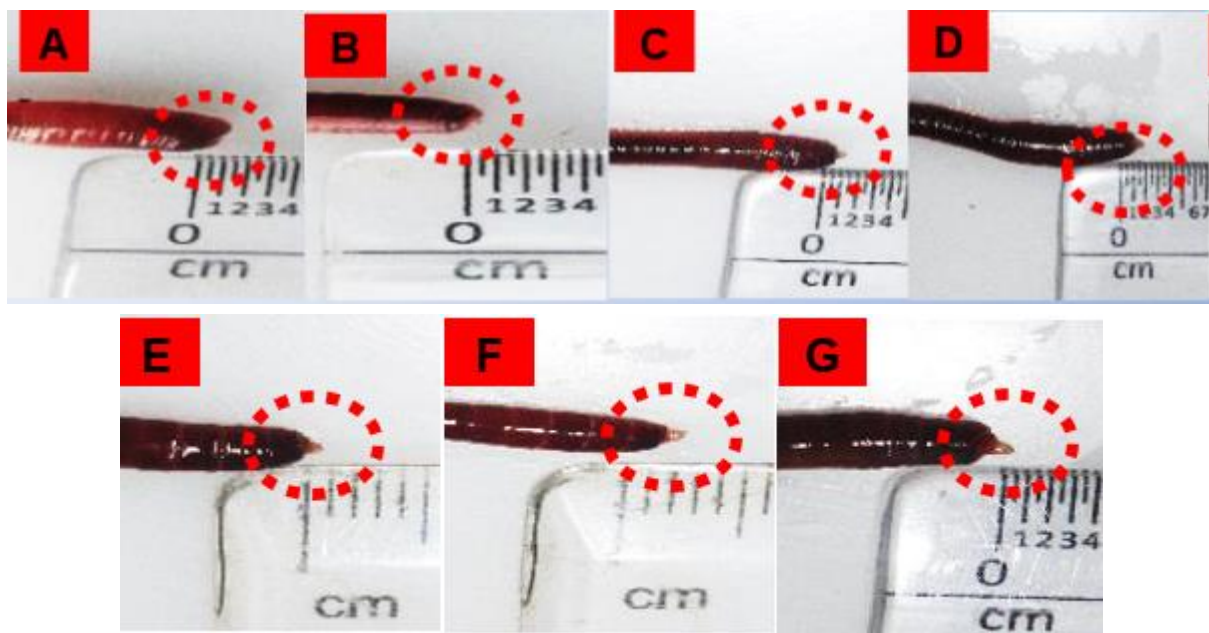


Fig 5.5 BLUE BUD GROWTH

A. Wound healing starts B. 24 hours after wound healing starts C. 48 hours after wound healing starts, bud formation starts D. Small protrusion found after wound heals

E. Bud growth on day 5 F. Visible bud formation can be seen G. Bud of about 0.15 cm can be observed

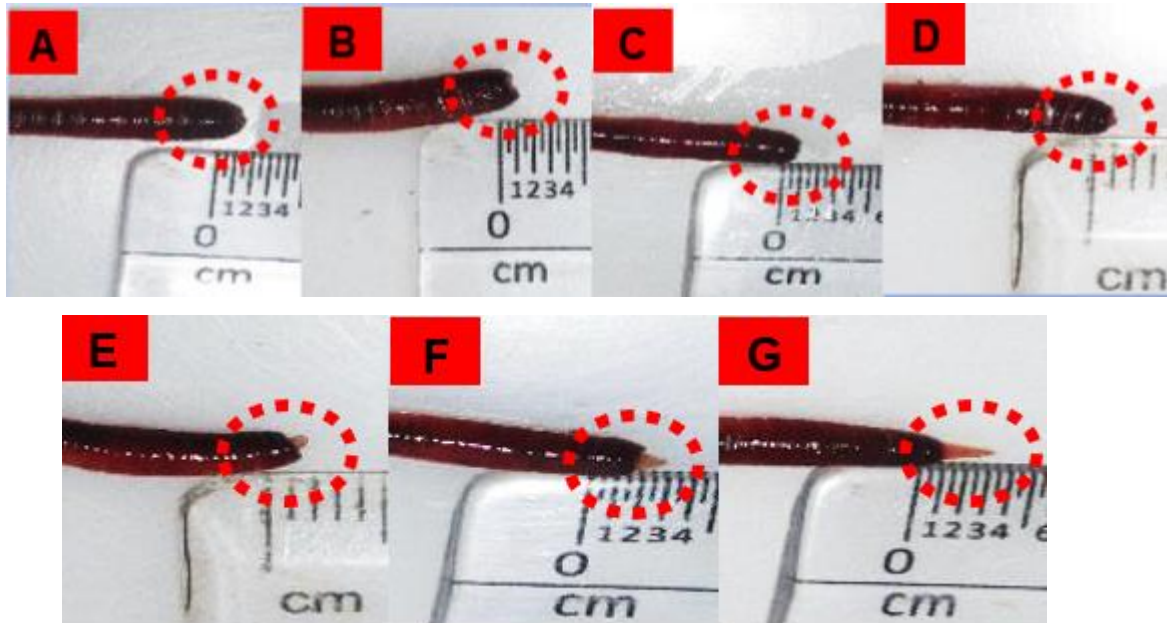


Fig 5.6 GREEN BUD GROWTH

A. Wound healing starts B. 24 hours after wound healing starts C. 48 hours after wound healing starts, bud formation starts D. Small protrusion found after wound heals

E. Bud growth on day 5 F. Visible bud formation can be seen G. Bud of about 0.25 cm can be observed

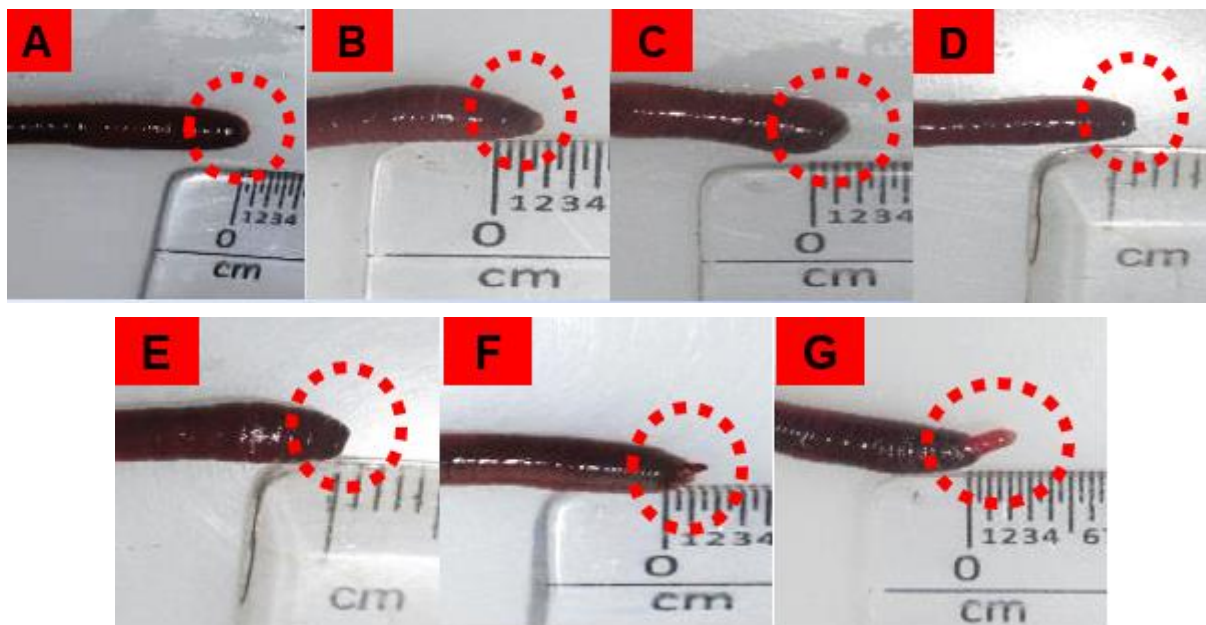


Fig 5.7 YELLOW BUD GROWTH

A. Wound healing starts B. 24 hours after wound healing starts C. 48 hours after wound healing starts, bud formation starts D. Small protrusion found after

wound heals

E. Bud growth on day 5 F. Visible bud formation can be seen G. Bud of about 0.30 cm can be observed

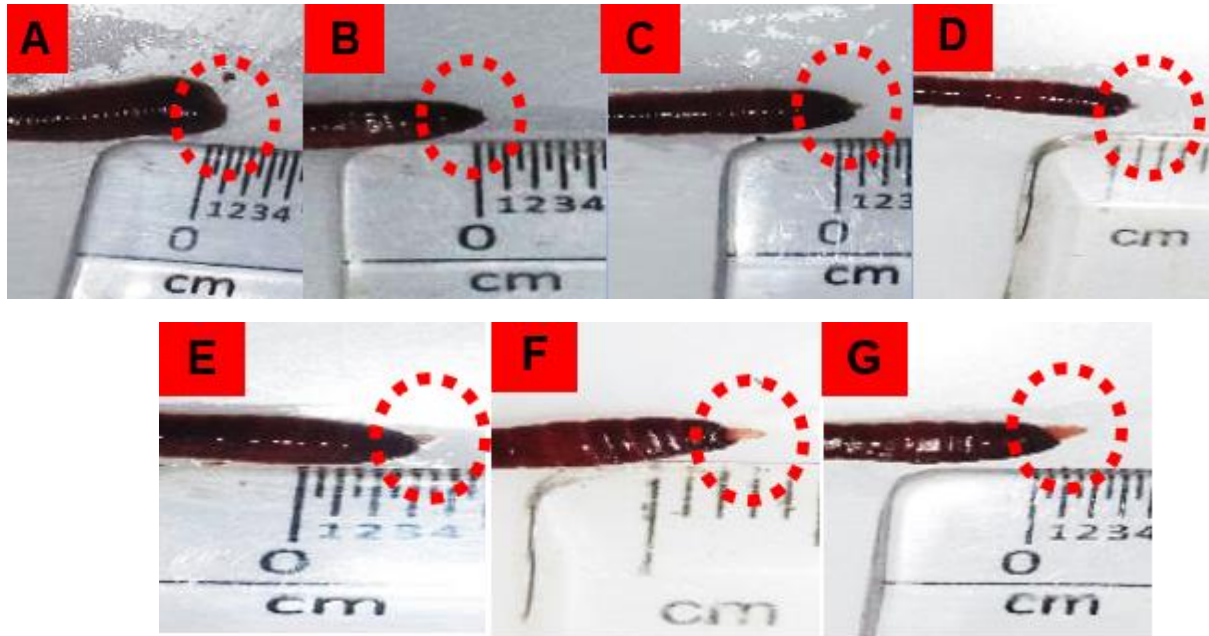


Fig 5.8 ORANGE BUD GROWTH

A. Wound healing starts B. 24 hours after wound healing starts C. 48 hours after wound healing starts, bud formation starts D. Small protrusion found after wound heals

E. Bud growth on day 5 F. Visible bud formation can be seen G. Bud of about 0.15 cm can be observed

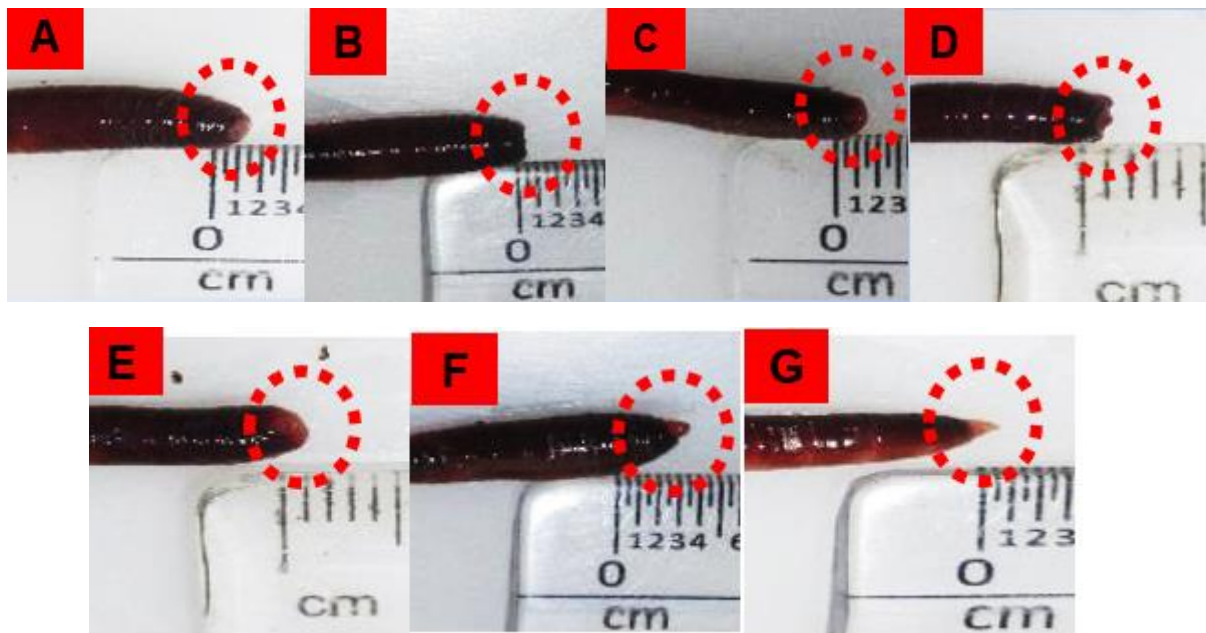


Fig 5.9 RED BUD GROWTH

A. Wound healing starts B. 24 hours after wound healing starts C. 48 hours after wound healing starts, bud formation starts D. Small protrusion found after wound heals

E. Bud growth on day 5 F. Visible bud formation can be seen G. Bud of about 0.10 cm can be observed

With these observations, we can say that yellow has the longest bud formation in the stipulated time and that by performing further intense studies, we can say that bud formation, and thus the regenerative capacity is the greatest in yellow, along with higher stressful environments.

The capacity of the clitellum factors to migrate to the wounded area or body segment and trigger the wound healing processes, (Selvan Christyraj et al., 2020) causing it to effectively regenerate until it entirely resembles a normal worm once again. One of the primary causes of effective regeneration is the migration of substances from the clitellum, which are thought to be stem cell factors. The stem cell niche, which may offer an environment required for the creation and maintenance of stem cells, may also be present in the clitellum segments.(Sivasubramaniam, 2021) In planarians, stem cells assemble at the location of the wound and wait for signs indicating tissue regeneration and healing.(Owlarn et al., 2017)

5.3 STATISTICAL ANALYSIS FOR SURVIVABILITY OF *E.eugeniae*

GraphPad Prism 8 is a popular statistical analysis and graphing software that may be used to create survival graphs. It is a powerful and versatile tool for data analysis and visualization in scientific research. It is designed for researchers in the fields of life

sciences, medicine, and engineering who need to analyze, graph, and present scientific data.

Survival analysis for earthworms are plotted in the form of a graph with the help of GraphPad Prism software. Fig 5.10 depicts the survival graph for two time durations – 30 mins and 2 hours. Tables 5.1 and 5.2 write down the survival figures of the earthworms in 30 mins and 2 hours respectively.

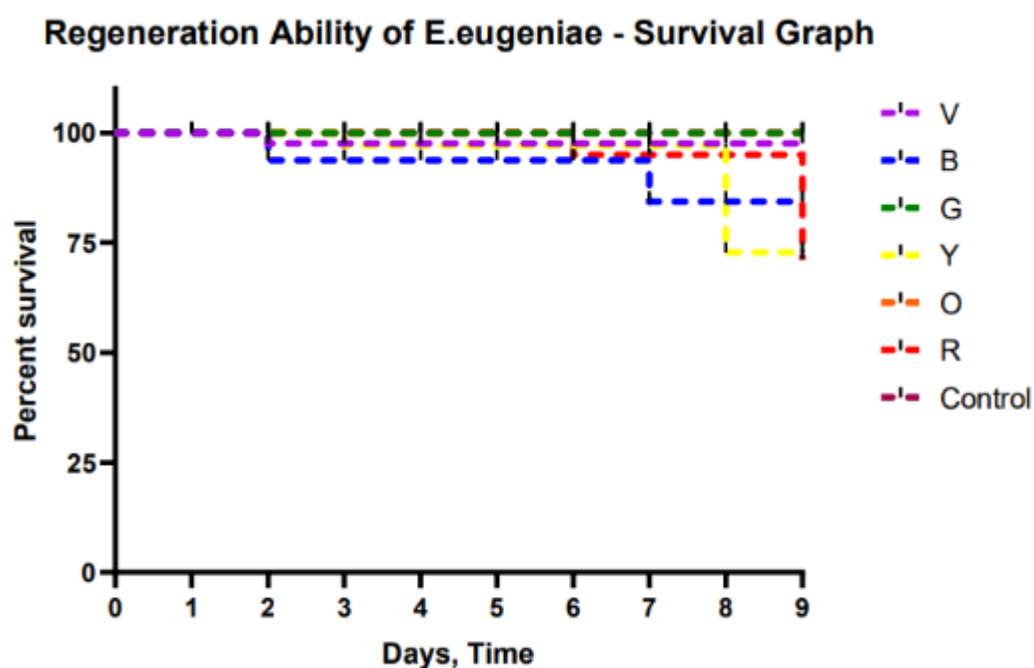


Fig 5.10 (a) A cumulative graph depicting the survival ability of earthworms in 30 mins exposure

Table 5.1 Survival data for 30 mins exposure

	V	B	G	Y	O	R	Control
0th day	6	6	6	6	6	6	6
1st day	5	4	6	6	6	6	6
2nd day	5	4	6	5	6	6	6
3rd day (amputation day)	5	4	6	5	6	6	6
4th day	5	4	6	5	6	6	6
5th day	5	4	6	5	6	5	6
6th day	5	3	6	5	6	5	6
7th day	5	3	6	3	6	5	6
8th day	5	3	6	3	6	3	6

Regeneration Ability of *E.eugeniae* - Survival Graph

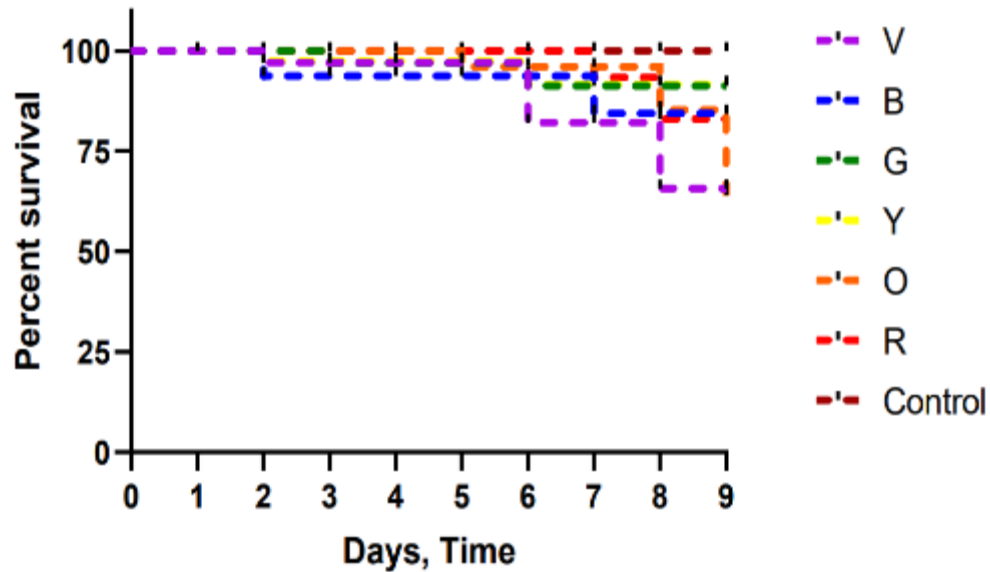


Fig 5.10 (b) A cumulative graph depicting the survival ability of earthworms in 2 hours exposure

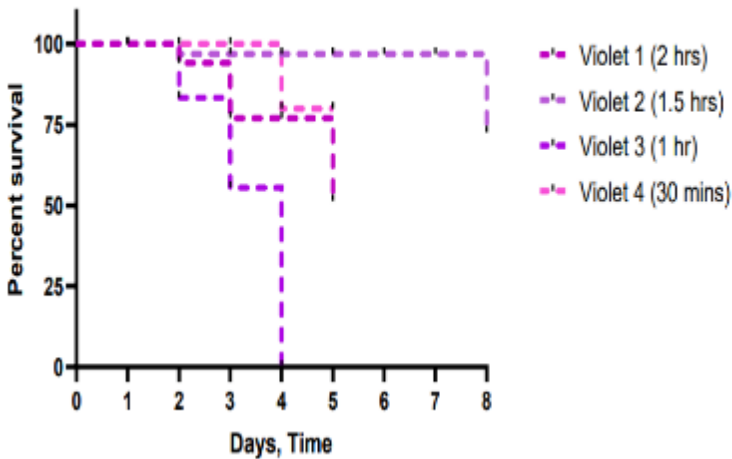
Table 5.2 Survival data for 2 hours exposure

	V	B	G	Y	O	R	Control
0th day	6	6	6	6	6	6	6
1st day	5	6	6	5	6	6	6
2nd day	5	6	5	5	6	6	6
3rd day (amputation day)	5	5	5	5	6	6	6
4th day	5	4	5	5	5	6	6
5th day	3	4	4	4	5	6	6
6th day	3	4	4	4	5	5	6
7th day	2	4	4	4	4	4	6
8th day	2	3	4	4	3	4	6

We can say that as the time of exposure increases, the intensity of light is increased and the earthworm feels extremely stressed. This results in faster wound healing and thus better regenerative capacities will be possessed by *E.eugeniae*. Since this is not in its natural environment and is additionally sensitive to external artificial light, they may not be able to survive for a long time and self-amputate themselves.

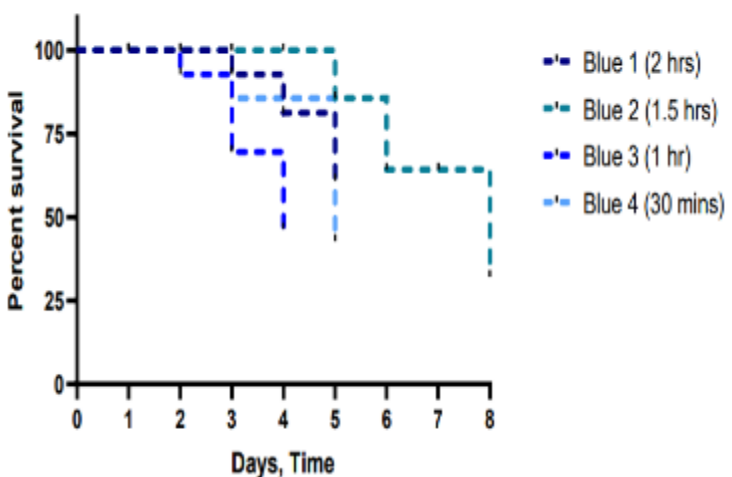
Survival graphs for the four times periods – 2 hours, 1.5 hours, 1 hour and 30 mins are compared and plotted as in Fig 5.11

Regeneration Ability of E.eugeniae - Survival Graph



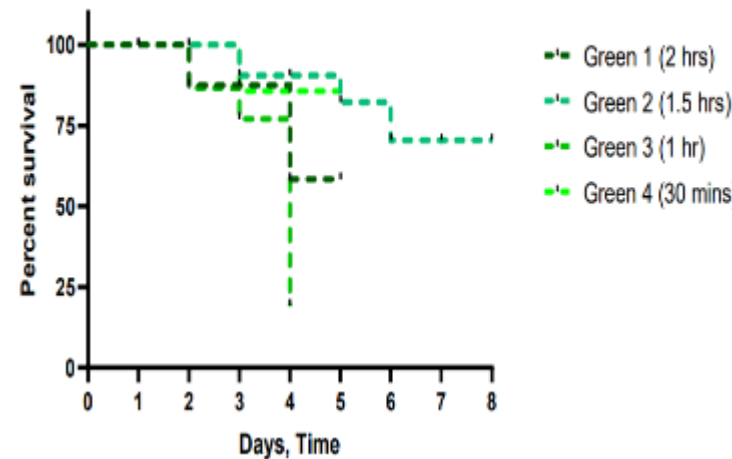
(a) Violet

Regeneration Ability of E.eugeniae - Survival Graph



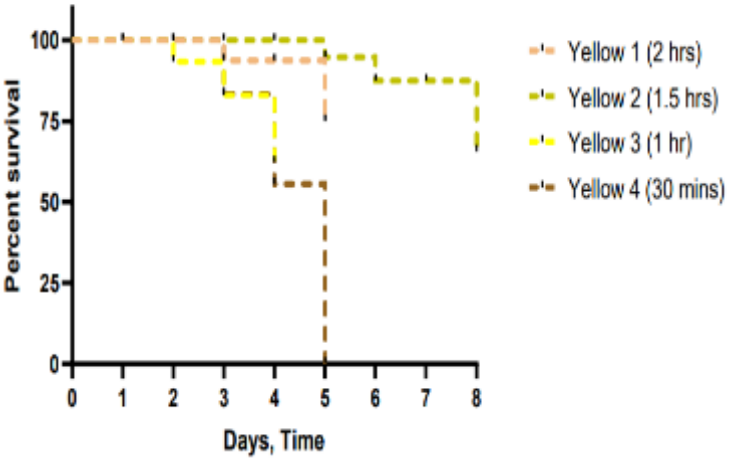
(b) Blue

Regeneration Ability of E.eugeniae - Survival Graph



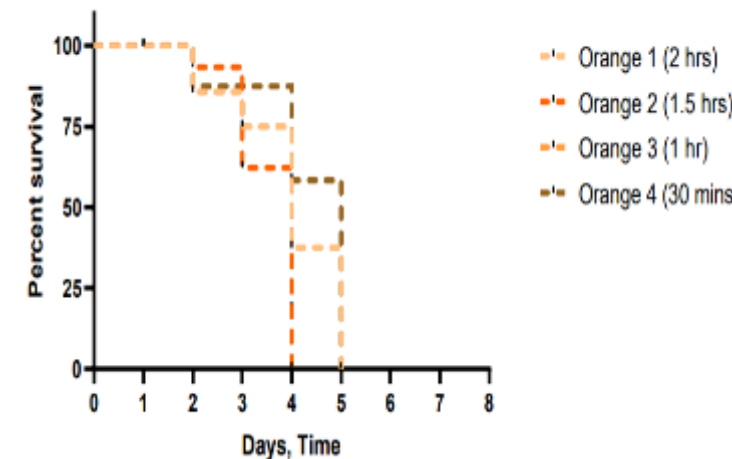
(c) Green

Regeneration Ability of E.eugeniae - Survival Graph



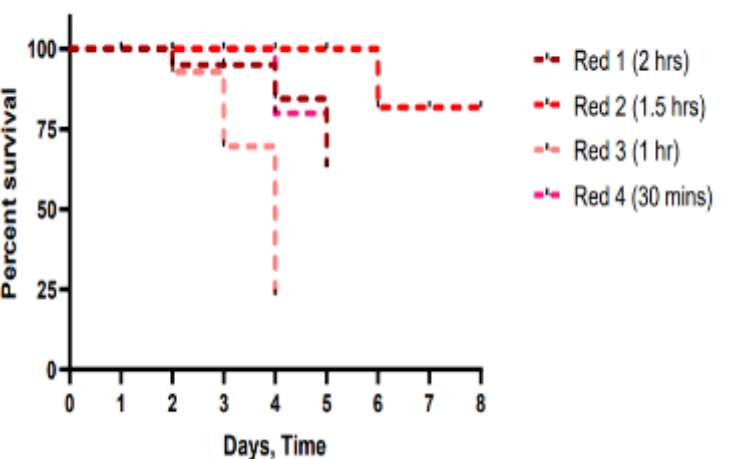
(d) Yellow

Regeneration Ability of E.eugeniae - Survival Graph



(e) Orange

Regeneration Ability of E.eugeniae - Survival Graph



(f) Red

Fig 5.11 Statistical graphs showing the survival of *Eudrilus eugeniae* under different time periods

5.4 WESTERN BLOTTING ANALYSIS

Western blotting was performed to study and differentiate between the expressions of various antibodies. It was performed for control, violet where the regeneration rate was slow, and yellow where there was high regeneration rate. In a nutshell, materials were generated under *in-vivo* conditions, resolved in 12% SDS-PAGE gel, and then subjected to western blotting analysis using antibodies against Actin, H2AX, WNT3a, and P53. The outcomes are shown in figures 5.12 and 5.13. The 3 samples may be distinguished from one another with ease.

H2AX is a stress protein that is produced in response to a variety of stresses, including radiation, physical harm, etc. The early DNA fragmentation during apoptosis promotes H2AX phosphorylation.(Rogakou et al., 2000) The significance of the histone variation H2AX in a number of additional biological processes, such as embryonic development, cell division, ageing and neural stem cell development, are supported by several investigations. The production of ssDNA and DNA double strand breaks (DSB) are the main causes of H2AX phosphorylation.(Turinetto & Giachino, 2015) According to several findings, nucleotide excision repair (NER) is necessary for the phosphorylation of H2AX. (Marti et al., 2006)(O'Driscoll et al., 2003)

The reinstatement of body axes is necessary for the patterning of regenerated tissue during whole-body regeneration. In many species, proper tissue regeneration along the major axis depends on Wnt signalling. Wnt-3 is necessary for posterior tissues to regenerate, according to functional investigations. Wnt-3 is a stem cell-specific protein that is produced in stem cells and required for their proliferation.(Ramirez et al., 2020) With mutations in Wnt genes exhibiting notable symptoms, Wnt proteins are now recognized as one of the primary families of developmentally significant signalling molecules. Intriguing processes including embryonic induction, cell polarity formation, and cell fate specification are among the roles carried out by Wnt proteins.(Moon et al., 1997)

In its role as a primary tumour suppressor, p53 controls a number of DNA-damage response (DDR) pathways to protect the genome. By stopping the cell cycle to give the repair mechanisms time to restore genomic integrity, p53 plays a significant

function as a DNA repair facilitator. Additionally, p53 adopted a variety of functions to directly influence the operation of different DNA-repair mechanisms.(Williams & Schumacher, 2016)

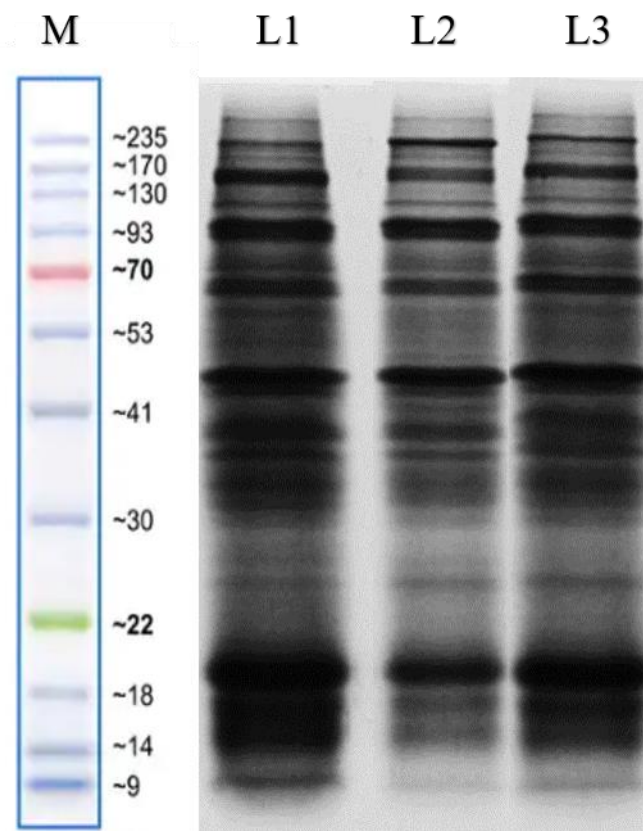


Fig 5.12 (a) SDS-PAGE results

L1. Control; L2. Violet; L3. Yellow

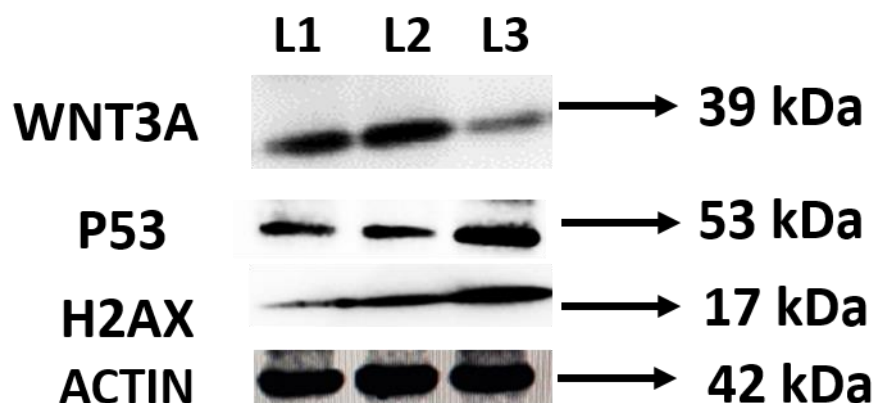


Fig 5.12 (b) Western Blotting results

L1. Control; L2. Yellow; L3. Violet

Our observations in this case indicate that the animal is stressed by the earthworm's amputation and subsequent regeneration. The same may apply to in-vitro research. The expression of H2AX is somewhat greater in yellow and maximum in violet when compared to control. This is due to violet's high levels of DNA damage and increased expression. When compared to control for WNT3a, violet exhibits the least expression while yellow exhibits the maximum. This is because, as was already established, violet earthworms repair wounds slowly, which suggests that there are less stem cells in the area than in the control earthworm, whose regeneration is normal. For yellow, this is the inverse situation. Greater expression results from more stem cells being present due to quicker wound healing.

Studies have shown that WNT3a signaling can affect DNA damage response and repair, which are processes in which H2AX is involved. For example, Wnt signaling can activate the expression of genes involved in DNA damage repair, such as Rad51 and Brca2. Additionally, H2AX can modulate the Wnt signaling pathway by regulating the expression of β -catenin, a key effector in the Wnt pathway. According to our results, the expression of WNT3a and H2AX is inversely proportional. While WNT3a is suppressed in violet, the expression is higher in H2AX. This indicates that both the proteins must be expressed at the same time for normal regeneration to occur, if not it will lead to abnormal regeneration of bud.

In case of p53, violet shows the maximum expression while yellow shows the least expression. This is due to increased stress experienced by earthworms in violet, which lies near to the UV region. Actin acts as a primary investigator to check whether there is any expression in the bud samples. So all the three samples have equal bands.

5.5 HISTOPATHOLOGICAL STUDIES OF *E.eugeniae*

Histology is the study of the microscopic structure of tissues and organs. There are several techniques that can be used to prepare tissue samples for histological analysis. The most common histology techniques are fixation, embedding, sectioning, staining, mounting and microscopy. The dyes used are hematoxylin and eosin. Hematoxylin is a purple-blue stain that exclusively stains only the tissue while eosin is a pink stain that stains only the proteins.

Histological studies reveal the structure and function of the circulatory and respiratory systems of *Eudrilus eugeniae*. The circulatory system consists of a dorsal blood vessel that runs the length of the body, along with a series of smaller vessels that supply

blood to various organs and tissues. The respiratory system consists of a network of moist, highly vascularized skin that allows for gas exchange with the environment.

There are 4 types of layers that is present in earthworms and are as listed below:

Earthworms have an outer layer of epithelial cells that makes up their skin. This layer of cells is called the epidermis and is made up of several types of cells, including gland cells, secretory cells, and sensory cells. The gland cells in the epidermis of earthworms produce mucus that helps keep the skin moist and provides a protective layer against physical and chemical damage. The secretory cells produce enzymes that are involved in breaking down food in the digestive system. The sensory cells are responsible for detecting touch, light, and chemicals in the environment. The epidermis of earthworms is also covered by a thin, transparent cuticle made of collagen. The cuticle provides additional protection against damage and helps prevent water loss from the earthworm's body.

The circular muscle layer of earthworms is one of the layers of muscles that are present in their body wall. This muscle layer is located just beneath the outermost layer of the skin and is responsible for the contraction and expansion of the body during movement. The circular muscles run around the circumference of the earthworm's body, forming a continuous layer. When these muscles contract, they become shorter in length, causing the body to become thinner and longer. This muscle action is important for helping the earthworm to move through soil and other substrates. The circular muscle layer is controlled by the nervous system of the earthworm, which sends signals to the muscles to contract or relax as needed. The nervous system also coordinates the movement of the earthworm's longitudinal muscles, which run along the length of its body, to produce coordinated movements and locomotion. In addition to movement, the circular muscle layer also plays a role in regulating the size and shape of the earthworm's body. For example, when the earthworm is at rest, the circular muscles may contract to reduce the size of the body and conserve energy. Conversely, when the earthworm needs to expand its body, such as during feeding, the circular muscles may relax to allow the body to expand.

The lumen of an earthworm refers to the central cavity or tube that runs the length of the worm's body. This tube is part of the digestive system and is lined with cells that secrete digestive enzymes and absorb nutrients from food. The lumen is divided into several distinct regions, each with its own specialized function. These regions include:

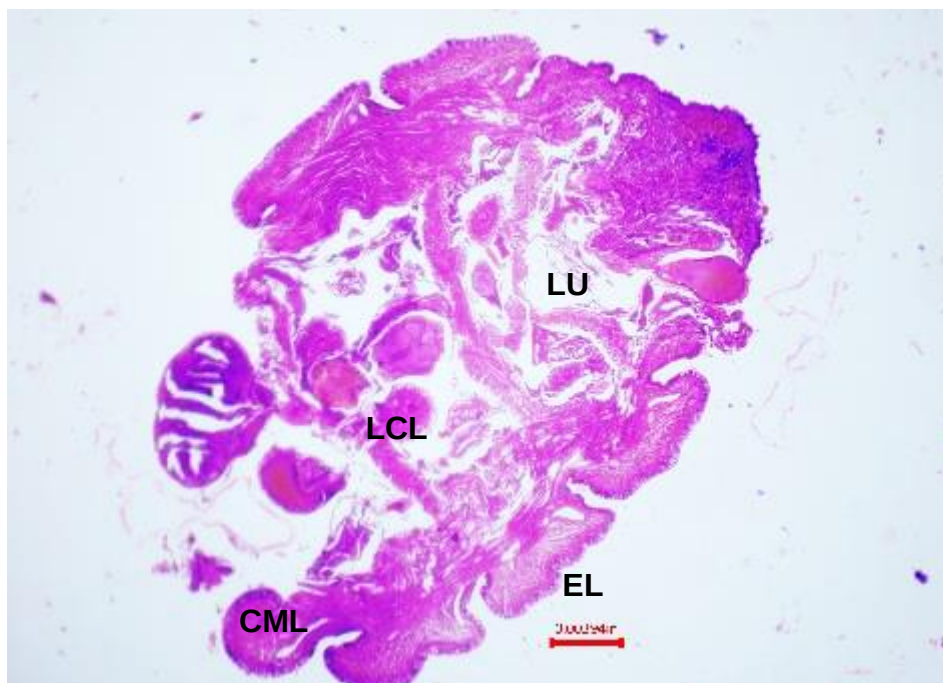
- Pharynx: The pharynx is the first region of the digestive system, located in the

head of the earthworm. It is a muscular tube that extends out of the mouth and helps to capture and grind up food.

- Esophagus: The esophagus is a short tube that connects the pharynx to the crop. It is lined with cells that secrete mucus to lubricate food and help it move through the digestive system.
- Crop: The crop is a thin-walled sac that stores food temporarily before it is passed on to the gizzard for grinding. The crop can expand to accommodate large amounts of food.
- Gizzard: The gizzard is a muscular organ that grinds up food using small stones and sand that the earthworm ingests. The walls of the gizzard are lined with cells that secrete digestive enzymes to further break down food.
- Intestine: The intestine is the longest part of the digestive system, and it is where most of the nutrients from food are absorbed into the bloodstream. The walls of the intestine are lined with cells that secrete digestive enzymes and absorb nutrients. The intestinal epithelium contains specialized cells called chloragocytes, which are thought to play a role in the detoxification of ingested materials.
- Anus: The anus is the final region of the digestive system, where undigested waste material is eliminated from the body.

The longitudinal cell layer of an earthworm is a layer of muscle cells located beneath the circular muscle layer in the body wall. These cells are oriented parallel to the long axis of the worm's body, and their contraction causes the body to elongate and shorten. The longitudinal muscle cells are smooth muscle cells, which means they lack the striations found in skeletal muscle cells. They are spindle-shaped cells with a single nucleus and are arranged in bundles running along the length of the earthworm's body. The cells are innervated by motor neurons and can contract independently of the circular muscle layer. The contraction of the longitudinal muscle layer helps to move the earthworm forward by extending the body in a wave-like motion. When the longitudinal muscle cells contract, they push the body forward, and when they relax, the circular muscle layer contracts, pulling the body forward. The combined action of these two muscle layers creates the characteristic undulating motion of the earthworm as it moves along the ground.

Fig 5.13 depicts the histology sectioning of the earthworm exposed under normal



(c) Histology sectioning of worm under yellow light

Fig 5.13 Histology sectioning of the 7th day bud of *E.eugeniae*

LU – Lumen; CML – Circular Muscle Layer; LCL – Longitudinal Muscle Layer; EL – Epithelial Layer

The epithelial layer, which is the outermost layer, determines the speed of regeneration, which also tells the rate of wound healing capacity. Both the longitudinal cell layer and the circular muscle layer are superbly characterized. These two layers are essential for regeneration when the worm is wounded because they cooperate to promote the growth of blastemal cells, which are produced when the longitudinal muscle layer elongates and/or expands to form the blastema. If the longitudinal layer is more, then that indicates that the speed of regeneration is more. As it is shown in Fig 5.13a, it is the control sample wherein the speed of regeneration is normal. When Fig 5.13b (violet light) and Fig 5.13c (yellow light) are compared, we can see that the longitudinal layer is less in violet compared to yellow which has a longitudinal layer greater than control as well as violet. So it can be suggested that the regeneration and wound healing capacity is the best in yellow light.

6. SUMMARY AND CONCLUSION

From the review of literature, it can be concluded that annelids, especially earthworms, have an amazing ability to regenerate. The regeneration is faster in earthworms than in other model organisms like *Danio rerio* (zebrafish), *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (nematode worm). On treatment with various wavelengths of visible light, it can be said that the earthworm *Eudrilus eugeniae* reacts differently to different wavelengths of light. Additionally, protein samples of the amputated bud segments are prepared. Gel electrophoresis, histology and western blotting techniques are carried out. The *H2AFX* gene, which produces the H2AX protein, and the WNT3a marker are used for the analysis of the regenerative potential of *E.eugeniae*.

We can find that the colour yellow (570 – 590 nm) is the most ideal light for wound healing and regeneration of the bud region. Violet (380 – 450 nm), being nearer to UV light, slows down regeneration of the bud. Histological results confirm the above statement.

As mentioned in a lot of reviews, bud formation post the amputation of the worm, as well as the partial or complete regeneration of the anterior (head) and the posterior (tail) segments of the earthworm can be easily observed in the earthworm *E.eugeniae*. It has a significantly higher amount of nutrients and proteins than other species of earthworms. This statistical data will be extrapolated for humans. Since prolonged exposure to UV light is dangerous for humans, visible light is chosen and its analysis is undertaken. The study is taken up to cure various ailments using regenerative medicine.

REFERENCES

- Anderson, F. E., Williams, B. W., Horn, K. M., Erséus, C., Halanych, K. M., Santos, S. R., & James, S. W. (2017). Phylogenomic analyses of Crassiclitellata support major Northern and Southern Hemisphere clades and a Pangaeian origin for earthworms. *BMC Evolutionary Biology*, 17(1), 1–18. <https://doi.org/10.1186/S12862-017-0973-4/FIGURES/4>
- Aztekin, C., Mekayla, J., Storer, A., & Storer, M. A. (2022). To regenerate or not to regenerate: Vertebrate model organisms of regeneration-competency and -incompetency. *Wound Repair and Regeneration*, 30(6), 623–635. <https://doi.org/10.1111/WRR.13000>
- Bae, J. M., Jung, H. M., Hong, B. Y., Lee, J. H., Choi, W. J., Lee, J. H., & Kim, G. M. (2017). Phototherapy for vitiligo: A systematic review and meta-analysis. *JAMA Dermatology*, 153(7), 666–674. <https://doi.org/10.1001/jamadermatol.2017.0002>
- Barros, N. de M., Sbroglio, L. L., Buffara, M. de O., Baka, J. L. C. e. S., Pessoa, A. de S., & Azulay-Abulafia, L. (2021). Phototherapy. *Anais Brasileiros de Dermatologia*, 96(4), 397–407. <https://doi.org/10.1016/j.abd.2021.03.001>
- Blakemore Vermecology, R. J. (2015). Eco-taxonomic profile of an iconic vermicomposter-the “African Nightcrawler” earthworm, *Eudrilus eugeniae* (Kinberg, 1867). *African Invertebrates*, 56(3), 527–548. <https://doi.org/10.10520/EJC181560>
- BRÜCKE, J., TANEW, A., ORTEL, B., HÖNIGSMANN, H., Lahti, A., Mester, E., Mester, A. F. A., Mester, A. F. A., HÖNIGSMANN, H., Barros, N. de M., Sbroglio, L. L., Buffara, M. de O., Baka, J. L. C. e. S., Pessoa, A. de S., Azulay-Abulafia, L., FARR, P. M., DIFFEY, B. L., HIGGINS, E. M., MATTHEWS, J. N. S., ... Chen, X. (2019). Phototherapy meets immunotherapy: A win-win strategy to fight against cancer. *ACS Nano*, 13(12), 11967–11980. <https://doi.org/10.1021/acsnano.9b06040>
- Chaudhuri, P. (2022). *UTTAR PRADESH JOURNAL OF ZOOLOGY NEUROSECRETION IN NEUROSECRETION IN OLIGOCHAETES - A LESS STUDIED SUBJECT IN INVERTEBRATE RESEARCH*. April.
- Chen, Q. Y., Costa, M., & Sun, H. (2015). Structure and function of histone acetyltransferase MOF. *AIMS Biophysics*, 2(4), 555–569. <https://doi.org/10.3934/biophy.2015.4.555>

- Durand, F. (1963). *Emergence as cornerstone in understanding evolution: examples from zoology*.
- Friend, H. (1919). Luminous Worms. *Nature* 1919 104:2613, 104(2613), 334–335. <https://doi.org/10.1038/104334f0>
- Gopi Daisy, N., Subramanian, E. R., Selvan Christyraj, J. D., Sudalai Mani, D. K., Selvan Christyraj, J. R. S., Ramamoorthy, K., Arumugaswami, V., & Sivasubramaniam, S. (2016). Studies on regeneration of central nervous system and social ability of the earthworm *Eudrilus eugeniae*. *Invertebrate Neuroscience* 2016 16:3, 16(3), 1–13. <https://doi.org/10.1007/S10158-016-0189-0>
- Hong, J. Y., Kim, M. H., Lee, J. H., Han, H. S., Seo, S. J., Park, K. Y., & Park, Y. M. (2020). Phototherapy May be a Useful Adjuvant Therapy for Retractable Chronic Spontaneous Urticaria: A Systematic Review. *Photochemistry and Photobiology*, 96(4), 738–740. <https://doi.org/10.1111/php.13260>
- Hönigsmann, H. (2013). Phototherapy. *Journal of Investigative Dermatology*, 133, E18–E20. <https://doi.org/10.1038/skinbio.2013.180>
- Hönigsmann, H., Szeimies, R., & Knobler, R. (2016). *Oral photochemotherapy with psoralens and UVA (PUVA): Principles and practice*. January.
- Johnson Retnaraj Samuel, S. C., Elaiya Raja, S., Beryl Vedha, Y., Edith Arul Jane, A., Amutha, K., Dinesh, S. M., Jackson Durairaj, S. C., Kalidas, R. M., Tharmaraj, V., Pitchumani, K., & Sudhakar, S. (2012). Autofluorescence in BrdU-positive cells and augmentation of regeneration kinetics by riboflavin. *Stem Cells and Development*, 21(11), 2071–2083. <https://doi.org/10.1089/scd.2011.0485>
- Kode, J. A., Mukherjee, S., Joglekar, M. V., & Hardikar, A. A. (2009). Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. *Cytotherapy*, 11(4), 377–391. <https://doi.org/10.1080/14653240903080367>
- Ma, Y., Zhang, Y., Li, X., Zhao, Y., Li, M., Jiang, W., Tang, X., Dou, J., Lu, L., Wang, F., & Wang, Y. (2019). Near-infrared II phototherapy induces deep tissue immunogenic cell death and potentiates cancer immunotherapy. *ACS Nano*, 13(10), 11967–11980. <https://doi.org/10.1021/acsnano.9b06040>
- Marti, T. M., Hefner, E., Feeney, L., Natale, V., & Cleaver, J. E. (2006). H2AX phosphorylation within the G1 phase after UV irradiation depends on nucleotide excision repair and not DNA double-strand breaks. *Proceedings of the National Academy of Sciences of the United States of America*, 103(26), 9891–9896.

- https://doi.org/10.1073/PNAS.0603779103/SUPPL_FILE/03779FIG9.PDF
- Matapurkar, B., & Rehan, H. (2023). New technique for repair of complex genitourinary rectal fistulae using peritoneum for urethral reconstruction and as an interpositional tissue. *Indian Journal of Urology*, 13(1), 36. <https://www.indianjurol.com/article.asp?issn=0970-1591>
- Moon, K. T., Brown, J. D., & Torres, M. (1997). WNTs modulate cell fate and behavior during vertebrate development. *Trends in Genetics*, 13(4), 157–162. [https://doi.org/10.1016/S0168-9525\(97\)01093-7](https://doi.org/10.1016/S0168-9525(97)01093-7)
- O'Driscoll, M., Ruiz-Perez, V. L., Woods, C. G., Jeggo, P. A., & Goodship, J. A. (2003). A splicing mutation affecting expression of ataxia–telangiectasia and Rad3–related protein (ATR) results in Seckel syndrome. *Nature Genetics* 2003 33:4, 33(4), 497–501. <https://doi.org/10.1038/ng1129>
- Owlarn, S., Klenner, F., Schmidt, D., Rabert, F., Tomasso, A., Reuter, H., Mulaw, M. A., Moritz, S., Gentile, L., Weidinger, G., & Bartscherer, K. (2017). Generic wound signals initiate regeneration in missing-tissue contexts. *Nature Communications* 2017 8:1, 8(1), 1–13. <https://doi.org/10.1038/s41467-017-02338-x>
- Paul, S., Balakrishnan, S., Arumugaperumal, A., Lathakumari, S., Syamala, S. S., Arumugaswami, V., & Sivasubramaniam, S. (2021). The transcriptome of anterior regeneration in earthworm *Eudrilus eugeniae*. *Molecular Biology Reports*, 48(1), 259–283. <https://doi.org/10.1007/s11033-020-06044-8>
- Pithawala, N. A., & Jain, B.. (2012). Journal of Advanced Scientific Research.of *Advanced Scientific* 1(2), 19–23. http://www.sciensage.info/journal/1359303580JASR_3006121.pdf
- Poss, K. D., Keating, M. T., & Nechiporuk, A. (2003). Tales of regeneration in zebrafish. *Developmental Dynamics*, 226(2), 202–210. <https://doi.org/10.1002/DVDY.10220>
- Rajesh, C., Kannan Balaji, S., Ramesh, P., Selvapalam, N., & Palanichelvam, K. (2021). Three Antimitotic Compounds Found in Banana Pseudo-Stem By Using Earthworms. <https://doi.org/10.21203/rs.3.rs-1170280/v1>
- Rajiv, P., Rajeshwari, S., & Rajendran, V. (2014). Impact of Parthenium weeds on earthworms (*Eudrilus eugeniae*) during vermicomposting. *Environmental Science and Pollution Research* 2014 21:21, 21(21), 12364–12371. <https://doi.org/10.1007/S11356-014-3149-4>
- Ramirez, A. N., Loubet-Senear, K., & Srivastava, M. (2020). A Regulatory Program for

- Initiation of Wnt Signaling during Posterior Regeneration. *Cell Reports*, 32(9), 108098. <https://doi.org/10.1016/j.celrep.2020.108098>
- Rodionova, N. S., Rota, E., Tsarkova, A. S., & Petushkov, V. N. (2017). Progress in the Study of Bioluminescent Earthworms. *Photochemistry and Photobiology*, 93(2), 416–428. <https://doi.org/10.1111/PHP.12709>
- Rogakou, E. P., Nieves-Neira, W., Boon, C., Pommier, Y., & Bonner, W. M. (2000). Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *Journal of Biological Chemistry*, 275(13), 9390–9395. <https://doi.org/10.1074/jbc.275.13.9390>
- Saint-Denis, M., Narbonne, J. F., Arnaud, C., Thybaud, E., & Ribera, D. (1999). Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil: effects of benzo(a)pyrene. *Soil Biology and Biochemistry*, 31(13), 1837–1846. [https://doi.org/10.1016/S0038-0717\(99\)00106-6](https://doi.org/10.1016/S0038-0717(99)00106-6)
- Sedelnikova, O. A., & Pilch, D. R. (2011). Molecular Determinants of Radiation Response. *Molecular Determinants of Radiation Response*, February, 1–4. <https://doi.org/10.1007/978-1-4419-8044-1>
- Selvan Christyraj, J. D., Azhagesan, A., Ganesan, M., Subbiah Nadar Chelladurai, K., Paulraj, V. D., & Selvan Christyraj, J. R. S. (2020). Understanding the Role of the Clitellum in the Regeneration Events of the Earthworm *Eudrilus eugeniae*. *Cells Tissues Organs*, 208(3–4), 134–141. <https://doi.org/10.1159/000507243>
- Sikes, J. M., & Newmark, P. A. (2013). Restoration of anterior regeneration in a planarian with limited regenerative ability. *Nature* 2013 500:7460, 500(7460), 77–80. <https://doi.org/10.1038/nature12403>
- Sivasubramaniam, S. (2021). The Earthworm *Eudrilus Eugeniae*: a Model Organism for Regenerative Biology. *Genetics & Genomic Sciences*, 6(1), 1–4. <https://doi.org/10.24966/ggs-2485/100023>
- Solier, S., Sordet, O., Kohn, K. W., & Pommier, Y. (2009). Death Receptor-Induced Activation of the Chk2- and Histone H2AX-Associated DNA Damage Response Pathways. *Molecular and Cellular Biology*, 29(1), 68–82. <https://doi.org/10.1128/MCB.00581-08/ASSET/56B7D5A9-CC44-4700-AEEF-C9FA1DF7536C>
- Srivastava, N., Gochhait, S., de Boer, P., & Bamezai, R. N. K. (2009). Role of H2AX in DNA damage response and human cancers. *Mutation Research/Reviews in*

<https://doi.org/10.1016/J.MRREV.2008.08.003>

- Sudalaimani, D., Retnaraj Samuel Selvan Christyraj, J., Ramamoorthy, K. M., Gopi, N. D., Durairaj Selvan Christyraj, J., Renganathan, K., Krishnan, S., Sivasubramaniam, S., Biol, T. J., Raja SUBRAMANIAN, E., Kumar SUDALAIMANI, D., Retnaraj Samuel SELVAN CHRISTYRAJ, J., Ramamoorthy, K., Gopi Daisy, N., & Durairaj SELVAN CHRISTYRAJ, J. (2017). Studies on organogenesis during regeneration in the earthworm, *Eudrilus eugeniae*, in support of symbiotic association with *Bacillus endophyticus*. *Turkish Journal of Biology*, 41(1), 113–126. <https://doi.org/10.3906/biy-1604-44>
- Tanaka, E. M., & Ferretti, P. (2009). Considering the evolution of regeneration in the central nervous system. *Nature Reviews Neuroscience* 2009 10:10, 10(10), 713–723. <https://doi.org/10.1038/nrn2707>
- Tian, R., Zeng, Q., Zhu, S., Lau, J., Chandra, S., Ertsey, R., Hettie, K. S., Teraphongphom, T., Hu, Z., Niu, G., Kiesewetter, D. O., Sun, H., Zhang, X., Antaris, A. L., Brooks, B. R., & Chen, X. (2019). Albumin-chaperoned cyanine dye yields superbright NIR-II fluorophore with enhanced pharmacokinetics. *Science Advances*, 5(9), 1–12. <https://doi.org/10.1126/sciadv.aaw0672>
- Turinetto, V., & Giachino, C. (2015). Multiple facets of histone variant H2AX: a DNA double-strand-break marker with several biological functions. *Nucleic Acids Research*, 43(5), 2489–2498. <https://doi.org/10.1093/NAR/GKV061>
- Vacanti, J.P., Cima, L.G. and Cima, M. . (1995). *Vascularized tissue regeneration matrices formed by solid free form fabrication techniques*.
- Williams, A. B., & Schumacher, B. (2016). p53 in the DNA-Damage-Repair Process. *Cold Spring Harbor Perspectives in Medicine*, 6(5). <https://doi.org/10.1101/CSHPERSPECT.A026070>

ANNEXURE

SUBMISSION OF REVIEW ARTICLE TITLED “Phototherapy – An emerging strategy in inducing cell response” in Cells Tissues Organs journal (IF: 2.20)

Status: In review

CTO-2023-1-1 Co-author manuscript submission confirmation

Inbox xUpdates x

Cells Tissues Organs <cto@manuscriptmanager.net>
to me

Tue, Jan 3, 3:00 PM

Manuscript: CTO-2023-1-1 - PHOTOTHERAPY – AN EMERGING STRATEGY IN INDUCING CELL RESPONSE
Authors: Srishti Raja (Co-author), Chandini Sengupta (Co-author), Jackson Durairaj Selvan Christyraj (Corresponding Author), Kamarajan Rajagopalan (Co-author), Inbathamizh L (Co-author), Sudha S (Co-author)
Date submitted: 2023-01-03

Dear Miss Raja

The above manuscript has been submitted online by Dr. Selvan Christyraj and you have been listed as a co-author.

Cells Tissues Organs

Karger

Srishti Raja is Author [CHANGE ROLE]

DASHBOARD

PROFILE

[SIGN OUT]

Dashboard

Submitting and resubmitting manuscripts
Read more ...

<https://orcid.org/0000-0003-0136-2073>

	Submission/Title/Type	Status	Action
Delete [Author files]	Confirmed as: Co-author Manuscript ID: CTO-2023-1-1 PHOTOTHERAPY – AN EMERGING STRATEGY IN INDUCING CELL RESPONSE Type: Review Article Authors: Srishti Raja (Co-author), Chandini Sengupta (Co-author), Jackson Durairaj Selvan Christyraj (Corresponding Author), Kamarajan Rajagopalan (Co-author), Inbathamizh L (Co-author), Sudha S (Co-author) Submitted: 2023-01-03	IN REVIEW	

Correspondence

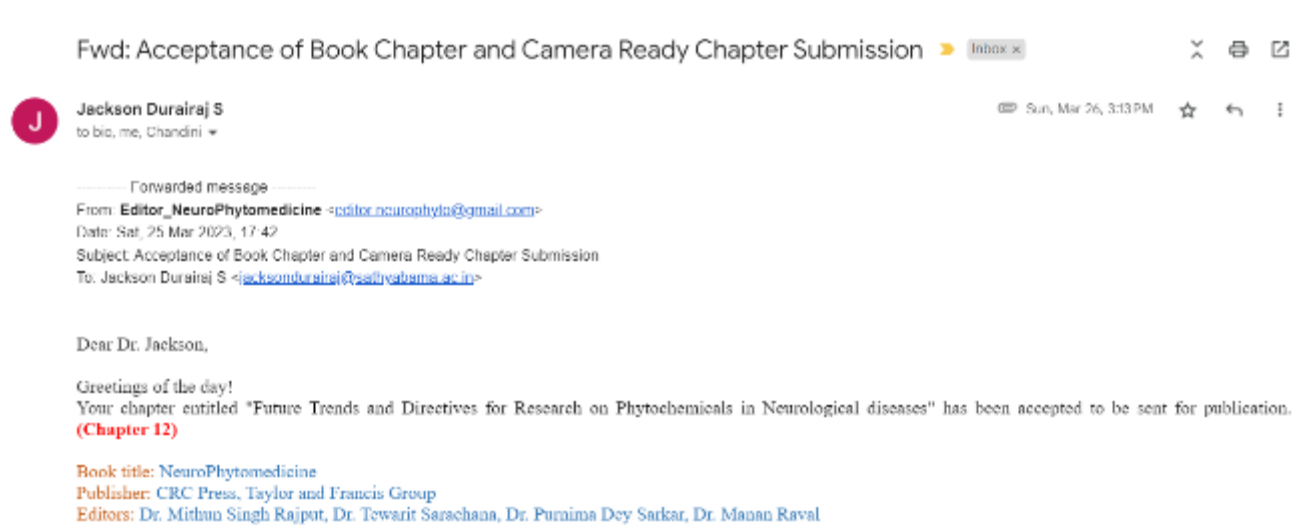
See messages sent to you

Company
About
Contact

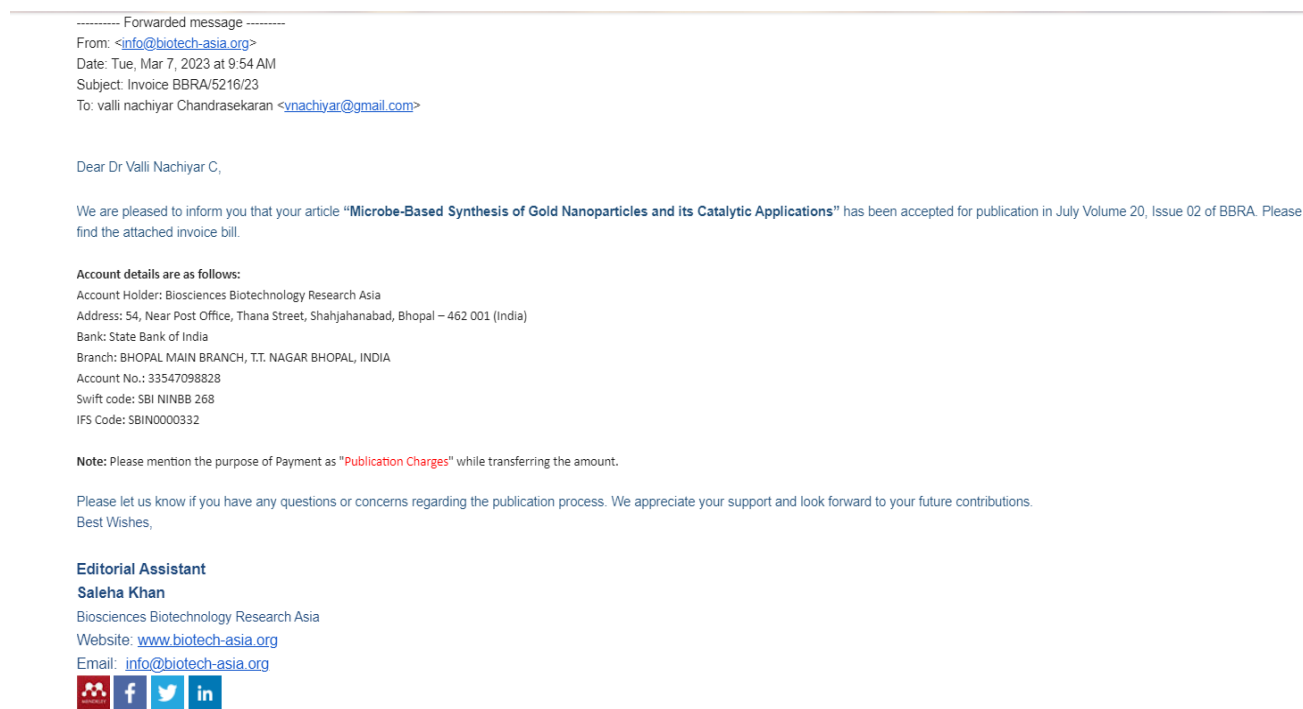
Support
ONLINE HELP
ASK A QUESTION
[Contact Us](#)

Session
Srishti Raja
Role: Author
[Sign out]

ACCEPTANCE OF BOOK CHAPTER TITLED “Future trends and Directives for Research on Phytochemicals in Neurological diseases” through Publisher: CRC Press, Taylor and Francis group



ACCEPTANCE OF BOOK CHAPTER TITLED “Microbe-Based Synthesis of Gold Nanoparticles and its Catalytic Applications” through Publisher: Bioscience Biotechnology Research Asia



PLAGIARISM REPORT



Document Information

Analyzed document	Srishti thesis final.docx (D147584134)
Submitted	2023-04-24 18:10:00
Submitted by	Dr Jackson Durairaj
Submitter email	jacksondurairaj@sathyabama.ac.in
Similarity	2%
Analysis address	jacksondurairaj.sathya@analysis.urkund.com

W

URL: https://www.researchgate.net/publication/10868024_Narrowband_UV-B_TL-01_Phototherapy_vs_Oral_8-Methoxypsoralen_Psoralen-UV-A_for_the_Treatment_of_Chronic_Plaque_Psoriasis
Fetched: 2020-10-18 02:01:14

1

W

URL: <https://www.dovepress.com/management-of-atopic-dermatitis-safety-and-efficacy-of-phototherapy-peer-reviewed-fulltext-article-CCID>
Fetched: 2021-12-10 13:12:19

2

57%

MATCHING BLOCK 1/30

W

NIR-II light may cause a more uniform production and circulation of DAMPs in the deeper regions of the

tumours. The NPs facilitate the photothermal effect to immediately eliminate the tumour upon NIR-II light irradiation and