

# ‘SLIME STORY’- EXPLORING THE POTENTIAL OF EARTHWORM COELOMIC FLUID: A REVIEW

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**Abstract:** It's a well known fact that earthworms are considered as farmers best friends. However, a lesser known truth is that earthworms were employed in traditional medicinal cures since centuries. The earliest account of such use dates back to 1340 AD. Extracts derived from earthworms were used to treat post partum weakness in women, to enhance hair growth, as toothpowder against gingivitis and to reduce pain in rheumatism. Earthworms even served as a powerful cure for small pox. Scientific research has uncovered the hidden potential of earthworm coelomic fluid and its extracts and shed light on their remarkable properties, which include, antiproliferative, antibacterial, fibrinolytic, wound healing, anticoagulative and antioxidative, to name a few.<sup>1</sup> This article intends to highlight the properties of earthworm coelomic fluid and emphasize on its importance in future research.

**Keywords :** Anticancer, anticoagulative, Earthworm coelomic fluid (ECF), *Eisenia fetida*, G-90

## INTRODUCTION

Nature has endowed us with plenty of unique natural resources, many of which have not yet been completely explored. Although history has ample evidence, lack of proper scientific research has discouraged their utilization. Of late, earthworms and their extracts which contain biologically active molecules have drawn much attention from many scientists and researchers all over the world. The coelomic cavity of earthworms is filled with a specialized kind of fluid, derived from its mesen-

chymal layer known as Earthworm coelomic fluid (ECF). It is rich in wandering coelomocytes, which includes namely 4 types of cells; the mucocytes, amoebocytes, circular cells and chloragogan cells. In addition, it also contains certain immunologically active compounds like lysenin, lumbricin, fetidin, eiseniapore, coelomic cytolytic factor and several growth factors. The ECF helps to maintain moisture and to aid in normal physiologic processes like cutaneous respiration, dessication, regeneration, circulation of nutrients and protection from pathogens.<sup>2,3,4</sup>

Scientific studies require collection of ECF from healthy earthworms. Coelomic fluid is extracted from earthworms by using 4 techniques.

- 1) Cold shock method
- 2) Warm water method
- 3) Electric shock method
- 4) Heat shock method.

The cold shock method is usually preferred as it is

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the least stressful for the earthworms. This method also yields the maximum amount of concentrated vermin wash (1.5 ml) when compared to other methods.<sup>2</sup> The collected ECF can be stored or subjected to purification and protein separation. The earthworms can be stored in plastic bins and fed on refuse.

### PROPERTIES ANTIFIBRINOLYTIC AND ANTICOAGULANT ACTION

ECF is a well known antifibrinolytic agent and anticoagulative agent. It has been used for years to treat cardiac diseases and cerebrovascular disorders. Lumbrokinase, a fibrinolytic enzyme isolated from the earthworm *Lumbricus rubellus* is a potent thrombolytic agent and is marketed as an orally administrable fibrinolytic agent to treat cardiac and cerebrovascular diseases.<sup>1</sup> Studies on fibrinolytic enzymes of earthworms have also paved way in the preparation of cosmetics as an 'age delaying' agent.<sup>5</sup> Studies on dogs and drawn human blood from volunteers suggests that the anticoagulative activity is due to 3 components namely; the glycolipoprotein mixture G-90, PI and PII<sup>1</sup>

### ANTIPROLIFERATIVE ACTION

The most feared six letter word 'Cancer' and its treatment, not only leaves the victim traumatized mentally and physically, but also devastates the patients' dear ones in many ways, including financially.

Facts and figures point out that 3,54,864 new oral cancer cases and 1,77,384 deaths were estimated in 2018.<sup>6</sup> Years of research have put in a lot of medicines derived from natural sources to combat cancer. As chemotherapeutic agents are expensive and trigger a lot of side effects, current research revolves around discovering a relatively cheaper drug with strong therapeutic potential, yet minimal side effects. Naturally occurring extracts of turmeric<sup>7</sup>, ginger<sup>8</sup>, honey<sup>9</sup>, green tea and pomegranate<sup>10</sup> thus became popular. Earthworm coelomic fluid, a naturally derived blend of components exhibiting cytotoxic, agglutinating, antioxidant and antipyretic actions also belongs to the same league of extraordinary compounds.

Coelomic fluid of the earthworm *Eisenia fetida* induced *in vitro* apoptosis of HeLa cells.<sup>11</sup> A study on the cytotoxic activity of coelomic fluid of the earthworm by

name *Eudrilus eugeniae* on Baby hamster Kidney 21 (BHK21) cells, yielded promising results.<sup>12</sup> The cytolytic protein 'Eiseniapore', isolated from the coelomic fluid of the earthworm *Eisenia fetida* induces cytolysis by creating pores, which was confirmed by electron microscopy of erythrocyte membranes.<sup>13</sup> Several other studies highlighted the ability of ECF to combat tumours of the liver, brain, breast and colon.<sup>14,15</sup> A recent *in vitro* study to compare the efficacy of ECF derived from 3 different species of earthworms on oral squamous cell carcinoma cell lines yielded very promising results.<sup>16</sup> Another study reported that a 24 hour incubation of oral cancer cell line (KB 3-1) with the ECF of *Perionyx excavates* (a species of earthworm), showed a percentage inhibition of 94.22% at 50 µg/mL, when compared to that of the control drug paclitaxel which had a maximum inhibitory percentage of 82.43 at 6.25 µg/mL.<sup>17</sup>

### ANTIBACTERIAL

Living organisms are prone to a hoard of infections from various pathogenic agents. These pathogens, most commonly bacteria and parasites, particularly the larval forms are present abundantly in soil and water and is introduced into the body through food or following an injury. As evolution progressed, earthworms devised measures to ward off pathogens using their highly specialized innate immunity. It is believed that earthworms thriving in the pathogen-riddled environments have peptides against bacteria and other parasites. Their innate immunity is maintained by various coelomocytes, housed in coelomic cavity whose fluid also contains several immunologically (antimicrobial) active molecules. An *in vitro* study on 4 different species of earthworms on various pathogens demonstrated promising antimicrobial activity.<sup>18</sup> Studies also suggest that earthworms possess both cellular as well as humoral immunity.<sup>19</sup> Six antibacterial peptides (antibacterial vermipeptide family; AVPF) were isolated and purified from ECF that showed efficacy against gram positive and gram negative bacteria and fungi.<sup>20</sup>

### WOUND HEALING

Studies emphasize on the fact that; by using preparations made from earthworm extracts, the wound healing time was significantly reduced as it accelerated epi-

thelization, granulation and collagen formation. This could be due to the influence of the mitogenic, haemostatic, antioxidant and antibacterial properties of ECF on healing and epithelization.<sup>21,22</sup> The Glycolipoprotein extract (G-90) from earthworm *Eisenia foetida* stimulated increased production of growth factors (EGF and FGF), thereby hastening the wound healing process.<sup>23</sup>

#### ANTIPYRETIC AND ANTIOXIDANT POTENTIAL

<sup>24</sup> and Balamurugan M et al<sup>25</sup> demonstrated the antipyretic and antioxidant potential of ECF on rats. His study also outlined the hepatoprotective effect of earthworm extract on paracetamol induced liver injury in Wistar rats. These properties are attributed to a glycolipoprotein mixture called G-90.

Metallothianins in ECF is considered to lower the toxins in human body due to pollutants in the environment. This study resulted from the fact that earthworms were found in decomposing matter and to overcome such adverse conditions, they must have evolved with a good immune system.<sup>18</sup>

#### CONCLUSION

Though earthworms were used extensively in traditional medicinal cure, it is only recently that detailed scientific research is being undertaken to explore the efficacy of its various extracts. Several in-vitro and in-vivo studies have successfully highlighted the antioxidant, fibrinolytic, antitumour, anticoagulative, antibacterial and wound healing properties of ECF. Recent studies highlighting the antiproliferative efficacy of ECF against oral cancer cell lines provides immense hope in the field of head and neck oncology and future research on oral cancers.

As the world is turning towards more safer and cheaper drugs with better therapeutic potential and lesser side effects, earthworm coelomic fluid holds great promise in future.

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#### REFERENCES

sources of fibrinolytic, anticoagulative, antimicrobial and anticancer molecules. International Jour-

1. Journal of Immunopathology and Pharmacology. 2004 Sep;17(3):237-44.
2. Patil SR, Biradar PM. Earthworm's coelomic fluid: extraction and importance. International Journal of Advanced Scientific Research. 2017;2(2):01-4.
3. Grdisa M. Therapeutic Properties of Earthworms. Bioremediation, Biodiversity and Bioavailability.;7(1):1-5.
4. Cooper EL, Kauschke E, Cossarizza A. Annelid humoral immunity: Cell lysis in earthworms, Adv. Exp. Med. Biol., 2001; 484:169-183.
5. Liu YQ, Sun ZJ, Wang C, Li SJ, Liu YZ. Purification of a novel antibacterial short peptide in earthworm *Eisenia foetida*. ACTA BIOCHIMICA ET BIOPHYSICA SINICA-CHINESE EDITION. 2004 Apr 1;36(4):297-302.
6. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018 Nov;68(6):394-424.
7. Farid RM. A Focus on Curcumin Local Application in Oral Diseases Management: Mini Review. IOSR Journal Of Pharmacy 2016; 6 (1);, 01. 2016;3.
8. Habib SH, Makpol S, Hamid NA, Das S, Ngah WZ, Yusof YA. Ginger extract (*Zingiber officinale*) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. Clinics. 2008;63(6):807-13.
9. Othman NH, Ahmed S, Sulaiman SA. Inhibitory effects of Malaysian tualang honey and Australian/New Zealand Manuka honey in modulating experimental breast cancers induced by N-methyl-N-nitrosourea (mnu): A comparative study. Pathology. 2016 Feb 1;48:S148.
10. Adhami VM, Mukhtar H. Anti-oxidants from green tea and pomegranate for chemoprevention of prostate cancer. Molecular biotechnology. 2007 Sep 1;37(1):52-7.
11. Yanqin L, Yan S, Zhenjun S, Shijie L, Chong W, Yan L, Yuhong G. Coelomic fluid of the earthworm *Eisenia fetida* induces apoptosis of HeLa cells in vitro. European Journal of Soil Biology. 2007 Nov

- 1;43:S143-8.
12. Rudrammaji LM, Dinesh MS, Sonole VG. Cytotoxic Effect of Coelomic Fluid of Earthworm *Eudrilus Eugeniae*. *Biomedical and Pharmacology Journal*. 2015 Jan 22;1(2):433-6.
  13. Lange S, Kauschke E, Mohrig W, Cooper EL. Biochemical characteristics of Eiseniapore, a pore forming protein in the coelomic fluid of earthworms. *European journal of biochemistry*. 1999 Jun 1;262(2):547-56.
  14. Liu CM, Chen XT, Pan YY, Liang H, Song SL, Ji AG. Antitumor studies of earthworm fibrinolytic enzyme component a from *Eisenia foetida* on breast cancer cell line MCF-7. *Indian Journal of Pharmaceutical Sciences*. 2017 Jul 31;79(3):361-8.
  15. Dinesh MS, Sridhar S, Chandana PG, Pai V, Geetha KS, Hegde N. Anticancer potentials of peptides of coelomic fluid of earthworm *Eudrilus eugeniae*. *Biosci Biotechnol Res Asia*. 2013;10:601-6.
  16. Augustine D, Rao RS, Anbu J, Murthy KC. In vitro antiproliferative effect of earthworm coelomic fluid of *Eudrilus eugeniae*, *Eisenia foetida*, and *Perionyx excavatus* on squamous cell carcinoma-9 cell line: A pilot study. *Pharmacognosy research*. 2017 Dec;9(Suppl 1):S61.
  17. Augustine D, Rao RS, Jayaraman A, Murthy KC. Anti-proliferative activity of earthworm coelomic fluid using oral squamous carcinoma KB 3-1 cells: An In vitro study with serine protease analysis. *Pharmacognosy Magazine*. 2018 Oct 1;14(59):528.
  18. Kathireswari P, Alakesan A, Abirami P, Sangeetha P. Antimicrobial activity of earthworm coelomic fluid against diseases causing microorganisms. *Int. J. Curr. Microbiol. App. Sci*. 2014;3(8):608-13.
  19. Field SG, Kurtz J, Cooper EL, Michiels NK. Evaluation of an innate immune reaction to parasites in earthworms. *Journal of Invertebrate Pathology*. 2004; 86:45-49.
  20. Wang C, Sun Z, Liu Y, Zhang X, Xu G. A novel antimicrobial vermipeptide family from earthworm *Eisenia fetida*. *European journal of soil biology*. 2007 Nov 1;43:S127-34.
  21. Prakash M, Balamurugan M, Parthasarathi K, Gunasekaran G, Cooper EL, Ranganathan L. Anti-ulceral and anti-oxidative properties of "earthworm paste" of *Lampito mauritii* (Kinberg). *Eur Rev Med Pharmacol Sci*. 2007;11:9-15.
  22. Li D, Wang P, Zeng Y. Study on lumbricus in promoting ligation hemorrhoidectomy postoperative wound healing. *Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi= Chinese journal of integrated traditional and Western medicine*. 2000 Dec;20(12):899-902.
- Hr•enjak T. Stimulation of growth factor synthesis in skin wounds using tissue extract (G 90) from the earthworm *Eisenia foetida*. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease*. 2004 Nov;22(6):373-8.
24. Grdisa M, Popovic M, Hrzenjak T. Glycolipoprotein extract (G-90) from earthworm *Eisenia foetida* exerts some antioxidative activity. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2001 Apr 1;128(4):821-5.
  25. Balamurugan M, Parthasarathi K, Cooper EL, Ranganathan LS. Earthworm paste (*Lampito mauritii*, Kinberg) alters inflammatory, oxidative, haematological and serum biochemical indices of inflamed rat. *European review for medical and pharmacological sciences*. 2007 Mar 1;11(2):77.