



THERAPEUTIC NATURE OF AN INDIGENOUS EARTHWORM *PERIONYX EXCAVATUS* (PERRIER)

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INTRODUCTION

The use of animals and their products is increasing in worldwide, and the public believe that combinations with herbal supplements are “safe” and “harmless”. Recent experimental evidences and clinical trials suggest that traditional medicines have a potent pharmacological value for the treatment of various diseases viz, inflammation, ulcer, alcoholism, microbial infection etc.

Preparations made of material of animal origin (Placenta, snake, and bees poison) were used extensively in folk and modern medicine for treating many human diseases. Renewable natural medical material of animal origin and pharmaceutical preparations on their basis as well as biologically active food supplements are the future medicine for diseases. Animals have been used as medicinal resources for the treatment and relieve of a myriad of illnesses and diseases in practically every human culture. Although considered by many as superstition, the pertinence of traditional medicine based on animals cannot be denied since they have been methodically tested by pharmaceutical companies as source of drugs to the modern medical science. Traditional Chinese medicine (TCM) has been increasingly practiced in many countries of the world. Some recent textbooks of TCM still recommend the formulae containing various animal tissues such as tiger bones, antelope, buffalo or rhino horns, antlers, testicles and on penis of the dog and bear bile (Alaves and Rosa, 2005; Alaves, 2009; Yibin Feng *et al.*, 2009)

Chemicals of nature have been a part of human civilization ever since our early ancestors began exploiting natural compounds to improve and enrich their own lives (Agasta, 1996). A major part of these chemicals come from animals. Indeed, animals are therapeutic arsenals that have been playing significant roles in the healing processes, magic rituals and religious practices of people from the five countries Indonesia, Burma, China, India and Malaysia (Costa-Neto and Marques, 2000). The healing of human ailments by using therapeutics that are obtained from animals or ultimately are derived from them is known as zootherapy (Costa-Neto, 2005).

The traditional medical knowledge of indigenous people throughout the world has played an important role in identifying biological resources worthy of commercial exploitation. Indeed, the search for new pharmaceuticals from naturally occurring biological material has been guided by ethnobiological data. El.Kamali (2000) has recorded 23 animals that are used as sources of remedies in the Sudanese traditional medicine. Adeola (1992) has also recorded 23 species in three ecological zones from Nigeria. He also added that most farmers in rural areas depend solely on wild animals and their by-products (hooves, tusks, bones, feathers, skins) for their daily animal protein supply and preparation of traditional medicine. Many tribes and people in remote villages were known to use earthworms for various kinds of ailments.

Immunity of invertebrate

Invertebrate immune system must rely on non-self-recognition molecules to ensure efficient defence responses against infectious pathogens that continuously threaten their survival. Invertebrates have developed innate immune mechanisms that detect pathogens by recognizing conserved molecular patterns. The recognition molecules for foreign material have been named as pattern-recognition proteins (PRPs) (Medzhitov and Janeway, 1997), because that host's primitive effector cells would recognize molecular patterns rather particular structures of the invading microorganisms. In recent years, antimicrobial peptides have become recognized as important contributors to nonspecific host defense for both vertebrates and invertebrates.

A natural immunity of annelids is formed by anatomical and chemical protective barriers preventing damage of underlying tissues, body fluid losses and microbial infections of the body cavity. The internal defense mechanism of the invertebrate involve phagocytosis, nodulation, encapsulation, blood coagulation, wound repair and antibacterial immune protein activation.

Earthworm – an Invertebrate

Earthworms (Oligochaeta) are the most ancient invertebrate animals on earth. They play an important role not only in the food chain of ecosystem, but they also

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influence physical chemical properties of soil composition and enforce microbiological processes in soil itself (Cooper *et al.*, 2002). Over 4000 Oligochaeta species are known, but at present only 12-15 species are used for vermiculture in different countries of the world (Titov and Anokhin, 2006). The vermiculture is particularly good for bioconversion of organic wastes into organic fertilizer (vermicompost) and earthworm biomass as the source of proteins, peptides, enzymes and physically active substances.

Apart from this earthworms play a vital role in curing various diseases. Earthworms have evolved in the environment replete with microorganisms, some of which threatening their existence. To survive in such environment, they have developed efficient defense mechanism against invading microorganisms. Earthworms and its products have been used as a drug (usually in dried powder for oral administration) to improve blood circulation and to treat apoplectic stroke from tenth century. Early in 1878, Frederiq found that the alimentary tract of earthworm excrete a type of protease. As described by Charles Darwin in 1883, earthworm digestive fluids can dissolve fibrin.

Although earthworms are not herbs, they are nevertheless a vital component of TCM. According to the concepts of TCM, earthworm is associated with the bladder, liver, lung and spleen meridians and has salty and cold properties (Cooper, 2005). It drains liver heat and clears lung heat and can also clear heat in the collateral channels. Typically, earthworm is used with other herbs to treat a wide range of conditions, ranging from spasms and convulsions to pain relief, treatment of fevers and certain type of arthritis. It is also used to treat some types of asthma and bronchitis.

Earthworms have been widely used in traditional Chinese medicine for thousands of years. However it is only during the past decades, either the development of biochemical technologies that research on the pharmaceutical effects of earthworm has been initiated. In china, research on medicinal use of earthworms has a history of nearly 4,000 years (Zhang *et al.*, 1992). Compendium of Materia Medica written by Li Schizhen in 1578 AD was a comprehensive summary of pharmacological knowledge accumulated in China up to his time. According to traditional Chinese medicine, earthworm possess antipyretic, antispasmodic, diuretic, antihypertensive, antiallergic, antiasthmatic, detoxic and spermatocidal effects.

Earthworms have been used to treat smallpox in Burma and Laos. First, worms are soaked in water and the patient is bathed in the liquid. The worms are then roasted, powdered, mixed with coconut water, and drunk. This treatment hasens the course of the disease and reduces mortality from 100 percent to 25 percent (Bristowe, 1932). Earthworm medicines are prescribed to treat over 80 diseases (eg., asthma, hypertension, mumps, ulcer, epilepsy, cancer etc), earthworms antipyretic properties are reportedly used in china and Japan in the dry form for reducing fever.

Goals and objectives

Earthworm coelomic fluid contains peptides which are the antibacterial components of the immune system and they can be induced by using different methods exhibiting non-specific immune responses to diverse environments; studying the induction of the antibacterial peptide from the earthworm with a non-specific immune response; assessing physicochemical and antibacterial properties of earthworm antibacterial peptides; developing the technology and methods for preparation, separation and purification of antibacterial peptides from earthworm coelomic fluid and tissue homogenate.

Biology of the indigeneous earthworm *Perionyx excavatus*

Perionyx excavatus is a dark pigmented and active worm. It is found in Himalayas, Assam, Darjeeling, Shimla, Calcutta, United provinces, Cochin, Travancore. Outside India the species has been met with in the Philippines, SriLanka, Myanmar, Indonesia, Thailand and China. They exhibit patchy distribution near their food sources in the plantations and agricultural lands.

Classification:

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|---------|---|------------------|
| Phylum | - | Annelida |
| Class | - | Oligochaeta |
| Order | - | Ophisthophora |
| Series | - | Megascolicina |
| Family | - | Megascolicidae |
| Genus | - | <i>Perionyx</i> |
| Species | - | <i>excavatus</i> |

It is long, segmented and cylindrical in shape and measures about 23-90 mm in length and 1.5 – 2 mm in width. The dorsal surface is deep purple to reddish brown in colour and pale brown ventrally. In mature worm 13 – 17 segments are found swollen with a glandular thickening of the skin forming clitellum. It has very high regeneration capacity

Preparation of Earthworm Powder and collection of coelomic fluid

The earthworm powder was prepared by following the method of Ishii and Mihara (1992) with slight modification. 1000 sexually mature clitellate worms (900 mg/worm) were washed with running tap water to remove any dirt from the body surface. Then the living earthworms were placed in fresh water. The living earthworms were left therein at a temperature of 25° C for a period of 72 hours. Thus the alimentary canal of the living earthworm was substantially freed of soil by their own excretory power. Thereafter the living earthworms were wet-ground by using chloroform and methanol in the ratio of 20:20 (v/v) and the resulting suspension was frozen at a temperature of -5° C or below. The temperature was increased stepwise from -10 to - 90° C, the suspension was freeze dried and then vacuum-dried under a vacuum of 100 mmHg for a period of 5 to 60 hours. Thus sterile and dried earthworm powder was obtained.



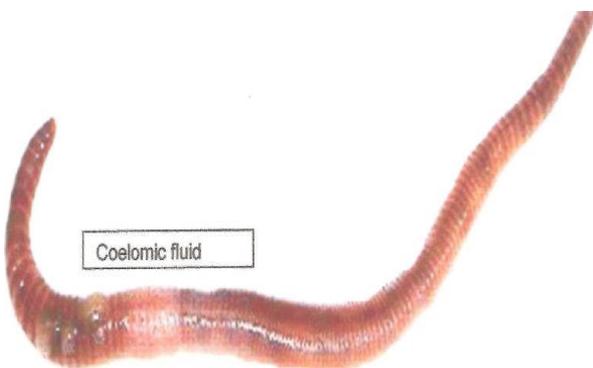
Individual earthworm



Worms taken for powder preparation



Earthworm powder



Earthworm as a medicine

Earthworms are used as a medicine in olden stages which supports our findings. In our research work earthworms were taken in two aspects i.e., earthworm powder and

earthworm coelomic fluid. Earthworm possess strong antibacterial, antifungal, antitumour, antiulceral, anti-inflammatory, analgesic, antipyretic, antioxidant and hepatoprotective activity.

Antibacterial activity

In vitro antimicrobial evaluation of earthworm powder were carried out against seven bacterial strains, two Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermids*) and five Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aureginosa*). The screening of the earthworm coelomic fluid, *P. excavatus* showed a significant activity with regard to the Gram-positive as well as Gram-negative bacteria which confirms the antibacterial activity of the earthworm

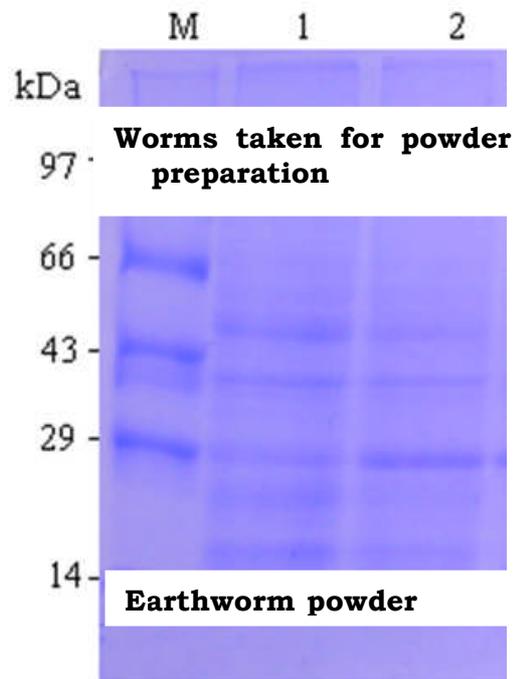


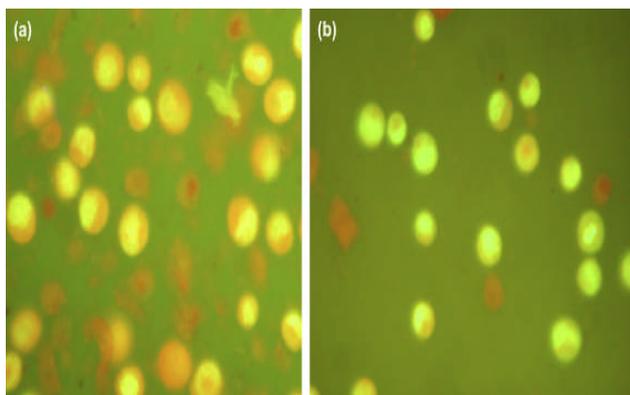
Fig shows the SDS-PAGE analysis of earthworm powder and earthworm coelomic fluid.

Antitumour activity

The coelomic fluid of earthworm was subjected to peptide isolation and the purified peptide was isolated and named as EP (earthworm peptide). The crude antimicrobial peptide (EP) specimens from earthworm coelomic fluid was tested on HeLa cells with the MTT staining method, AO/EB fluorescent staining method and DNA agars electrophoresis. The results showed that the effects of EP5 on HeLa cells lead to the cancer cell's apoptosis and break down (see Fig. 2, yellow color)

Antiulceral activity

Aspirin, a known ulcerogenic drug and pyloric ligation had significantly increased the gastric juice volume (64%), free acidity (32%) and total acidity (28%) except pH compared to normal control. These symptoms of ulcer were brought to near normal condition when the standard drug-ranitidine was administered. "Earthworm paste" administration,



Effects of EP5 to HeLa cell under fluorescence microscope, (a) 1.5 mg/mL, and (b) control

particularly 160 mg/kg, had significantly decreased the gastric juice volume, free acidity and total acidity in the ulcerated rats. These values were better than treatment with ranitidine. The ulcer index in aspirin treated rat was 15.05 ± 0.17 . This was found to get reduced in the "earthworm powder" treated animal which shows the antiulcer activity.

Antioxidant activity

Aspirin plus pyloric ligation had significantly increased the peroxidation level indicator-TBARS and decreased the anti-oxidants-SOD, CAT, GPx and GSH leading to oxidative stress compared to normal control. Administration of "earthworm powder" brought a significant reduction in TBARS and increase in the level of SOD, CAT, GPx and GSH. Similar results were observed in the ranitidine treated animal. Much better to ranitidine, treatment with 160 mg of "earthworm powder"/kg showed better results.

Anti-inflammatory and antipyretic activity

In clinical study induction of histamine induce acute phase rat hind paw oedema volume and turpentine induced chronic phase granuloma pouchweight. Administration of earthworm powder was found to exhibit better results in a dose-dependent manner. Administration of 200mg/kg earthworm powder was found to reduce the above parameters and brought to near normalcy and this results was found to be followed by administration of 50 and 100mg/kg, respectively which confirms the anti-inflammatory activity of the earthworm powder

For analgesic study the subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration to rats. Treatments with earthworm powder at the doses of 50, 100 and 200mg/kg decreased the rectal temperature in a dose-dependent manner. The antipyretic effect started from the first hour and was maintained for 4 h, after administration of earthworm powder.

Earthworm powder ameliorates alcohol-induced injury and the mechanism may involve the prevention of cell membrane disturbances and reduction of oxidative stress by free radical scavenging and antioxidant activity (Grdisa *et al.*, 2004), this in turn prevents kupffer cell activation and normalization of the altered redox state in addition to hastened the elimination of ethanol.

CONCLUSION

The usefulness of earthworm *P. excavatus* powder against various ailments in indigenous system of medicine was found to be fruitful, as evidenced from the observations of present investigation. The observations and results of the present study have opened up a new vista and paved the way to explore the usefulness of earthworms.

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