

**LABORATORY SETTING TO INVESTIGATE
THE PROTECTIVE EFFECTS OF *EISENIA
FOETIDA* COELOMIC FLUID IN BREAST
CANCER CELLS**

A thesis Submitted by

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BSZGM-F20-016



SUPERIOR UNIVERSITY

BS ZOOLOGY

**Department of Biological Sciences
Faculty of Sciences
Superior University Lahore**

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SUPERIOR UNIVERSITY

**In the Partial Fulfillment for the Award of
BS Zoology**

Supervisor: Dr. Usman Elahi

**Department of Biological Sciences
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2024**

UNDERTAKING BY STUDENT

I Sayed Akhtar Regd. No. bszgm-f20-016 declare that the contents of my research project entitled “LABORATORY SETTING TO INVESTIGATE THE PROTECTIVE EFFECTS OF *EISENIA FOETIDA* COELOMIC FLUID IN BREAST CANCER CELLS” are based on my own research findings and have not been taken from any other work except the references and has not been published before.

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SUPERVISOR CERTIFICATE

I Usman Elahi certify that the contents and the form of research project submitted by Sayed Akhtar Regd. No. bszgm-f20-016 have been found satisfactory. I hereby accept the full responsibility of the supervisor ship of mentioned above student and recommend it for the award of the degree of bs zoology).

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Declaration of Originality

I, Sayeda Akhtar, solemnly affirm the following about this project:

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Dedicated To My Family

In the world of academically-minded individuals, where the pursuit of explanation is often a solitary one, to ascribe one's research to his or her family is a influential gesture. This gesture is one of the solid support and inspiration constantly coming from the family's way, creating an environment of cultivation around the intelligence. It is also a gesture of gratitude – it concedes that any successes claimed by the paper are not the academician's own, but rather a collective one. Furthermore, it is to acknowledge that the family's support is not just emotional – many times they have shared in the struggle, the triumph, and the creation of new knowledge. This expresses the idea that "this is not mine - this is ours" while also feeling a lot like an emotional credit. This moving tribute serves as a reminder that we all deserve credit for this, not just I. In many respects, it serves as a poignant reminder to always remember the rich, beautiful, and resilient pattern of family and love that lies underneath the vast surface of survey and study.

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LIST OF ABBREVIATIONS

ECF	Earthworm coelomic fluid
CF	Coelomic fluid
NAEs	Nature available extracts
NSCLC	Non-small cell lung cancer

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Abstract

Earthworms are tubular, metameric worms then have a dual conveyance organization involving of earthworm coelomic fluid (ECF) poignant inside the filled tube in their body. As one of the by nature available natural extracts (NAEs), ECF applies numerous biotic ways, such as hindering the propagation of cancer cells. Recent research has target on the anticancer activity of different areas of earthworms as a new probable anticancer agent. In topical times, obviously happening constituents such as extracts from earthworms have effectively been used to treat wounds by fighting against microbes (germs) and reducing inflammation. It has also shown auspicious antitumor activity in cancer. This study aims to evaluate the disinfectant activity of worms in contradiction of two cell lines, breast cancer (MCF-7). ECf has been shown to have antitumor effects on breast, liver, colon, and brain cancers, but has not been acceptably intentional verbally.

CHAPTER NO I

Introduction

Worms are multipart invertebrates, many defensive disease particles. They can association and changed types of white blood cells can be twisted. They have some dwindling system-related actions (rejection of allodapic tissue) as well as protection in the body. Earthworms are usually found in moist, compost-rich soil and feed on a variability of carbon-based matter, counting debris, live protozoa, rotifers, nematodes, bacteria, fungi and other creatures [1] [2].

Worms have been extensively used in antique Chinese medicine for thousands of years. However, with the expansion of biochemical knowledge in current years, research on the medical use of soil bacteria has not commenced. Fibrinolytic enzymes were first unapproachable from soil bacteria in the 1980s. Coelomic fluid contains aqueous plasma containing proteins, salts, and small bodies such as phagocytes, white blood cells, and lymph nodes. Earthworm coelomic fluid (CF) has many molecular elements and cells that play a part in the immune system. Lectin-like glycoproteins bind to carbohydrates to recognize foreign material and then immobilize and destroy it by agglutination and lysis [3][5].

The fluid inside the earthworm, called coelomic fluid, encompasses several beneficial materials like proteins. This fluid has different effects on living things, such as fighting bacteria, preventing cancer, breaking down blood cells, being toxic to cells, causing blood clumping, and breaking down proteins. Among the approaches used to collect this fluid from earthworms, the cold shock method is a new and practical approach. It doesn't need any special equipment, and importantly, the earthworms used in this method remain unharmed and continue their normal activities even after the fluid is collected multiple times. [4][6].

Cancer is a significant health problem worldwide, causing illness and death. To reduce the death rate from cancer, numerous effective treatments are used, counting surgery, chemotherapy, radiation, and immunotherapy. In recent years, with advancements in biotechnology, researchers have been working on emerging handlings that can destroy cancer cells while causing minimal destruction to healthy cells. Many studies have explored the potential anti-cancer effects of extracts from earthworms. Extracts from the entire body of earthworms have been found to slow

Down the growth of cancer cells in the lab and prevent the development of cancer in living organisms. Lessons have also shown that vermination can increase the efficiency of chemotherapy and radiation therapy [7].

From sympathetic cancer, it is clear that the incessant development and construction of cancer cells is a composite procedure involving of numerous events. Throughout the development of malicious tumors. In fresh years, natural singularities such as worm extract have involved consideration for their role in averting the propagation and partition of cancer cells, production this a fascinating eight-host for investigate. Current research needs to be deliberate to organize biomolecules accomplished of constraining cancer cells. Determining how to avoid the growth of cancer cells subversive could be the first step in emerging treatment and cancer treatment in the future. Consequently, this study intention to control the anti-cancer effect of earth bacteria on cancer over a qualitative analysis, concentrating on the features of the extracts and cancer representations used [5].

1 of the chief growths is lung malignance, characterized by lung cancer. Most non-small cell lung cancer (NSCLC), counting lung glandular carcinoma, is caused by acquaintance to toxins in the sniff, smoking (secondhand smoke), and other toxins. In today's significant atmosphere, the main elements in the air that have a negative effect on the lungs remain sulfur oxides, nitrogen oxides or dirt slighter than two or three micrometers in diameter. CF is an exceptional grounding encompasses substances like metabolites, proteins, enzymes, peptides, and polysaccharides are found in the fluid inside an earthworm, soil bacteria exhibitions numerous biological happenings such as antibacterial, proteolysis, hemolytic, hemagglutination, fungicidal and anticancer activities.

Even though 5-10% of tumor is triggered by inheritances, 90-95% of tumor progression is due to atmosphere, liquor/smoking, overweightness, lack of diet, lack of carnal movement and poor routine (such as high figure mass). Breast cancer is one of the maximum communal cancers in women in current years. With the inspiration of lifestyle, genetics, environment and other factors, breast cancer has become the leading cause of death. The pathogenesis of breast cancer is long-lasting procedure. Diet and superiority of lifetime serve as carcinogenic hazard modulators in the progress and evolution of cancer [9].

OBJECTIVES

The current study aims to explore the anti-growth effects of earthworm coelomic fluid (ECF). This involves investigating the anti-cancer activity of the fluid in living organisms, which is crucial for understanding its effects both inside the body (in vivo) and in controlled laboratory settings (in vitro).

CHAPTER NO II

Literature review

Earthworms, scientifically known as Crassicitellata, are a type of oligochaetes that inhabit the soil. In numerous temperate ecosystems, they constitute the largest portion of animal biomass. They play a crucial role in shaping the physical, chemical, and biological properties of the soil [1].

Earthworms obtain their nutrition from a variety of organic sources. These include fallen leaves, decaying plants, and other organic debris. Additionally, they feed on microorganisms such as bacteria, fungi, and algae [1].

Earthworms, belonging to the class Annelida, exhibit a fascinating body plan. Earthworms are segmented worms with identical right and left sides, displaying bilateral symmetry. Each segment contains specific structures, including the clitellum (an external gland) responsible for producing the egg case or cocoon. Earthworms are hermaphroditic, meaning both male and female reproductive organs exist within the same individual. Their reproductive process involves the formation of egg cocoons. A sensory lobe (prostomium) is located in front of the mouth. The mouth is positioned at the front end, while the anus is at the tail end of the animal. Earthworms lack limbs but possess a small number of bristles called setae on each segment. Their body follows a remarkable “tube within a tube” arrangement [10].

Earthworms, those fascinating invertebrates, synthesize a variety of immunoprotective molecules and produce different types of leukocytes. They possess both innate immunity and some functions associated with adaptive immunity (such as allogeneic tissue rejection).

These molecules exhibit diverse activities, including fibrinolytic, anticoagulative, anticancer, and antimicrobial properties. As a result, they hold promise for treating various diseases.

Coelomic fluid, found within the coelom of earthworms, is a milky white, dense, and gelatinous substance. It is secreted by the earthworms themselves. This fluid contains water plasma, along with proteins, salts, and various cellular components such as phagocytes, leukocytes, and mucocytes. Notably, the coelomic fluid plays a crucial role in facilitating the locomotion of earthworms [5].

Earthworm coelomic fluid (CF), composed of numerous molecular elements and cells, plays a crucial role in innate immunity. Lectin-like glycoproteins within this fluid bind to carbohydrates, allowing them to recognize foreign materials. Subsequently, they immobilize and destroy these invaders through processes like agglutination and lysis. The biological functions of earthworm coelomic fluid include bacteriostatic, proteolytic, cytolytic, and mitogenic activities [11].

Notably, research has demonstrated that earthworm coelomic fluid has antiproliferative effects against cancers affecting different organs, such as breast, liver, digestive tract, and brain. Natural origin substances play a crucial role as medicinal compounds and have been utilized for centuries to treat various ailments. Among these sources, earthworm coelomic fluid (CF) stands out as a potential reservoir of valuable bioactive molecules [12].

While earthworm coelomic fluid shows promise as an anticancer agent, its non-selective activity affects both cancer cells and normal cells. For instance, a study found that coelomic fluid extracted from the earthworm species *Eisenia foetida* exhibited anti-proliferative effects on breast cancer cells, potentially by inducing apoptosis or programmed cell death [13].

Breast cancer, which ranks as the second most frequent cancer among women globally, is most common in developed nations. It was projected that by 2000, breast cancer would be responsible for about 500,000 deaths each year. A lot of literature exists on the risk factors that increase the likelihood of breast cancer, but there is less emphasis on methods to mitigate these risks and prevent the disease. Prevention is a complex task because many of the risk factors are internal (endogenous) and hence, hard to control. Notably, the incidence rates of breast cancer specific to age groups escalate quickly with age. However, unlike other prevalent cancers, this rate of increase slows down after the age of 50, typically coinciding with menopause [14].

The risk of breast cancer is significantly influenced by family history. Relatives often have shared genetic and environmental factors. The risk of breast cancer increases two to three-fold if a first-degree relative (parent, sibling, or child) is affected. The risk also increases, though less so, if a second-degree relative (aunt, uncle, or grandparent) is affected. The risk is further heightened if two first-degree relatives are affected, if the relative has cancer in both breasts (bilateral breast cancer), or if the

relative was diagnosed before the age of 40-45. The risk is particularly pronounced for younger women if a first-degree relative was diagnosed at an early age. However, after the age of 50, the high-risk period for familial disease decreases. An early onset of breast cancer is a potent sign of genetic predisposition. Family history accounts for about 10-15% of breast cancer cases, with about half of this risk associated with genes that are dominantly inherited. [14].

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related deaths among women. It represents 23% of all cancer diagnoses and 14% of all cancer-related deaths. Therefore, research in this area is vital to reduce the economic and psychological impacts of breast cancer. The MCF-7 cell line, a commonly used breast cancer cell line, has been the focus of numerous research groups for over forty years. In 1973, Dr. Soule and his team at the Michigan Cancer Foundation developed the MCF-7 cell line. These cells were derived from the pleural effusion of a 69-year-old woman with metastatic disease. [15].

Earthworms, also known as Dilong, have a rich history in traditional Chinese medicine (TCM) and other ancient cultures. In Burma, people applied ashes from burnt earthworms to alleviate fever symptoms. In Laos, earthworms were used to treat smallpox. Ancient Chinese medicine employed earthworms to ease conditions such as fever-associated convulsions, hemiplegia, and blood clots. Contemporary studies have revealed beneficial effects of earthworms in both in vitro (laboratory) and in vivo (living organism) models [16]

Its advantages include easy availability, low cost, and minimal side effects. Given its medical value, pharmacological significance, and cellular effects, investigating its curative potential against the toxic effects of cancer drugs is worthwhile [17].

Sodium butyrate (NaBu), a byproduct of dietary fiber digestion in humans, serves as a treatment agent for breast cancer, which is the most common cancer among women worldwide. As a histone deacetylase inhibitor, NaBu has been found to suppress growth and induce structural changes in breast cancer cell lines, resulting in halted growth, differentiation, and cell death. Exploring protective effect of coelomic fluid from *Eisenia foetida* against the toxicity of NaBu in breast cancer. We examined the cytotoxic and protective effects of this fluid on the survival of breast cancer cells and its role in mitigating the oxidative toxicity caused by NaBu in MCF-7 cells, including

Alterations in ROS production and the expression of genes associated with cell death and ROS [18].

CHAPTER NO III

METHODOLOGY

Research design

Earthworms, specifically *Eisenia foetida*, were bought from a commercial supplier. These worms were raised and fed in a compost box unit with cattle manure under specific conditions: 65% humidity, darkness, and a temperature of 25 °C. Before collecting their fluid, about 400 mg of earthworms were placed on damp filter paper to clear their digestive tracts. The coelomic fluid was then collected using an electric shock method.

Clinical setting

Humane treatment of earthworms, appropriate living conditions, balanced nutrition, avoiding harmful substances, monitoring and intervention.

Sample size

Approximately 400 mg of earthworm was placed in a container and kitchen waste, vegetables fruits as a food source.

Sample technique

Method of collection of coelomic fluid

Cold shock method

The earthworms will be imperiled to cold shock by ice wadding, and then the fluid will be composed in a dehydrated fresh test tube. In the cold shock process, the worms will be alive and vigorous, however they will secrete a moderately greater volume of fluid (1.5 ml) than extra procedures. The liquid collected will be clear brown in color deprived of any remains, as seen in the heat and electric shock process.

Duration of study

Adult earthworms will be fed on kitchen waste for about 3 months. To reproduce their cocoon till the next life cycle, case study maybe prolonged for about 91 days.

Selection criteria

Adaptability, rapid reproduction rate, feeding habits of *Eisenia foetida*, ease of handling.

Inclusion criteria

Climate suitability, substrate availability, adequate space, moisture control, ventilation, pH level, protection from predators, light avoidance, waste management.

Data collection procedure

Human sources, articles, internet.

Ethical considerations

Ethical handling of earthworms, suitable habitat conditions, well-rounded diet, steering clear of detrimental substances, and vigilant observation and action.

Materials and methods

***Eisenia foetida* coelomic fluid preparations**

Earthworms of the species *Eisenia foetida* was cared for and fed in livestock dung within an EkosolFarm Compost Box unit. It was done under specific conditions: 65% relative humidity, a temperature of 25°C, and in darkness. These worms were obtained from a commercial company. Before collecting their fluid (ECF), the worms were gathered using the cold shock method, transferring them to damp filter paper to empty their digestive tracts. The collected ECF was then undergoing pasteurization through filtration (0.22 mm diameter) and centrifugation at 1000 rpm for 5 minutes at 4°C. The protein concentration of the collected ECF was measured using the Qubit protein assay kit and stored at -20°C for further research.



Figure 1 Feeding of earthworm



Figure 2 Eggs of earthworm

Conditions of cell culture

The study used MCF-7 breast cancer cells to assess how well ECF protected against cell damage caused by a substance called NaBu. These MCF-7 cells were obtained commercially from the American Type Culture Collection (ATCC). The cells were kept in a specific cell culture medium called Dulbecco's Modified Eagle Medium (DMEM), which included 0.1% penicillin and streptomycin, 10% fetal bovine serum (FBS), and they were grown at 37°C with 5% carbon dioxide (CO₂). In simpler terms, the research used breast cancer cells to see how effective ECF was in protecting against damage caused by NaBu. The cells were obtained from a commercial source and were grown in a specific medium under controlled conditions for the experiments.

NaBu exposure and pre - treatment with ECF

To evaluate the impact of ECF on MCF-7 breast cancer cells, the WST-1 assay was utilized. Initially, a certain number of cells were placed in 96-well plates (1x10⁴ cells/well) and were allowed to grow for 24 hours. Subsequently, varying concentrations of ECF (1, 5, 10, 20, and 40 µg/mL) were introduced to the cells and incubated for 24 and 48 hours at 37 °C with 5% CO₂. After this incubation period, a substance known as WST-1 reagent was added to each well and left for 40 minutes. The optical density of the solution was then measured at 450 nm using a microplate reader.

In a similar manner, another batch of cells was placed in 96-well plates (1 × 10⁴ cells/well) and was allowed to grow for 24 hours. Following this, the cells were treated with different concentrations of NaBu (0.5, 1, 2, 3, 4, and 8 nM) for 24 and 48 hours at 37 °C with 5% CO₂. The cell viability was then assessed using the WST-1

assay. In a pre-treatment approach, MCF-7 cells were pre-treated with ECF (1, 5, and 10 $\mu\text{g/mL}$) for 24 hours prior to exposure to NaBu (2 and 8 nM). After 24 hours, the cell viability was measured using the WST-1 assay, and the specific concentrations of ECF and NaBu used were recorded for further experiments.

Microplate assay of reactive oxygen species (ROS)

To ascertain the protective effect of ECF against oxidative damage induced by NaBu in MCF-7 cells, the levels of reactive oxygen species (ROS) were measured using the ROS microplate assay. Here's a simpler explanation:

- MCF-7 cells were placed in 96-well plates (25×10^3 cells/well) and were allowed to grow for 24 hours.
- The cells were then pre-treated with different concentrations of ECF (1, 5, and 10 $\mu\text{g/mL}$) for 24 hours.
- After this pre-treatment, the cells were exposed to NaBu (2 nM) for an additional 24 hours of incubation.
- Following incubation, the ROS levels were measured using the DCFDA/H2DCFDA-Cellular ROS Assay Kit (Abcam). This kit helped detect ROS levels in cells.
- The optical density of each well was measured at specific wavelengths using a microplate reader (Ex/Em = 485/535 nm).
- The ROS levels for each group were compared, and the protective effect of ECF against oxidative damage induced by NaBu was assessed. The term "doubling change" referred to the comparison of ROS levels between different groups.

CHAPTER NO IV

Results

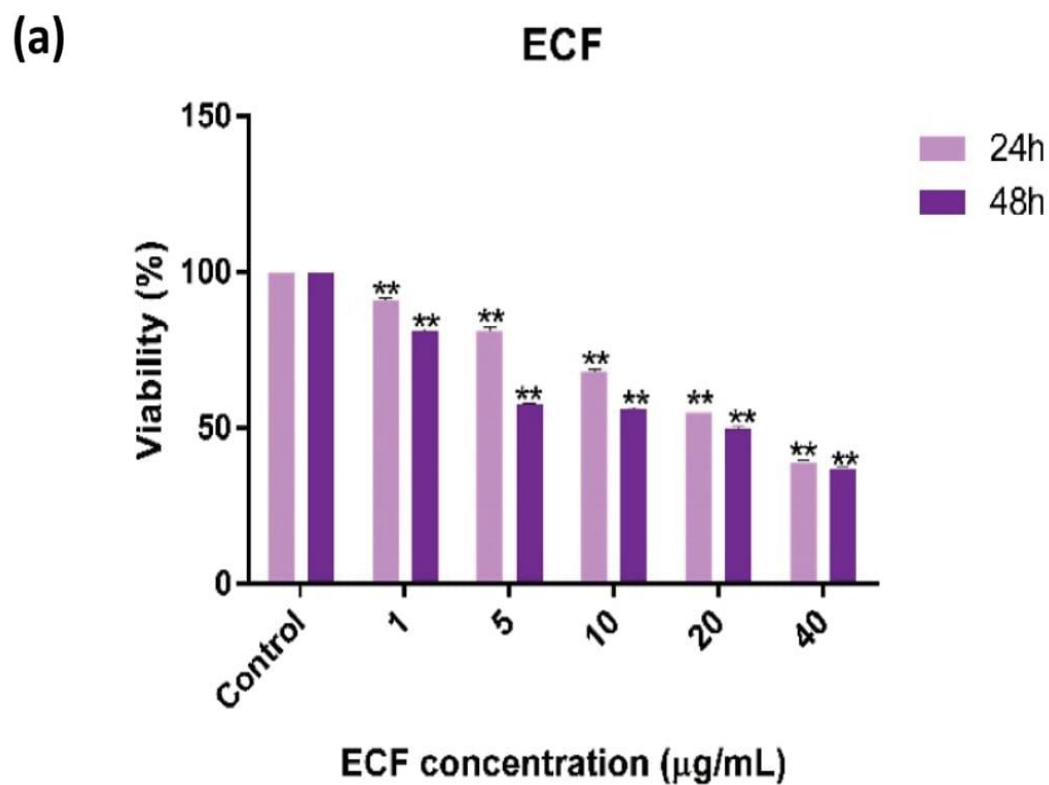
The WST-1 assay revealed that ECF and NaBu individually exert a cytotoxic influence on MCF-7 cells. When assessing the impact of NaBu on cells pre-treated with ECF, a clear dose-response relationship emerged. Specifically, cell viability rates declined progressively when exposed to ECF at concentrations of 1, 5, 10, 20, and 40 $\mu\text{g/mL}$ over periods of 24 and 48 hours. Notably, a more pronounced reduction in cell viability was observed after 48 hours. In particular, ECF at 20 and 40 $\mu\text{g/mL}$ significantly reduced MCF-7 cell viability after 24 hours, as compared to the control group that received no treatment ($p < 0.05$). Consequently, ECF concentrations of 1, 5, and 10 $\mu\text{g/mL}$ were identified as the most effective dosages for ECF treatment.

The viability of HUVEC cells was observed to diminish in correlation with increasing concentrations of NaBu (0.5, 1, 2, 3, 4, and 8 nM). Specifically, cell viability hovered between 40% and 51% following 24 hours of treatment with 2 and 8 nM NaBu, indicating a substantial oxidative impact on the cells ($p < 0.05$). To further investigate, ECF at doses of 1, 5, and 10 $\mu\text{g/mL}$ was administered to MCF-7 cells 24 hours prior to introducing NaBu at 2 and 8 mM concentrations. It was noted that the 8 mM NaBu group exhibited a reduction in cell viability when compared to the 2 mM group. Interestingly, cells pre-treated with ECF demonstrated significantly greater viability than those treated with 2 mM NaBu alone ($p < 0.05$). These findings suggest that ECF has a protective effect on MCF-7 cells, mitigating the oxidative damage caused by NaBu.

To assess the potential chemo-protective properties of ECF, ROS levels were measured in the context of NaBu-induced oxidative stress in MCF-7 cells. It was observed that treatment with NaBu (2 mM) led to a significant elevation in ROS levels, approximately 4.7 times the baseline ($p < 0.01$) as depicted. However, when cells were pre-treated with varying concentrations of ECF (1, 5, and 10 $\mu\text{g/mL}$) prior to NaBu exposure, there was a notable reduction in ROS levels in a concentration-dependent fashion, compared to the group treated with NaBu alone. These findings suggest that ECF pre-treatment effectively mitigates ROS generation in MCF-7 cells subjected to NaBu-induced oxidative stress.

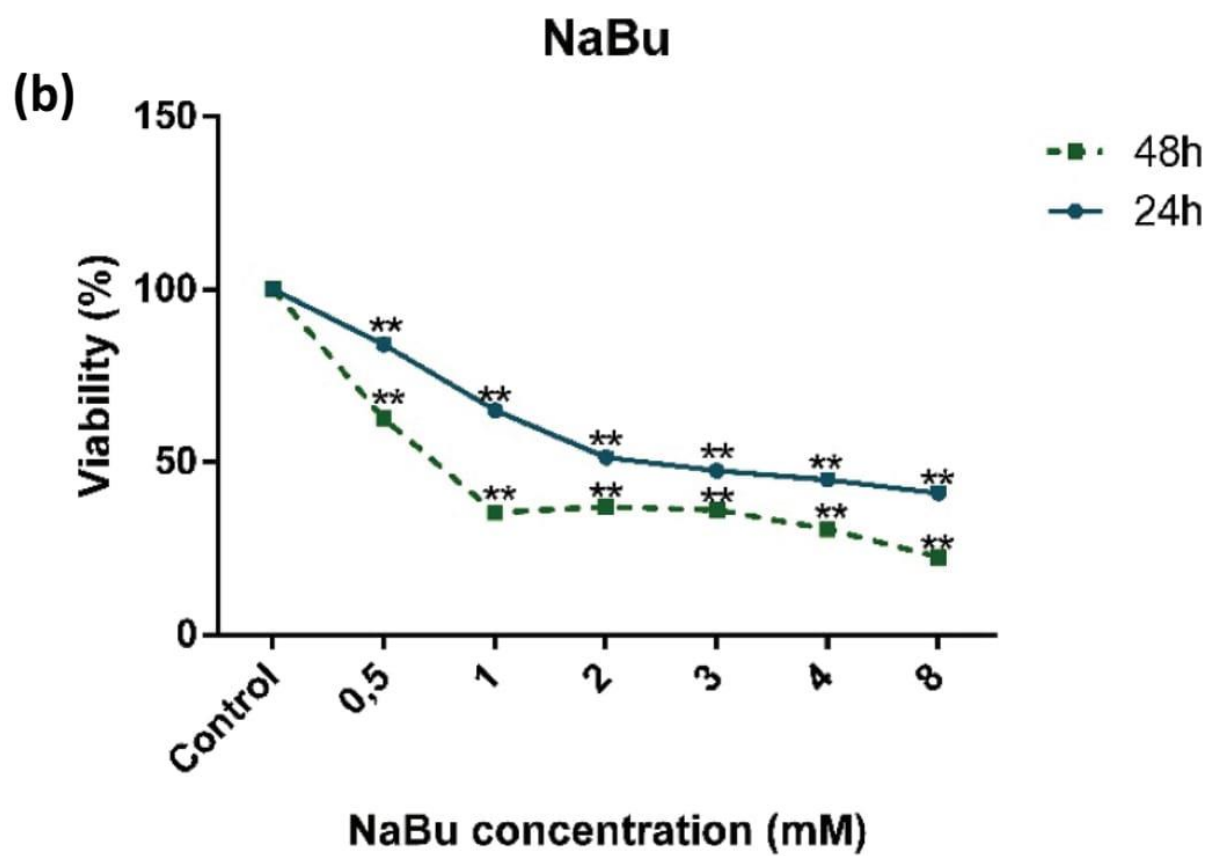
Table 1

Earthworm species	Cancer type	Extract	Protein characterization	Protein composition	Antiproliferative Potency	Assay	Cell line	Adjunctive test
<i>Eisenia foetida</i>	Breast cancer	Coelomic fluid	Qubit protein assay kit	Lysenin Lumbrokinase	1,5 and 10 μ g\ mL with NaBu	In Vitro	MCF-7	NaBu exposure, ROS micro plate assay



Direct and chemo-protective effects of ECF on cell viability on MCF-7 cells.

(a) The effect of different concentrations of (1, 10, and 20 $\mu\text{g/ml}$) ECF on the viability of MCF-7 cells



(b) The effect of different concentrations of (0.5, 1, 2, 3, 4 and 8 mM) NaBu on the viability of MCF-7 cells.

CHAPTER NO V

Discussion

Moreover, we hypothesize that the significant reduction in ROS levels, when compared to the control group that did not receive ECF treatment, suggests that pre-treatment with ECF shields MCF-7 cells from ROS production and the oxidative toxicity induced by NaBu.

The Bax protein, encoded by the Bax gene, is a member of the Bcl-2 protein family and acts as an activator of apoptosis (Lessene et al. 2008). The Bax gene triggers apoptosis and is overexpressed in cancerous tissues relative to Bcl-2. The presence of proapoptotic proteins in a cell makes it more susceptible to apoptosis, while cells abundant in antiapoptotic proteins are less prone to apoptosis (Zhang et al. 2000). Bax is proapoptotic, whereas Bcl-2 is antiapoptotic (Opferman and Kothari 2018; Alam et al. 2019). Thus, the intracellular ratio of Bcl-2 to Bax is a critical determinant of whether a cell will undergo apoptosis. A high Bax level leads to apoptosis, while a high Bcl-2 level inhibits apoptosis. Our data revealed an increase in the mRNA level of Bax and a decrease in the expression levels of Bcl-2 following NaBu treatment alone. We infer that the expression levels of the apoptosis-related genes Bax and Bcl-2 remained stable in MCF-7 cells exposed to NaBu after pre-treatment with ECF, suggesting that ECF exerts a chemoprotective effect in MCF-7 cells.

Prolonged release of reactive oxygen species (ROS) can cause DNA damage and elevate the mutation rate, potentially leading to oncogenic transformation in cancer-related genes and alterations in DNA transcription and replication, activation of signal transduction pathways, and genomic instability (Ozkan and Yuksel 2022). Cells have developed a variety of antioxidant mechanisms to counteract ROS-induced oxidative damage and preserve their genomic stability.

Our examination of the gene expression data of SOD and CAT enzymes, which catalyze the oxidative stress product, superoxide, and hydrogen peroxide, revealed a decrease in these enzymes only in MCF-7 cells treated with NaBu. Conversely, an increase in related genes in cells treated with ECF was observed, which is noteworthy. This increase in cancer cells may be attributed to the therapeutic efficacy of ECF, which possesses biological functions such as anticancer activity. This underscores the potential of ECF as a unique and promising therapeutic agent in cancer treatment.

CHAPTER NO VI

Conclusion

The necessity for alternative therapeutic strategies is underscored by the significant challenges associated with current cancer treatments, including toxicity, severe side effects, and a lack of cell specificity. As the search for a definitive cure for various types of cancer continues and the incidence of the disease increases, research in this field is not only persistent but also evolving with the latest scientific breakthroughs.

Our investigation highlights the significance and innovation of a more thorough examination of the potential therapeutic properties of *Eisenia foetida* coelomic fluid (ECF) in the treatment of cancer. This research will add new perspectives to the existing literature, addressing a crucial knowledge gap. The findings from this study will aid in gathering data that could be pivotal in the future pharmacological development of ECF.

The potential of ECF to act as a less toxic alternative to current treatments could significantly enhance the quality of life for cancer patients by alleviating the impact of treatment-related side effects.

In conclusion, our study not only adds to the existing knowledge on cancer treatment but also uncovers new possibilities for future research and development in this area. The potential advantages of ECF in cancer treatment emphasize the need for ongoing research and innovation in the pursuit of more effective and less harmful cancer treatments. The path towards a cure for cancer is lengthy, but each new discovery brings us one step closer to that objective.

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