

Evaluation of anti-inflammatory activity in extracts of three epigeic earthworm species

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Abstract: Earthworm tissue and their coelomic fluid contain many bioactive compounds that have potential medicinal properties. The present study was carried out to compare and evaluate anti-inflammatory activity in extracts of three epigeic earthworm species *Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus* through protein denaturation assay. The results of the present study revealed that the standard drug, Aspirin showed highest percent inhibition activity with minimum IC₅₀ value as compared to the extracts of three epigeic earthworm species. Among three epigeic earthworms, *Eisenia fetida* showed strong percent inhibitory activity with minimum IC₅₀ value followed by *Perionyx excavatus* and *Eudrilus eugeniae*. The percent inhibitory activity was directly proportional to the increase in the test concentration of all samples of earthworm extracts and even in standard drug, Aspirin.

The results of the present study highlights the importance of further research on epigeic earthworm species for isolation of bioactive compounds present in it and their possible utility in the formulation of new drugs against many diseases with low cost treatment and least side effects.

Keywords Anti-inflammatory activity, Epigeic earthworms, *Eudrilus eugeniae*, *Eisenia fetida*, *Perionyx excavatus* and standard drug, Aspirin.

1. INTRODUCTION

Earthworms are terrestrial invertebrates play an important role in decomposition of organic matter, recycling of nutrients, soil structure and soil productivity, through their feeding, and fragmentation. The importance of earthworms in environment and organic waste management is well documented and understood by various researchers [1,2,3,4,5]. Earthworms are also known as biological indicators of soil quality, the presence of these creatures indicate that the availability of large population of bacteria, viruses, fungi, insects, spiders and other microorganisms that leads to a healthy soil system [6,7].

The earthworms have been known to be the source of important biomolecules such as protein, peptides, enzymes and other physiologically active substances. The literature suggests that the extracts prepared from earthworms have been used for the treatment of numerous diseases. Earthworms have been used in medicine for various remedies since 1340 A.D. [8].

The traditional use of earthworms in medicine is supported by the findings showing anti-diuretic, anti-spasmodic, anti-microbial and anti-inflammatory properties as reported by Cooper and Balamurugan [9]. Earthworms have also been equally valued as an anti-ulcer, anti-oxidant and anti-inflammatory activity in India. Some of the investigations such as an earthworm paste prepared from *Lampito mauritii* demonstrated the biological activity comparable to the anti-ulcer drug, Ranitidine in animal studies [10].

Earthworm tissue/fluid contains many bioactive compounds, effective against inflammatory, hematological oxidative and nerve diseases etc [11,12,13]. Metabolites extracted from earthworms tissue or coelomic fluid have been reported as anti-microbial [14], anti-inflammatory, anti-pyretic [15] and anti-cancer [16] agents. It has also been reported that biological activities and medicinal properties of earthworm may vary depending up on the species and their living environment or habitat.

Therefore, it is necessary to study and demonstrate various biological activities and medicinal properties of different epigeic earthworm species like *Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus* used in vermiculture. These three species are extensively used in vermiculture for vermicomposting and worm biomass production as they are voracious feeder and breeder throughout the year. Hence, the present study was undertaken to evaluate anti-inflammatory activity in the tissue extracts of three epigeic earthworm species.

2.METHODOLOGY

Collection of earthworms

Adult epigeic earthworms such as *Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavates* were obtained from stock culture maintained at Department of Studies in Zoology, Karnatak University, Dharwad (Karnataka), India.

Preparation of earthworm powder

The earthworm powder was prepared as per the method prescribed by Shradha and Pratik [17] with slight modifications. About 30 sexually matured all three epigeic earthworm species were washed separately in running tap water to remove any dirt from the body surface, then these earthworms were fed with soaked tissue paper for about 24 hours so as to clean their gut properly. The gut cleared earthworms were again washed in distilled water, then kept them in tightly covered petriplates and were exposed to sunlight for drying. The whole dried earthworms were crushed using mortar and pestle so as to produce brown colored powder, then it is stored in an air tight plastic cover and used it for evaluation of anti-inflammatory activity through in-vitro protein denaturation assay.

In-vitro protein denaturation assay

Protein denaturation assay is the process in which proteins lose their tertiary and secondary structure by the application of external stress like heat or chemical compounds such as strong acid and base or inorganic salts. Most of the proteins lose their biological functions, when denatured. Denaturation of proteins is well documented cause of inflammation. *In-vitro* protein denaturation assay of different epigeic earthworm species was assessed by using a standard drug, Aspirin for anti-inflammatory activity.

Protocol

The reaction mixture consists of 1ml of PBS+50µl of BSA were added to 1.5 ml centrifuge tube with different concentrations of 25, 50, 100, 200 and 400 µl/ml of earthworm tissue extract samples with standard solution were incubated for 15 minutes at room temperature. Denaturation of protein was induced by keeping the samples in hot water bath at 70°C for 15 minutes. The absorbance was measured at 660nm through Labmann UV-Visible spectrophotometer.

The percent inhibition of protein denaturation assay of different concentration of test samples and standard drug, Aspirin was calculated by using the following formula:

$$\text{Percent protein denaturation assay} = [(Ac - At) / Ac] \times 100$$

Where, Ac and At are the absorbance of control and test samples respectively. Aspirin was used as standard drug as positive control for test concentrations of different epigeic earthworm species samples. The concentration of Standard drug/sample need to inhibit the protein denaturation by 50% (IC₅₀ value) was generated from the dose response curve.

Statistical analysis

Two-way ANOVA was used to analyze the significant difference in mean values of anti-inflammatory activity among and between three epigeic earthworm samples and standard drug. The data were expressed in Mean ± SE.

3.RESULTS

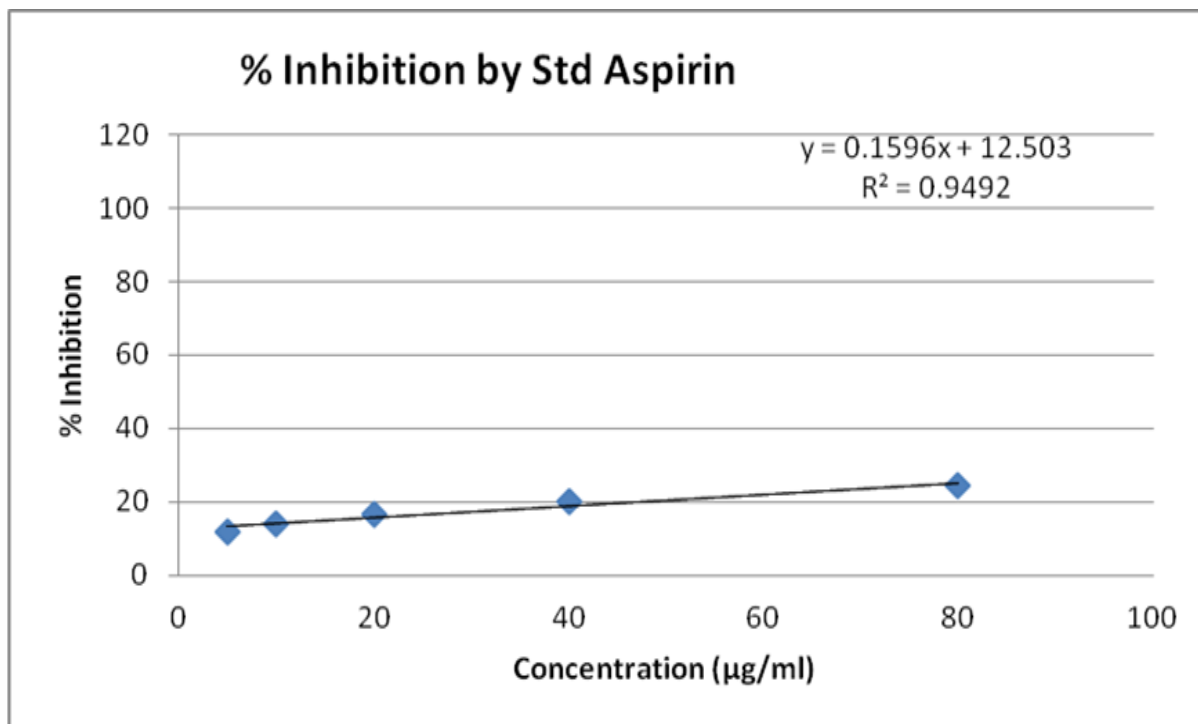
The anti-inflammatory activity of the selected three epigeic earthworm species was determined/tested through protein denaturation assay as per Leelaprakash and Dass [18]. The results of the present assay were represented in the Table-1 and Figure 1-5. The percent inhibition activity was analyzed with various test concentrations i.e. 5, 10, 20, 40 and 80µg/ml for standard control drug (Aspirin) and 25, 50, 100, 200 and 400µg/ml of epigeic earthworm species samples. The percent inhibition activity increased gradually as the concentration of the test sample increased in both standard control drug (Aspirin) as well as in earthworm extract samples (Table-1). The result revealed that the percent inhibition was comparatively more in *Eisenia fetida* (43.26%) followed by *Perionyx excavates* (38.66%) and *Eudrilus eugeniae* (22.13%) at 400µg/ml, where as the percent inhibition was comparatively more 24.54% with standard drug, Aspirin at 80µg/ml as compared to earthworm samples (Table-1).

Among all the test samples, including the standard drug, the IC₅₀ value (Half the maximum inhibitory concentration) was minimum in standard drug (Aspirin) with 235.31±5.14µg/ml followed by *Eisenia fetida* (476.54±4.02µg/ml) and *Perionyx excavates* (526.16±5.13µg/ml) and it was maximum in *Eudrilus eugeniae* with 844.00±6.65µg/ml (Table-1 and Figure-5).

Table-1: Results of protein denaturation assay for anti-inflammatory activity with respect to percent inhibition and IC50 values of Standard drug, Aspirin and various test concentrations of three epigeic earthworm species (EE, EF and PE).

Sl. No.	Test samples	Conc. of test samples (µg/ml)	ODD at 660nm	Percent inhibition	IC50 Value*	Significant value at P≤0.05
1	Std. drug Aspirin (control)	5	0.348	11.86	235.31±5.14 µg/ml	F - Value = 2211.00 and P - Value = 0.00
		10	0.340	14.04		
		20	0.329	16.84		
		40	0.316	19.95		
		80	0.298	24.54		
2	<i>E. eugeniae</i>	25	0.395	0.10	844.00±6.65 µg/ml	
		50	0.389	1.64		
		100	0.375	5.14		
		200	0.339	14.28		
		400	0.308	22.13		
3	<i>E. fetida</i>	25	0.395	0.16	476.54±4.02 µg/ml	
		50	0.390	1.19		
		100	0.292	26.09		
		200	0.289	26.89		
		400	0.224	43.26		
4	<i>P. excavatus</i>	25	0.387	2.15	526.16±5.13 µg/ml	
		50	0.386	2.40		
		100	0.359	9.09		
		200	0.331	16.32		
		400	0.242	38.66		

*Data are in Mean ± SE



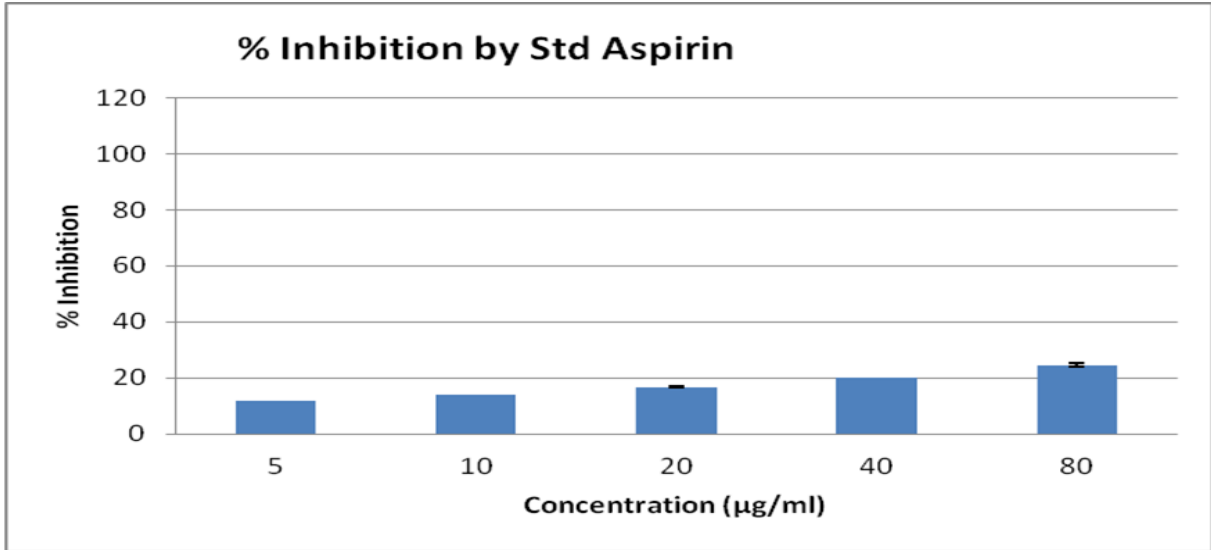


Figure-1: Percent inhibition of protein denaturation assay with respect to different test concentrations of the standard anti-inflammatory drug, Aspirin (Control).

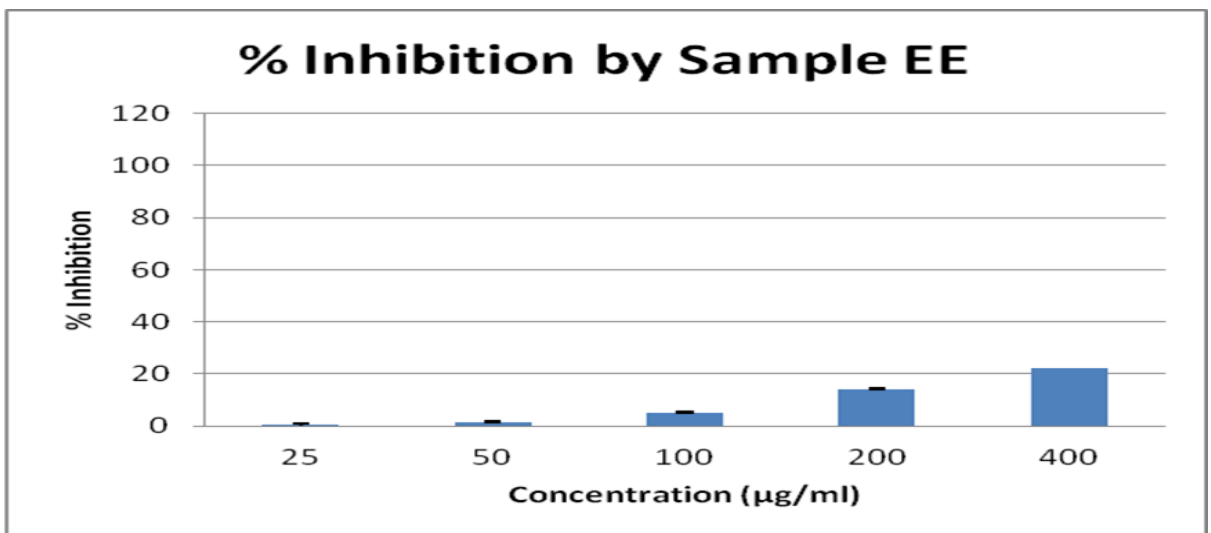
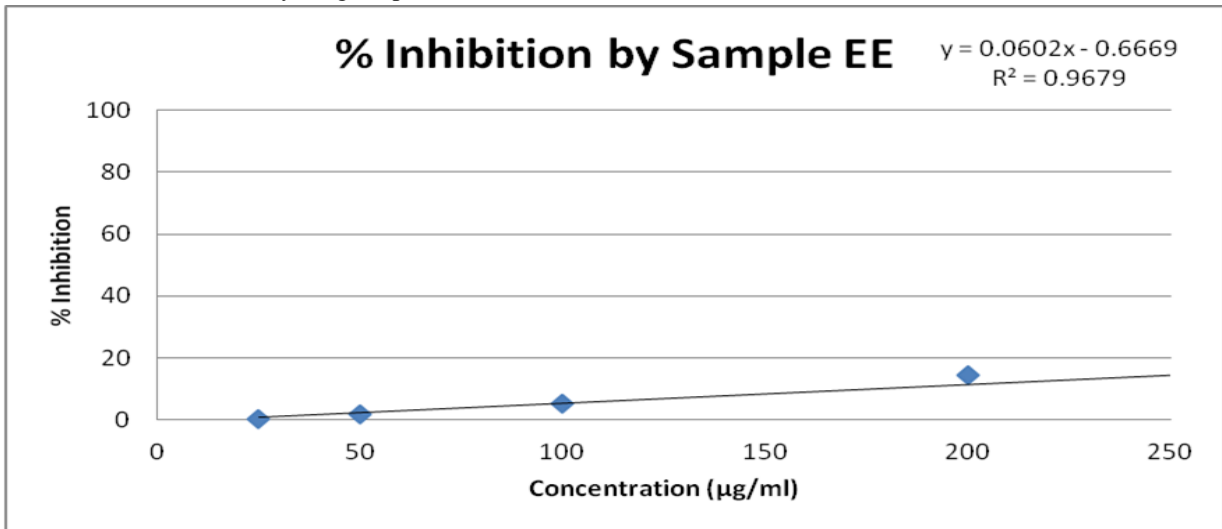


Figure -2: Percent inhibition of protein denaturation assay with respect to various test concentrations of the epigeic earthworm, *E. eugeniae*.

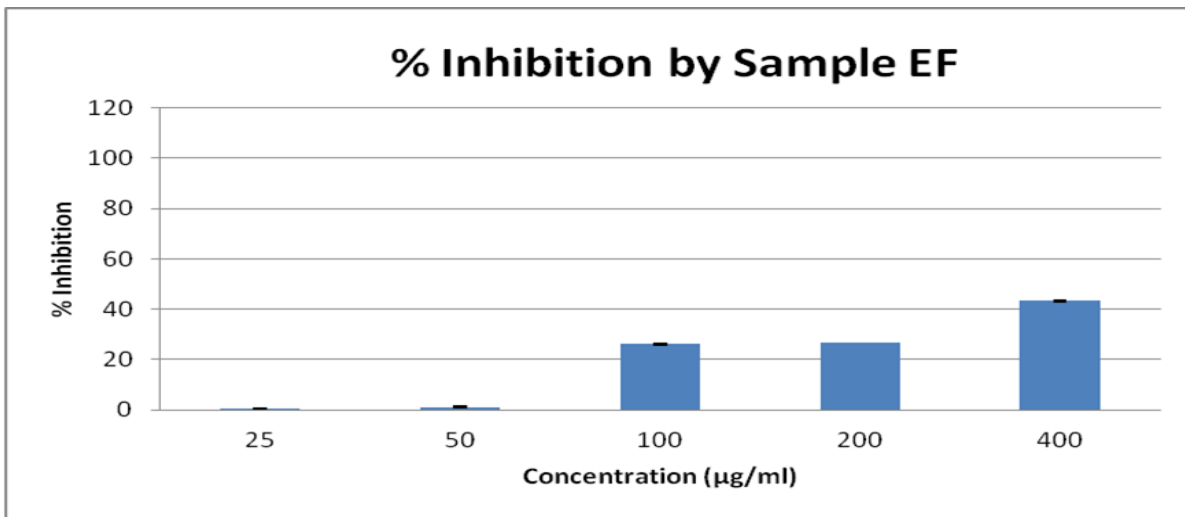
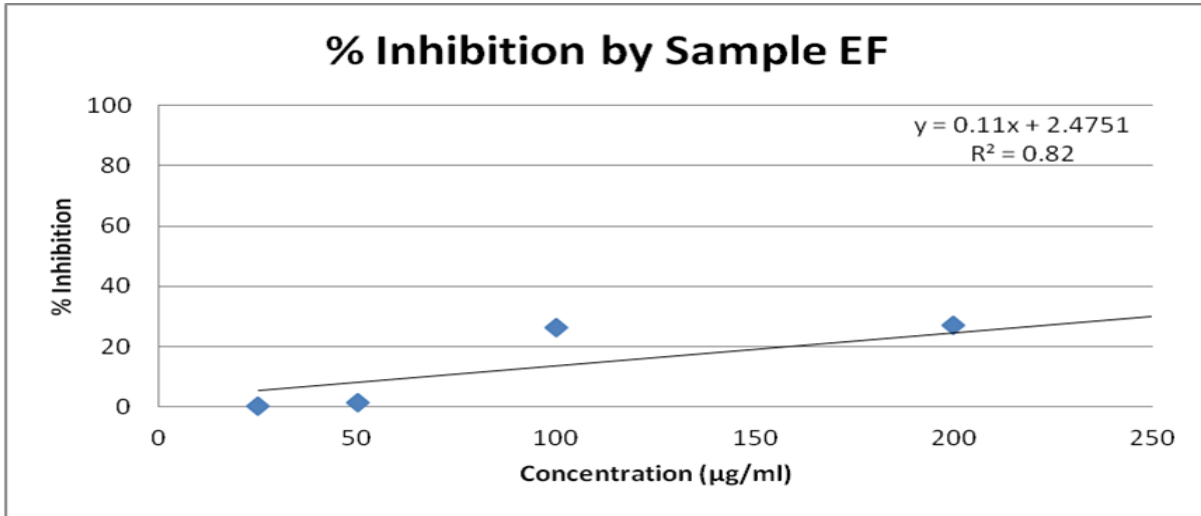
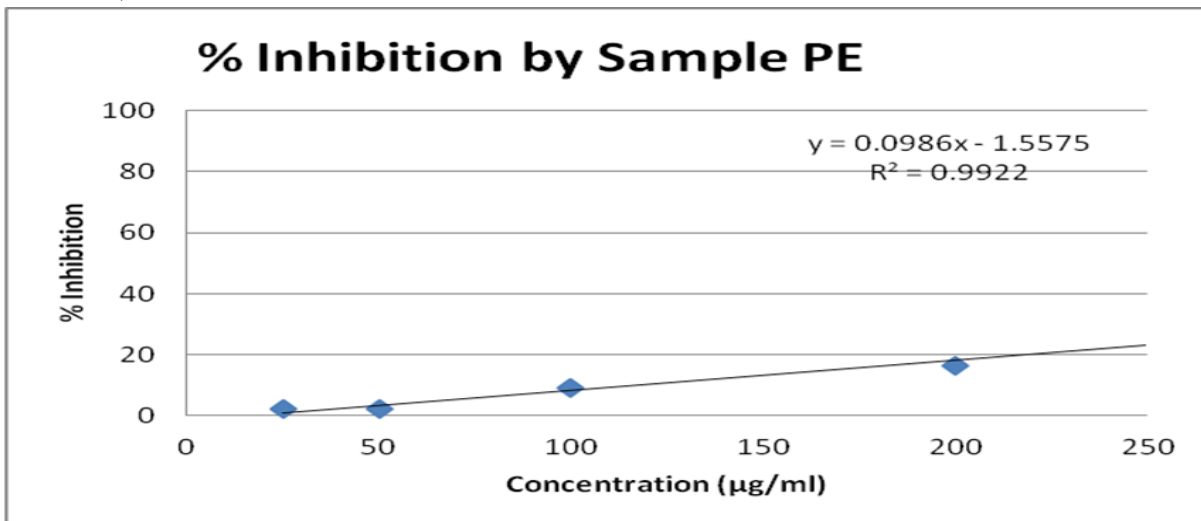


Figure -3: Percent inhibition of protein denaturation assay with respect to various test concentrations of the epigeic earthworm, *E. fetida*.



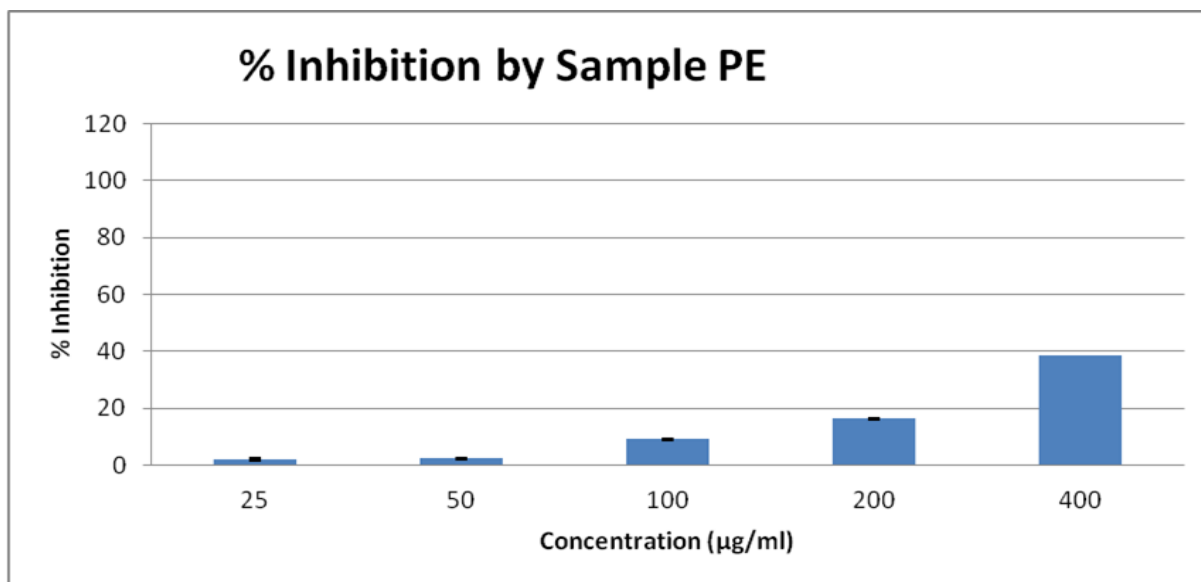


Figure -4: Percent inhibition of protein denaturation assay with respect to various test concentrations of the epigeic earthworm, *P. excavatus*.

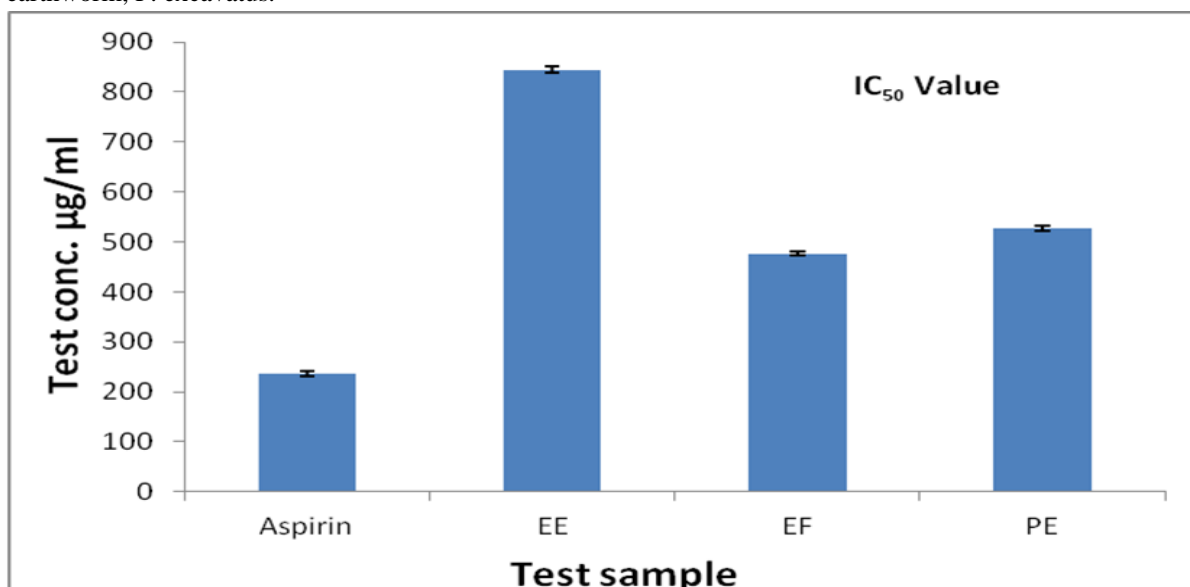


Figure - 5: IC₅₀ value of anti-inflammatory protein denaturation assay with respect to standard drug, Aspirin and samples of different epigeic earthworm species (EE, EF and PE).

4.DISCUSSION

The results of the anti-inflammatory assay indicate that the epigeic earthworm *Eisenia fetida* had maximum inhibitory activity with minimum IC₅₀ value. But the inhibitory activity was minimum in *Eudrilus eugeniae* with maximum IC₅₀ value. As per the data of the present study, the percent inhibition of *Eisenia fetida* and *Perionyx excavates* were more than that of the standard drug, Aspirin and the IC₅₀ value

was minimum (Table-1). However, there is a significant variation ($P \leq 0.05$) in IC₅₀ among various test concentrations of different samples carried out in this study.

Inflammation is due to denaturation of protein is well documented and several anti-inflammatory drugs have shown concentration dose dependent ability to inhibit thermally induced protein denaturation. The ability of different earthworm extract to inhibit denaturation of protein is a reflection of its anti-inflammatory activity.

The extracts of different epigeic earthworms and the standard drug, Aspirin displayed concentration dependent anti-inflammatory activity at different test concentrations from 5 to 80µg/ml for standard drug and from 25µg/ml to 400µg/ml with respect to earthworm extracts in our studies.

The percent inhibition and IC₅₀ values were significantly differed ($P \leq 0.05$) among different epigeic earthworm samples along with standard drug, Aspirin, this may be attributed to the difference in presence of various bioactive compounds like phenol, steroids, flavonoids, glycosides present in the tissues of different epigeic earthworm species (EE, EF & PE). Some biomolecules present in the earthworm tissue have been known to inhibit enzymes as well as mediators of inflammation process like C-reactive protein and adhesion molecules. The anti-inflammatory activity of different earthworms has been reported by Ismail et al.[19] and Balamurugan et al. [20]. However, complete analysis of these biomolecules is very much necessary to assess and correlate properly with respect to anti-inflammatory activity. Further, such detailed studies are essential to refine the techniques so as to isolate and test the active principles/ molecules involved in such activities to get rid of various diseases in future.

5.SUMMARY

The results of the present study revealed that the standard drug, Aspirin shows highest percent inhibition (24.54%) with minimum IC₅₀ value ($235.31 \pm 5.14 \mu\text{g/ml}$) as compared to the extracts of different epigeic earthworm species (*Eisenia fetida*, *Eudrilus eugeniae* and *Perionyx excavates*). The percentage inhibition was directly proportional to the increase in the test concentrations of all the samples of earthworm extracts including standard drug, Aspirin. Among the extracts of different epigeic earthworm species, *Eisenia fetida* showed highest percent inhibition (43.26%) with minimum IC₅₀ value ($476.54 \pm 4.02 \mu\text{g/ml}$) as compared to *Perionyx excavates* ($526.16 \pm 5.13 \mu\text{g/ml}$) and *Eudrilus eugeniae* ($844.00 \pm 6.65 \mu\text{g/ml}$).

6.CONCLUSION

From the results of the present study, it can be concluded that of the three tested species of epigeic

earthworms, *Eisenia fetida* extract seems to have a relatively higher level of anti-inflammatory activity as compared to other two earthworm species, *Perionyx excavates* and *Eudrilus eugeniae*. The results of the present study also highlight the importance of bioactive compounds present in the earthworm tissue/extracts for isolation and extraction of different biomolecules and their possible usage as medicine in near future. This is a preliminary study to evaluate anti-inflammatory activity present in different epigeic earthworms, still many in-vitro and in-vivo tests are necessary to validate this research.

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