Study on the Antibacterial Activities of Coelomic Fluid of Local Earthworms against Disease Causing Microorganisms

ABSTRACT

The study was carried out on the antibacterial properties of coelomic fluid of local earthworms (*Pontoscolex corethrurus, Megascolex konkanensis, Drawida ghatensis*) against selected pathogens like *Vibrio cholera, Vibrio parahaemoliticus, Staphylococcus aureus, Salmonella typhi and Escherichia coli*. The study was conducted in School of Environmental Science and College of Veterinary and Animal Science in May 2017 to November 2017.The earthworm was collected on the basis of hand sorting method and Coelomic fluid was collected due to cold shock drips. Antimicrobial activity of coelomic fluid of earthworms was assessed by agar well diffusion method.The result revealed that the maximum inhibition zone of 18mm and 16mm showed by coelomic fluid of *P.corethrurus* and *M. konkanensis* against *S. aureus*. The *Drawida ghatensis* shows least antibacterial activities against selected pathogens. The *staphylococcus aureus* shows highest inhibition zone against coelomic fluid of all the selected earthworms and least result showing in bacterial isolate *vibrio parahaemoliticus. From the study coelomic fluid activity is good for pathogen like salmonella typhi and E.coli and have medicinal values.*

Keywords: Antibacterial activity, Coelomic fluid, Microorganisms.

1.INTRODUCTION

The Earthworms (Anelida, Clitellata, Oligochaeta) are familiar to almost everyone. The role of some species in organic matter decomposition and mineral cycling may be important[9,11], and a great deal has been written concerning earthworm farming[2]. The activities of earthworms that affect the soil involve the ingestion of soil and the mixing of the main soil ingredients of clay, lime, and humus; the construction of burrows that enhance aeration, drainage, and root penetration. The influence of earthworms on the translocation of soil material may be quite considerable. The body of earthworm is divided into a serious of uniformly placed segments. This nature of division of the body both externally and internally has enabled animal to have flexibility and for the initiation of development of good musculature. Earthworms are the first group of animals to have complete digestive system, closed circulatory system with haemoglobin in the plasma as carrier of oxygen and carbondioxide.

The pathogens are firstly bacteria living in water or soil that are ingested during feeding or introduced into the body following injury. During the course of evolution, earthworms have developed defense strategies against these living pathogens. Earthworms lack true antibodies and hence an adaptive immune response and instead have efficient innate immunity system to defend themselves against invading foreign materials. In living organisms, peptides are an important defense component, many peptides were found in various living organisms.

The earthworms are coelomate animals and filled with coelomic fluid – a milky alkaline liquid that helps the worm in locomotion nutrition, excretion, detoxification of tissues, heavy metal accumulation and protects internal organs from external jerks, destroys bacterial attack, prevents desiccation, promotes cutaneous respiration and internal acclimation. (Bilej et al., 2000; Weidong et al., 2003) [11,12]The coelomic fluid of earthworms and their body extracts were known to have antimicrobial and many medicinal properties since 1340. Studies done by many workers have strongly pointed out that coelomic fluid of earthworm like *Eisenia foetida, Eudrilus euginae, Polyphertima elo-ngate, Perionyx excavates, Lampito maurtii, L. rubellus and Perionyx sansibaricus* have medicinal and antibacterial properties. Earthworm has been recognized in prenatal medicine and anti-inflammatory, analgesic and antipyretic agent.

Except for congenital diseases, all other diseases are caused by certain microorganisms. Such microbes are called pathogens. They may affect or damage the whole body system or some specific tissues or organs. Some human diseases caused by pathogens are polio, cholera, mumps, rabies, malaria etc. Plants and animals are also victims of these microbial effects.Some microorganisms need vectors or agents for their transmission. Malaria is an example of the harmful microorganism.

It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious*, S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections. Typhoid fever, also known simply as typhoid, is a bacterial infection due to *Salmonella typhi* that causes symptoms. Symptoms may vary from mild to severe and usually begin six to thirty days after exposure. Often there is a gradual onset of a high fever over several days. Weakness, abdominal pain, constipation, and headaches also commonly occur. Cholera is an acute, diarrheal illness caused by infection of the intestine with the bacterium *Vibrio cholerae.*

Vibrio parahaemolyticus is a self-limiting, enterotoxic bacterium, typically causing acute gastroenteritis in humans. More severe cases of infection can occur in immune-compromised individuals, which can lead to septicemia and death, although this is very rare. Moderate to severe skin infections can also result from open wound exposure to *V. parahaemolyticus* in warm seawater, although this occurs less frequently than illness following ingestion of the organism. *E. coli* refers to a wide range of bacteria that can cause various diseases, including pneumonia, urinary tract infections, and diarrhea. Most strains of *E. coli* are harmless to humans. Some strains of *E. coli* infection can include nausea, vomiting, and fever.

- To study the antibacterial activities of coelomic fluid of local earthworms against disease causing microorganisms.
- To study the antibacterial on pathogenic bacteria using well diffusion method.

2.MATERIALS AND METHODS

2.1.Collection of earthworm

Hand sorting method was used for collecting earthworms. This method is widely used for sampling earthworms in India (Edward and Lofty,1977; Reynold,1977). The quadrate is provided on 20*20*30cm2 and are gently broken and the worms are hand sorted (Julka,1993). The earthworm samples were collected from the field were pesticides cannot be used. The collected earthworms were identified with the help of standard manual and experts. The study mainly focused on four species of earthworms both exotic and native such as *Pontoscolex corethrurus* (Muller, 1856), *Megascolex konkanensis* (Fedarb,1898) and *Drawida ghatensis* (Michaelsen,1910).

Pontoscolex corethrurus *Megascolex konkanensis* *Drawida ghatensis*

Figure1: Earthworms selected for the study

2.2.Microorganisms used

Test organisms were collected from the Environmental Microbiology Lab, School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala. These include the standard cultures of *Vibrio cholera, Vibrio parahaemoliticus, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli.*

2.3.Coelomic fluid collection method

The selected local earthworms were washed in distilled water and they were placed on ordinary wet filter paper in plastic tough which is covered by alluminium foil with fine pin holes. After 48 hrs, the gut was cleared of organic matter as they feed on filter paper. Coelomic fluid was collected by placing the earthworms in petri plates held in a slanting position in the palm. Their body surface was rubbed with wet finger and later with ice cubes taken in a beaker. The coelomic fluid released due to cold shock drips and gets collected at the lower side of the Petriplate. The released fluid from their body was collected by Pasteur pipette.

2.4. Antimicrobial activity (Well diffusion method)

The young culture of selected pathogens *Vibrio cholera (1), Vibrio parahaemoliticus (2), Staphylococcus aureus(3), Salmonella typhi(4)* and *E.coli (5)* were prepared in nutrient broth(1.3gm in 100ml and inoculated in 10ml) and lawn culture of different pathogens were prepared by swabbing young culture (16-18hrs) in Muller Hinton agar and waited for 15 minutes to absorb the culture to the medium. Agar wells (3mm) in diameter were punched in the plates using a sterile gel puncture. 30µL of coelomic fluid were pipetted into the well and plates were incubated for 24hrs in an incubator. Zone of inhibition around the wells were recorded in mm.

3. RESULTS

The coelomic fluid of selected earthworms *Pontoscolex corethrurus, Megascolex konkanensis and Drawida ghatensis* was collected by cold shock method were tested for antibacterial activity against 5 pathogenic bacteria using well diffusion method. The antibacterial property of coelomic fluid against selected five bacterial strains was found that *M. konkanensis* shows high zone of inhibition for *staphylococcus aureus* compare with all the three earthworms. The *Pontoscolex corethrurus* shows high antibacterial activity against *staphylococcus aureus* then *vibrio cholera* and the least activity shows in *vibrio parahaemoliticus.*In *Pontoscolex corethrurus* no activity shown in *S typhi. Megascolex konkanensis* shows higher zone of inhibition against *S. aureus*, follows *E.coli, then S typhi no ativity shown in V.parahaemoliticus. Drawida ghatensis shows higher zone of inhibition in S.typhi, follows S.aureus and E.coli, no activity shown in both V.cholera and V.parahaemoliticus. A maximum inhibition zone of 18mm and 16mm showed by coelomic fluid of P.corethrurus and M. konkanensis against S. aureus .*

Table 1: Zone of inhibition of earthworms against selected bacterial strain

Bacterial isolates	Zone of inhibition(mm)		
	P.corethrurus	*M. konkanensis*	*D.ghatensis*
V. cholera	14	10	0
V.parahaemoliticus	8	0	0
S. aureus	16	18	9
S typhi	0	14	10
E.coli	11	15	0

4. DISCUSSIONS

The extraction of coelomic fluid from earthworms, cold shock method is a novel method as it won't require any apparatus and more importantly used earthworms are unharmed and show normal activities even after collection of coelomic fluid several times.The coelomic fluid of selected earthworms shows good antibacterial properties and have good medicinal value. The antibacterial property of coelomic fluid in the *megascolex konkenensis* shows high medicinal values against *staphylococcus aureus* and *e.coli*. The selected pathogens are common disease causing bacteria. The coelomic fluid contains several bioactive compounds such as proteins, exhibits a variety of biological functions such as antibacterial, anticancer, haemolytic, cytotoxic, hemagglutinating andproteolytic activities etc.

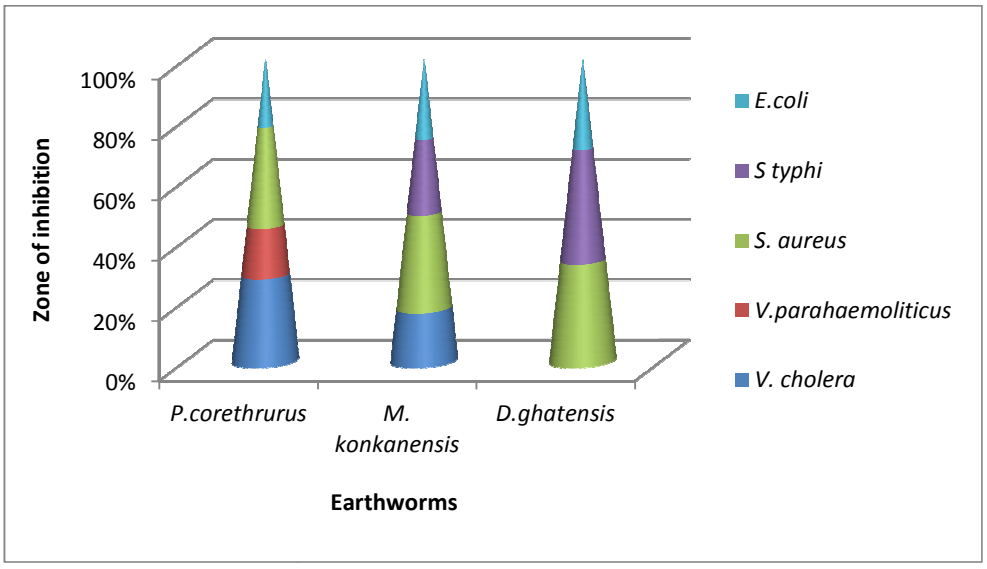

Figure2: Antibacterial activity of earthworms against selected bacterial strain

CONCLUSION

The present work deals Study on the Antibacterial Activities of Coelomic Fluid of Local Earthworms against Disease Causing Microorganisms. The coelomic fluid of *Pontoscolex corethrurus, Megascolex konkanensis, Drawida ghatensis* shows antibacterial properties against Vibrio cholera, *Vibrio parahaemoliticus, Staphylococcus aureus, Salmonella typhi and Escherichia coli*. The *Megascolex konkanensis* shows high antibacterial activity for these two bacterial strains. *Drawida ghatensis* shows least antibacterial activity. So this work suggest some of the coelomic fluid components might be useful for pharmaceutical applications and it can pave way for purifying the biomolecules to introduce in the field ofpharmaceuticals.

ABBREVIATIONS

P.corethrurus - *Pontoscolex corethrurus*

M.konkanensis - *Megascolex konkenensis*

D.ghatensis - *Drawida ghatensis*

V.cholera -*Vibrio cholera*

V.parahaemoliticus -*Vibrio parahaemoliticus*

S.aures -*Staphylococcus aureus*

S.typhi -*Salmonella typhi*

E.coli -*Escherichia coli*

REFERENCES

1) Cooper, E., Balamurugan, M., Huang, C.Y., Tsao, C.R., Heredia, J., and Tommaseo-ponzetta, M., et al. Earthworms dilong: Ancient, inexpensive, non controversial models my help clarify approaches to integrated medicine emphasizing neuroimmuno systems. Evid. Based complement. Alternative medicine, 2012. 20(12): 152-164.
2) Ismail, S.A. 1997.Vermitechnology The biologyof Earthworms,Hyderabad.Orient Longman
3) Julka, J.M. 1993. Earthworm Resource and Vermiculture. Zoological Survey of India earthworms. Journal of microbiology residence technology. 47(44):237-253.
4) Lavelle, P. 1988.Earthworm activities and the soil system. Biology of fertile soil fertile, 6: 237-251.
5) Reynolds, J.W., and Reynolds, W.M. 1972. Earthworms in medicine. Am. Journal of Nursing, 120(72): 1273-1283.
6) Stevenson, J. 1930. Oligochaeta. Claredon press oxford.
7) Talashilkar, S.C. 2005. Earthworm in Agriculture. Agrobios, Jodhpur, India, pp: 48-77.
8) Waksman, S.A., Bugie, E. and Schatz, A. 1944. Isolation of antibiotic substances from soil microorganisms, with special reference to streptothricin and streptomycin. in: Proc Staff Meet Mayo Clin. pp: 6:537–548.
9) Barnett, H.L. and Hunter, B.B. (1972). Illustrated Genera of Imperfect Fungi. 3rd Edition, Burgess Publishing Co., Minneapolis, 241 .
10) Edwards, C. A. and Lofty, J. R. 1972. Biology of earthworms. Chapman & Hall, London. 283 pp
11) Weidong Pan, Sianghui Liu, Feng Ge, Tao Zheng.Reconfirmation of anti-microbial activity in the coelomicfluid of the earthworm, Eisenia fetida andrei bycolorimetric assay. J Biosci. 2003; 28(6):723-731.
12) Bilej M, De Baetselier P, Beschin A. Anti-microbial defence of the earthworms. Folia Microbiol. 2000; 45:283-300.