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MITOGENIC, ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITIES OF G-90 AND COELOMIC FLUID FROM EARTHWORM AS A THERAPEUTIC AGENT, A REVIEW

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ABSTRACT: The mitogenic activity in earthworms should be accompanied by antioxidative and antimicrobial activities during the healing or regeneration of their amputated part. These activities are achieved by bioactive macromolecules in their tissue and coelomic fluid. The antioxidative activity applied to prevent the cellular function from oxidative damage and assist cell multiplication. The antimicrobial activity prevents the wounded area from bacterial infection and enhances cell division. This review aims to promote cheap natural products of earthworms to be used widely in human medicine. Thus, we reviewed how to extract coelomic fluid, preparation of tissue homogenate (G-90) and application of mitogenic, antioxidative, antimicrobial activities on experimental animal rats, mice and rabbits. These products were efficient for hasty wound healing, the proliferation of damaged bone, boosting of immune cells and nerve cell regeneration without carcinogenic and mutagenic characteristics. Therefore, the biotechnological application of tissue homogenates and coelomic fluid should get due attention.

INTRODUCTION: Earthworms are metamerically segmented organisms that belong to class Oligochaeta of phylum Annelida. The coelomic cavity of an earthworm is filled with coelomic fluid that oozes out through dorsal pore as a mechanism of defense in response to the body stress. Coelomic fluid from earthworms has been reported to possess several biological properties, like cytolytic, proteolytic, antimicrobial, hemolytic, haemagglutinin, tumorlytic, antiinflammatory, antioxidant, bacteriostatic, mitogenic and anticancer activities [1, 2].

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It was identified to display biological activities including anticoagulation, fibrinolysis, bacteriostatic [3, 4], antioxidative [5], anticancer [6] and mitogenic activity [7]. Both coelomic fluid and glycolipoprotein (G‑90) were reported to contain immunoglobulin growth factor, insulin-like growth factor, coelomic mitogenic factor, coelomic cytolytic factor and many other peptides which facilitate basic physiological processes in earthworm [5-8].

They helped earthworm for mitogenic [7] antioxidative and antimicrobial activities [3, 6] for their survival. The mitogenic activity helped in the regeneration of earthworm's amputated parts, either anteriorly or posteriorly [7]. Antimicrobial activity aided earthworms to prevent microbial infection of wounded body parts during regeneration [3, 7]. The medicinal properties of earth worm's coelomic

fluid and G‑90 had been applied by different researchers [9, 10]. The best example was the utilization of bioactive molecules of fibrinolytic activities in the treatment of heart attack and blood vessel-related diseases [11]. Similarly, the bioactive molecules governing mitogenic activity in earthworm could also be produced and applied pharmaceutically to treat wounds during surgery and damage [10].

During regeneration, the wound of amputated body parts in earthworms could be healed allowing cells to divide around amputated body parts [7, 10]. The mitogenic bioactive molecules also showed antioxidative and antimicrobial activities [6]. Further, the mitogenic activity could not be facilitated without antioxidative activity [6]. Several studies showed that bioactive molecules derived from earthworms' coelomic fluid and tissue homogenate were applied in wound healing [12], bone [13] and nerve cell proliferation [14]. The significance of tissue homogenate and coelomic fluid had not been promoted to be applied widely. The mitogenic, antimicrobial and antioxidative activities were displayed in the body of earthworm to facilitate normal physiological activities of the animal [15]. The objective of this review was to promote the importance of bioactive molecules in G‑90 and coelomic fluid of earthworms as a therapeutic agent.

1. Methods of Extracting Coelomic Fluid: Two main methods of coelomic fluid extraction were applied *viz.*, electric shock, and puncturing methods. It was extracted from earthworm by oozing through its dorsal pore by a sort of electric shock as a mechanism of defense [16]. The other method of coelomic fluid extraction involved the puncturing method which was applied to extract fresh and pure coelomic fluid. It involved puncturing and sucking up coelomic fluid from the coelomic cavity of earthworm with a thin glass capillary tube [17].

1.1. Methods of Preparing Tissue Homogenate (G-90): Tissue homogenate of earthworm was identified to contain a mixture of glycolipoprotein referred to as G-90, which can be applied pharmaceutically and medically [6]. G-90 was prepared from earthworm's different body parts, *viz.*, anterior, middle and posterior body regions, and midgut or from epidermal parts of the skin or whole body parts by mincing the body part into fine homogenous solution [5, 6, 11]. The concentration of bioactive molecules extracted from different body parts of an earthworm differed depending on the role of the organ from which it was extracted. As an example, G-90 prepared from earthworm's mid-body showed a significant mitogen property that promoted fibroblast proliferation and stimulated vascular endothelial growth factor and keratinocyte growth factor in wounded areas [11]. This was possibly due to the concentration of mitogenic bioactive of G-90 extracted from the middle area of the body part to started up and promoted regeneration of cut body part of earthworm [11].

2. Mitogen Activities: The mitogenic activity was reported to be vital for the regeneration and healing of amputated body parts of earthworm [18]. It was reported that organogenesis and regeneration were controlled by a similar principle of mitotic cell division, which began from the zygote and then to embryo [19, 20]. Mitotic cell division occurred in multicellular animals as a function of organo-genesis, regeneration, and wound healing [18]. Mitotic cell division needs to be activated by certain kinds of mitogenic molecules [7]. Glycolipoprotein (G-90) was identified to possess the mitogenic bioactive molecules that could instigate cell division in multicellular organisms during wound healing and tissue regeneration [7]. The property of bioactive protein molecules of G-90, acting as a mitogen, played vital roles in promoting cell division because of the unique structure of their molecules to bind together molecules of different substances [1]. G-90 and coelomic fluid possessed the ability to trigger cell division [7, 14]. It was demonstrated that bioactive molecules in G-90 acted as adhesive substance between adjacent cells. This indicated that G-90 had a significant effect on mitogenic activity by providing resources essential for joining cells that helped in the healing process of a wound [10, 15].

Cell proliferation in multicellular organisms was governed by mitogenic molecules, which influenced the growth factor either from the external or internal environment of cells [1]. Mitogen instigated kinase family (MAK) was first sequenced on yeast and human cells [21]. Cyclins were important protein structures that were

influenced by mitogenic molecules and in turn, triggered kinases to facilitate mitotic cell division [22]. MAK was the main component applied to control the pathways of embryogenesis that initiated cell division of the zygote into a multicellular embryo, cell differentiation and specialization, cell proliferation and cell death [21, 23]. Without activation of kinases by a cyclin, cell division couldn't be commenced [23]. The dual phosphorylation of both threonine and tyrosine facilitated the activation of Kinases to instigate cell division through multistep protein kinases cascade [23]. Cyclindependent kinase (CDK) was termed due to its determinant effect of cyclins on kinases [24]. It was reported that G-90 exhibited a significant effect on regeneration and wound healing by its effect on cells as a supply of protein building block, specifically threonine and tyrosine [1, 7].

It was also reported that G-90 possessed two serine peptidases with a tyrosine code, which was received by epidermal growth factor (EGF) in mammals for enhancing cell proliferation on the damaged skin [12]. Furthermore, G-90 instigated the synthesis of fibroblast growth factor (FGF) and epidermal growth factor (EGF), which increased cell division, but it did not contain growth factors directly; instead, it instigated their synthesis in the culture of cell [1].

Macromolecules purified from earthworm's coelomic fluid and G-90 helped in the regeneration of cut body parts and wound healing in mammals such as mice [12]. There was a report that G-90 instigated synthesis of fibroblast growth factor (FGF) used for initial stabilization of wound healing and epidermal growth factor to replace injured skin during the wound healing process on mice skin [12]. G‑90 extract from earthworm had initiated the growth of epithelial cells and human fibroblasts without mutagenic and carcinogenic effects [1, 7]. The mitotic cell's nucleus index in the experimental group was also higher than that of the control group. Further, G-90 treated wounds displayed rapid healing and granulation filling than wounds of the control group [7]. This extract was shown to enhance the phase of proliferation and maturation of the wound healing process and stimulated injured epidermis to regenerate. Wound healing undergoes three stages *viz*., inflammation, and proliferation of the cell, remodeling and maturation, which carried out through interaction of many cells, growth factors, and cytokines [25]. When the body part of an organism cut off physically, the wound should be healed before the cut part started to regenerate, indicating that regeneration and wound healing were interlinked processes [12, 23]. Besides its mitogenic activity, G-90 and coelomic fluid played a vital role in many different activities. It was suggested that investigation of tissue homogenate of earthworm (G-90) as an ability of wound healing agent directly linked with mitogenic activities were antioxidative, bacteriostatic and anti-inflammation [6]. It was also suggested that wounds treated with G-90 inhibited bacterial infection as well as inflammation [10].

2.1. Wound Healing Efficiency of G-90: It was demonstrated that earthworm's G-90 was effective in the diabetic wounded rat in minimizing wound from infection in comparison to treatment with D-panthenol [15]. In their study, they divided 36 animals into six groups, each containing six animals [15]. Group (A) were treated by using D-Panthenol as a positive control. Group (B) treated with an injection of G-90. Group (C) were treated with G-90 by ingesting along with their diet and Group (D) treated with G-90 on site of the wound.

Group (E) diabetic rats that were left without any treatment as negative control Group (F) the healthy rats which were untreated and served as control. They found that G-90 minimized the wound infection as compared to D-panthenol. It also enhanced the formation of extracellular matrix and proliferation of fibroblast as well as G-90 hastened synthesis of collagen and formation of the epithelial layer in rats treated by it. They suggested G‑90 as a new wound healing agent in a novel method of therapeutic approach in both veterinary and human medicine to treat the wound of a diabetic patient [15]. The efficiency of G-90 from *E. foetida* was checked to stimulate signal for transduction pathways of the production of epidermal growth factor (EGF) and fibroblast growth factor (FGF) in wound treatment [12]. They also evaluated whether G-90 activated EGF and FGF in healthy skin and wounds of mice [12]. Results showed that the abundance of EGF increased 2-fold and FGF 1.5-fold in wound healing under normal physiological conditions. However, in the case of G-90 treatment, both growth factors increased by

10-fold and 5-fold, respectively in comparison to normally grown healthy skin. Growth factors could be stimulated by G-90 to achieve synthesis and tissue regeneration as well as stimulating the proliferation of fibroblasts and epithelial cells [12]. In addition to the EGF, which was found in transmembrane protein, tyrosine, a biomolecule in G-90, also possessed a polar side group used by cells to make proteins through an anabolic reaction in the processes of mitogenic activity. Tyrosine was identified to show anabolic activity that initiated the synthesis of protein [24].

2.2. The Efficiency of G-90 for Bone Cell Regeneration: The G‑90 also initiated bone cell regeneration during injury [13]. They reported that G-90 displayed important effects on cell cultures and showed a potential effect on bone cell regeneration [13]. It was identified that the effect of different concentrations of G-90 on bone cells. In their experiment, the cellular effect of different concentrations of G-90 on osteoblasts and osteoclasts respectively were identified by using macrophage cells, namely osteoblast‑like MG 63 cells and RAW 264.7 [13]. The optimum concentration of G-90 was examined by mitochondrial assay, western blotting, acid phosphatase 5, tartrate-resistant activity, deposition of calcium matrix and alkaline phosphatase activity. The result indicated that the maximum effective concentration of tissue homogenate (G-90) was 3 mg/ml [13].

At this maximum concentration, it could greatly increase differentiation and proliferation of osteoblast, matrix calcium deposition, osteopontin, expression levels of alkaline phosphatase and osteocalcin. Inversely, 3 mg/ml maximum concentration of earthworm extract was negligibly reduced tartrateresistant acid phosphatase activity of osteoblast without affecting the viability of cells [13].

2.3. The Efficiency of G-90 for Nerve Cells Regeneration: It was reported that the extract of tissue homogenate of earthworm had a potential benefit to treat injury to peripheral nerves as it was demonstrated on experimental animal rat [26]. They used experimental and control groups to see the effect of tissue extract of Lumbricus (G‑90). The experimental group's left sciatic nerve was surgically damaged by clamping. Control groups were surgically operated without clamping the sciatic nerve. Control groups subjected to 2 ml of 0.9% NaCl and experimental groups were treated by Lumbricus tissue extract (1 g/ml), sequentially after six weeks of operation once daily. The result suggested that Lumbricus extract enhanced regeneration of sciatic nerve and functional restoration of the injured nerve. The clinical application of Lumbricus extracts to treat injured peripheral nerves in humans was recommended [26].

2.4. Mitogenic Agent in Coelomic Fluid and G-90: The bioactive mitogenic macromolecule in earthworm's coelomic fluid was first identified in *E. foetida* by [27] with a molecular weight of 60 kDa. It was a semi-purified fraction of the component obtained by using antiserum polyclonal and was termed as CMF (coelomic mitogenic factor). They applied CMF to induce spleen cell proliferation, and it was trimer of 20 kDa protein [27]. The proliferation of cells was not only facilitated by specific molecules in G 90 and coelomic fluid, but other molecules also assisted in the mitogenic activity. The other specific protein structure that enabled G 90 to possess the essential biological activity and acted as a mitogen was an insulin-like protein that had high anabolic characteristics [12]. Besides, insulin-like growth factor (IGF like) and immuno-globulin-like growth factor (IgFG-like) were identified from G-90 to stimulate proliferation of cell in cell cultures [6, 14] **Table 1**.

TABLE 1: BIOACTIVE MACROMOLECULE THAT FACILITATES MITOGENIC ACTIVITY IN COELOMIC FLUID AND G-90

Bioactive molecule	Molecular weight in kDa	Mechanism of activity	First isolated (Source)
Coelomic mitogenic factor (CMF)	60 kDa	Instigate cell proliferation	Coelomic fluid [27]
Insulin-like growth factor (IGF like)	14-45 kDa	Plays anabolic role	Tissue homogenate (G-90) [6, 8, 12]
Immunoglobulin-like growth factor (IgGF-like)	45 kDa	Stimulate growth factor	Tissue homogenate (G-90) [6, 8, 12]

Coelomic mitogenic factor (CMF) purified from coelomic fluid with 60 kDa identified to instigate cell division, Insulin-like growth factor (IGF like) play the anabolic role which was purified from tissue homogenate of earthworm with 14-45 kDa and Immunoglobulin-like growth factor (IgGF-like)

stimulate growth factor which directly involved in cell proliferation [27].

3. Anti-oxidative Activities:

3.1. The Function of Antioxidative Activity in Earthworm: The antioxidative activity of earthworm tissue homogenate (G-90) and coelomic fluid primarily helped the earthworm to carry out the normal physiological activity [5]. As a result of exposure of earthworms to oxidative toxic molecules, the concentration of anti-oxidative enzymes increased to catalyze several oxidative molecules from their bodies [28]. The accumulation of toxic phenanthrene in the body of earthworm, *Eisenia foetida*, stimulated their antioxidative system when exposed to a higher concentration of it in soil [29]. Besides, the toxic substance accumulation in the soil, such as azoxystrobin, caused oxidative stress in earthworm [30]. Effect of oxidative substances, interfering with normal physiological activities of worms, should be prevented by enhancing their antioxidative activity [30]. Drilodefensine, which helped earthworms as an antioxidative agent to detoxify oxidative polyphenol from plant material they feed [31]. Drilodefensins are those rare metabolites that belong to the class of dialkyl furan sulfonic acids and found in the anterior portion of the gut of earthworms [32]. It is with the general structural formula indicated by **Fig. 1**. It is this unique chemical that may provide tissue homogenate (G-90) to play a role as an antioxidative agent. Earthworms adapted to stress from oxidation compounds for two weeks after exposure to a strange environment that disrupted their normal physiological activity due to oxidative components in the soil [31].

The antioxidative property was important in earthworms to secure their survival. It prevented them from plant materials to catalyze toxic polyphenol, which synthesized in all higher plants as a secondary metabolite [16]. The presence of antioxidant enzymes, glutathione and glutathione related enzymes and catalase in the extract of *Eisenia foetidaandrei* are applied as an anti-oxidative agent in earthworms [33]. These enzymes were very important to counteract oxidative compounds like polyphenol. Though oxidation was an important chemical reaction in organisms, a balancing oxidation mechanism existed against damage to cellular function by free radicals [34]. It was achieved by catalase, dismutase and glutathione formation which could function as antioxidants [34, 35]. It was suggested that the mechanism of oxidative bioactive substances was by inhibiting lipid peroxidation, scavenge free radicals and by chelating transition metal [35].

The phenolic compounds present in the diet of earthworm didn't support the synthesis of larger molecules. It could disrupt the normal metabolic activities of the organism because polyphenolic compounds possessed free radicals of a hydroxyl group [36]. Earthworms were well adapted to detoxify plant's polyphenols by their antioxidative activity [37]. Any bioactive molecules with mitogenic activity such as coelomic mitogenic factor (CMF), insulin-like growth factor (IGF like) and Immunoglobulin-like growth factor (IgFG-like) should be accompanied by the antioxidative property without which mitogenic process couldn't be achieved [5]. The antioxidative activity supported the synthesis of a larger molecule, which in turn directly influenced mitogenic activity positively [5]. G-90 showed antioxidative activity, which was essential for the manifestation of mitogenic activities [5].

3.2. Experimental Application of G-90 to Prevent Oxidative Compound: The effect of G-90, extracted from *E. foetida*, as an antioxidant in cultured epithelial cells and human fibroblasts by incubating the cells with oxidative hydrogen peroxide and G-90 for 48 h [5]. The results indicated that G-90 significantly protected the cell from oxidative damage [5]. An excessive number of oxidative compounds were poison to cellular function and structure [38]. Morphological examination of oxidative compounds of linoleic acid hydroperoxide on cultured endothelial cells of human umbilical vein showed that higher concentration of oxidative compounds resulted in dilation and enlargement of the rough endoplasmic reticulum [38]. It caused slight increases in electron density of the mitochondrial matrix, indicating that at its excess amount, it could disrupt physiological activities of the cells that needed antioxidation activity to scavenge -OH group of the oxidative compounds [38]. The tissue homogenate (G-90) prevented cells from oxidative damage in a culture caused by an oxidative substance such as hydrogen peroxide, and the cells were restored and multiplied as it incubated by G-90 [5].

These were very important resources to salivary synthesis enzyme, which detoxified the effect of oxidative activities of phenolic compounds in mammals. Tannin, a family of the polyphenolic compounds in plant product foods [39], disrupted the physiological activity of humans and found to be precipitated by two salivary proteins Histidine and Proline. Histidine and proline‑rich salivary peptide acted as the first-line defense against precipitate tannins 40. Arginine acted as a direct antioxidant by scavenging oxygen-derived free radicals [39].

It was observed that the 5-carbon amino acid glutamine was essential to fuel for the gut, liver, and cells of immunity due to its important role as anti-oxidant and served as a precursor to reducing oxidation [41]. Glutamine functioned as an anabolic precursor for the growth of muscle-building block for protein synthesis [42,] which also functioned directly as a mitogenic agent. Proline, histidine, arginine, glutamine, and glutamate contained in food prepared from earthworm, and they acted as a component of the antioxidant [43, 44]. Research indicated that the presence of antioxidative and antiulcer properties in earthworm paste (G-90) derived from *L. mauritii*. They tested the appropriate dose of 160 mg of earthworm paste/kg found to possess greater therapeutic properties [43].

The oxidative substances could damage organs like the heart, which was tested using Wistar rat as an experimental animal by treating with earthworm powder [44]. It was shown that earthworm powder effectively protected against heart attack by its antioxidative components as compared to Wistar rats treated by oxidative components. When the rats were orally ingested with dried earthworm powder, these parameters were reversed to normal.

These results suggested that *P. excavates* powder could prevent significantly from liver damage occurring due to oxidative compounds like alcohol. Furthermore, antioxidative substances were important in protecting damaged essential macromolecules such as protein, DNA and lipid that contributed to degenerative diseases and aging [45].

Antioxidative substances also protected essential organs such as the heart, liver, nervous tissues and vital macro-molecule DNA from oxidative toxic components [5, 30, 35].

4. Anti-microbial Activities:

4.1. Adaptation of Earthworm against Soil Pathogen: Earthworms were exposed to an environment of pathogens because they fed on the decomposing dead organic matter [8]. Earthworms defend themselves against pathogenic micro-organisms from the soil by a variety of humoral cell and structure [46]. Humoral method of defense mechanism, the anti‑microbial activity, a basic vital biological activity in earthworms (skin protection, mucus) secured their survival and protected them from pathogenic bacteria in a variety of ways. Possibly, an evolutionary adaptation enabled them to protect themselves from pathogens [47-51]. The variety of ways through which they prevent from pathogens is a humoral method that was assisted by several bioactive peptide chains of different amino acids [3]. The humoral method could be achieved through lysis cells by forming pore on the cell membrane [52, 53] and hemolysis [54, 55].

4.2. Experimental Application G-90 and Coelomic Fluid against Microbes: Earthworms (Eudriluseugeniae) tissues extract (G-90) were effectively acted against four species of bacteria and two fungi species with significant results [55]. Antimicrobial macromolecules were identified and had referred to as antimicrobial vermipeptide family (AVPF) from coelomic fluid and tissue homogenate of earthworm [56]. They examined and separated using crude peptide preparation, ultra-filtration purification, ion-exchange chromatography, gel filtration, and HPLC chromatography. They have determined the antibacterial activity for Gram ve, Gram +ve bacteria, and anti-fungal property with significant positive results [56]. It was investigated that the coelomic fluid extracts from *Eisenia foetida* andrei to act against gram-ve bacteria [53]. Coelomocyte concentrations in *Lumbricus terrestris* using as the independent variable was evaluated to determine parasitic load and it was indicated that as invading parasite increased, the coelomocyte concentration also increased [57]. The coelomic fluid was experi-mentally identified as an antimicrobial activity that benefited the animal as an agent of immunity [57]. The coelomic fluid of *E. foetida* possesses a short peptide, coded as OEP3121, which acted against bacterial species of *Pseudomonas aeruginosa*,

Escherichia coli and *Staphylococcus aureus* [58]. One experiment reported that antimicrobial activity of vermi-extract of *P. excavates* against four human pathogenic microorganisms (*E. coli*, Proteus, Providencia, Morganella species), which caused urinary tract infection in children [59]. They concluded that the continuous taking of earthworms as a meal could recover people from urinary tract infections. Earthworm was able to defend the pathogenic bacteria by bioactive macromolecules secreted from their organs and the organs were mostly exposed to make in contact with soil and harbor varieties of pathogens. There was a report that earthworm's coelomocytes prevented pathogenic bacteria not only by phagocytosis and intoxicating them but also by lysing the bacteria extracellularly [60, 61]. *In-vivo* analysis of the antimicrobial activity of tissue homogenate (G-90) extracted from *E. foetida* on non-pathogenic bacteria and facultative pathogenic bacteria demonstrated that the bacteriostatic influence of G-90 was higher for facultative pathogenic bacteria than non-pathogenic bacteria [62]. A higher concentration of G-90 showed a higher effect on inhibiting the facultative-pathogenic bacteria than non-pathogenic bacteria [62]. It was demonstrated that the immune system of earthworm encouraged a mutual relationship between them and their gut microbes, which assisted in the digestion process. The activity of different bioactive molecules of coelomic fluid against different bacterial species was identified by the recognition of lysozyme [63]. There was the mechanism that invertebrate could identify the chemical structure on either cell wall in case of bacteria by detecting peptidoglycan and phospholipid or cell membrane by detecting sphingomyelin in higher animals [63]. Aforesaid studies on bioactive biomolecules were carried out on different species of earthworms as given in **Table 2**.

TABLE 2: MOZAIC MODEL OF HUMORAL METHOD OF DEFENSE MECHANISMS IN EARTHWORM

Bioactive molecule	Molecular weight in kDa	Mechanism of defense	Source
Lysozyme (*E. foetida and E. andrei*)	39 kDa	Extracellular lysis acts to recognize between microbes	Coelomic fluid [5, 46]
Lumbricin I and lumbricin I (6-34) (*Lumbricus rubellus*)	7.2 kDa	Acts against a broad spectrum of microorganisms without hemolytic activity	Coelomic fluid [46]
Lysenin (*E. foetida*)	33 kDa and 41 kD a	Lysis act on the cell wall of both Gram-positive and Gram –ve and Pore-forming	Coelomic fluid [46, 52]
Coelomic cytolytic factor (CCF) (*E. foetida*)	42 kDa	Lysis bacterial cells and it is pore-forming	Mesenchymal lining cell Coelomic fluid [46, 50]
lumbricin-PG *(Pheretima guillelmi)*	Short peptide	Acts as a primary defense fungistatic, bacteriostatic and antiviral	Skin secretion of earthworms [8]
Eiseniapor (*E. foetida*)	38.6 kDa	Cytotoxin, Pore-forming	Coelomic fluid [46]
Fetidins (*Eisenia andrei*)	40-kDa & 45kDa	Hemolytic and anti-bacterial and Pore-forming	Coelomic fluid [3, 4]
OEP3121 *(E. foetida)*	Short peptide	Lysis the cell	Coelomic fluid [58]
Bacteriostatins *(E. fetidaandrei*)	45, 40, 20 kDa	Lysing bacterial cell and prevent the bacteria from growth and Pore-forming	Coelomic fluid [47]

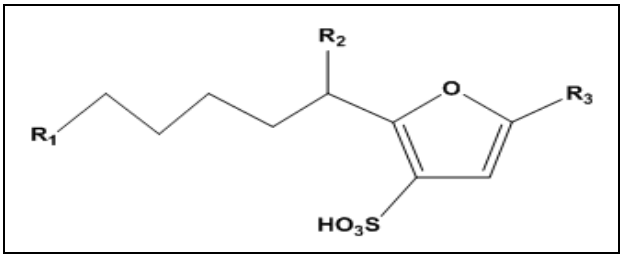

FIG. 1: STRUCTURES OF EARTHWORM 2, 5-DIALKYLFURAN-3-SULFONIC ACID METABOLITES 1-6 TERMED AS DRILODEFENSINE

4.3. Bioactive Applied as Antimicrobial Activity:

Lysenin lysed and killed cells by the sphingomyelin-dependent mechanism in eukaryotic mammals and sphingomyelin-independent mechanism in bacteria as the later lack sphingomyelin [52].

An earthworm protein (38.6 kDa) from the coelomic fluid of *E. foetida* was isolated and purified by using different isolation procedures [46]. Eiseniapore (38.6 kDa) also needed a reaction with sphingomyelin to lyse cells by ion formation with glycoproteins and phospholipids binding action and to lyse of bacterial cells through the formation of pores [46]. Lysenin and eiseniapore were toxic to mammalian cells because they directly developed a link reaction with sphingomyelin to lyse cells. Lumbricin PG was identified from the skin secretion of *Pheretimagullerie*, which acts as a primary defense to prevent earthworm from pathogenic soil bacteria. The bioactive molecules such as lysenin (33 kD a and 41 kDa), cytolytic

eiseniapore (38.6 kDa), and fetidin (40 kDa), hemolysin and fetidin (45 kDa), lysozyme (39 kDa), Lumbricin I (7.2kDa), Lumbricin-PG coelomic cytolytic factor (42 kDa), OEP3121, bacteriostats were obtained from coelomic fluid of earthworm which is responsible for defending earthworms from pathogen [3, 4, 47, 58] **Table 2**.

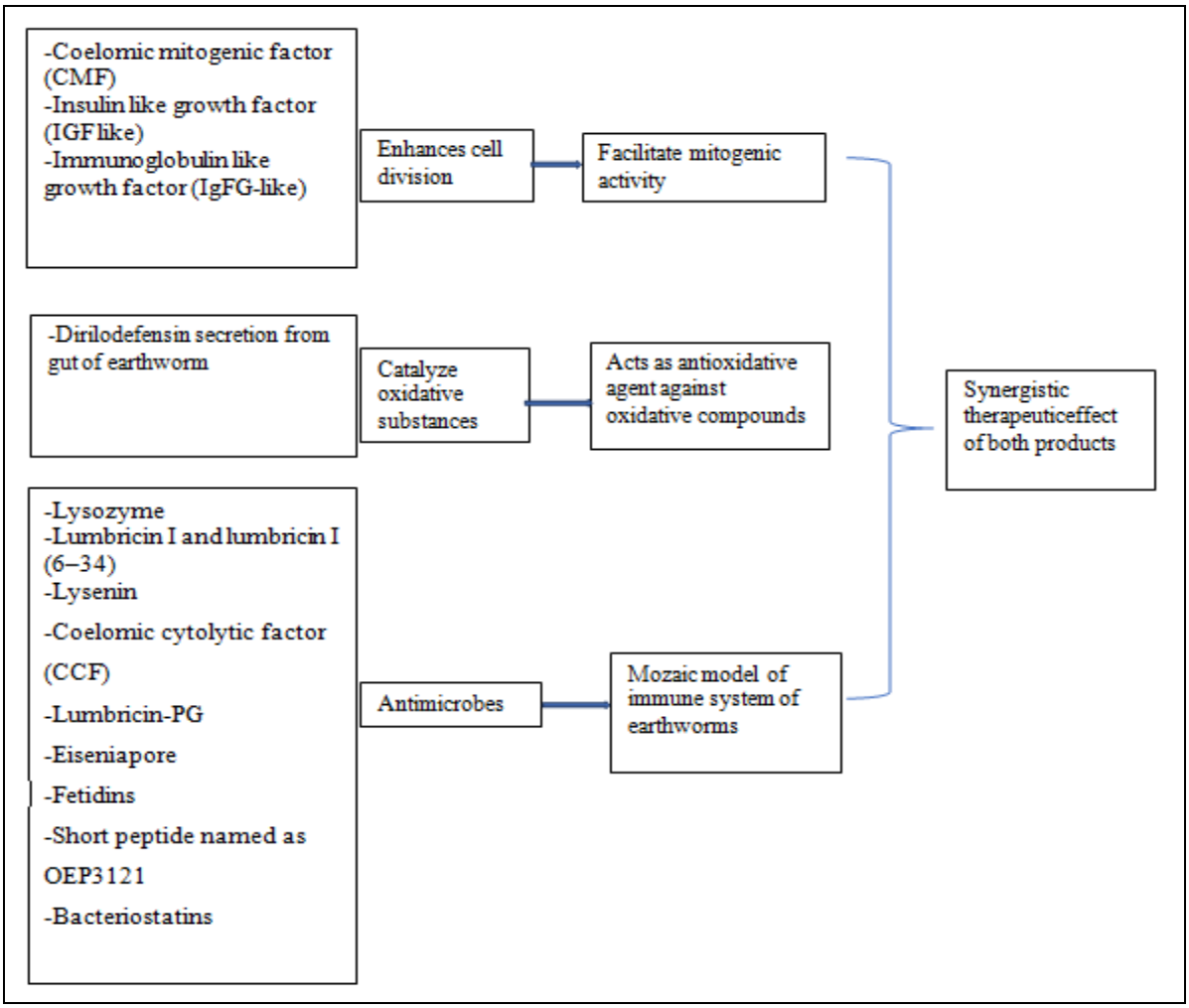

FIG. 2: SUMMARY OF THE SYNERGISTIC THERAPEUTIC ROLE OF BOTH COELOMIC FLUID AND EXTRACTS OF EARTHWORM (G-90) INDICATING THAT THE MITOGENIC ACTIVITY NEEDS AN ANTIOXIDATIVE ACTIVITY TO PREVENT THE CELLULAR FUNCTION FROM OXIDATIVE DAMAGE TO ASSIST CELL MULTIPLICATION [5, 14, 30]. ANTIMICROBIAL ACTIVITY APPLIED TO PREVENT THE WOUNDED AREA FROM BACTERIAL INFECTION AND ENHANCES CELL DIVISION

CONCLUSION AND FUTURE DIRECTIONS:

The mitogenic activity was maintained through an anabolic reaction, which was supported by anti-oxidative activity to prevent unnecessary oxidative reactions during wound healing, regeneration, and proliferation of different cells at an injury or surgery.

The anti-microbial activity was also very important to prevent microbial infection of a wound so that the healing process could be hastened. Therefore, the gene that code for earthworm's bioactive molecules responsible for mitogenic, antioxidative and microbial activity in G-90 and coelomic fluid should be assayed, amplified for identification and genetically engineered to produce pharmaceutical products.

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