IJPSR (2011), Vol. 2, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 September, 2010; received in revised form 25 November, 2010; accepted 13, January 2011

ANTI-INFLAMMATORY ACTIVITY OF EARTHWORM EXTRACTS

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Keywords:

Eudrilus eugeniae,
Anti-inflammatory activity,
95% Ethanol,
Petroleum ether,
0.2 M Phosphate buffer (pH, 7.0)

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ABSTRACT

Earthworm plays a major role in the proper functioning of the soil ecosystem. It acts as scavenger and helps in recycling of dead and decayed plant material by feeding on them. Earthworm increases the soil fertility and is often referred to as a farmer's friend. Earthworms have been used in medicine for various remedies. In the present investigation, various solvent extracts of an earthworm, *Eudrilus eugeniae* were prepared and anti-inflammatory activity of these extracts were determined. The petroleum ether fraction possessed maximum anti-inflammatory activity in carrageenan induced albino rats in comparison to 95% ethanol and 0.2 M phosphate buffer (pH, 7.0) extracts. The paw volume was determined and was compared with that of aspirin, a standard anti-inflammatory drug. The results indicates that petroleum ether fraction of earthworm extract possessed similar anti-inflammatory activity as that of aspirin.

INTRODUCTION: Earthworms have been used in medicine for various remedies since 1340 AD [1]. Earthworm has been recognized in oriental medicine as anti- inflammatory, analgesic and antipyretic agent [2]. It shows anticancer effect by preventing excess glucose uptake[3]. Microorganisms are known to play a major role in soil characteristics, invertebrates are believed to act as regulators of antimicrobial activity. Earthworm surface excreta were found to have potent antimicrobial activity[4]. It is also having anti-coagulatory or fibrinolytic activity which results in the facilitation of blood circulation[5]. The earthworm has been suspected to contain proteases which dissolve the fibrin clots or anticoagulants which selectively interfere with the intrinsic pathway of blood coagulation cascade [6-10]. Medicinal properties of earthworm have been described [11-15].

Earthworms have largely been used internally and externally as powerful aphrodisiacs [16]. Anti-inflammatory activity of earthworm extracts was studied [17]. The anti-inflammatory and antipyretic activities of biologically active extract isolated form whole earthworm, *Lampito mauritii* were determined [18]. Antimicrobial potency of *Eudrilus eugeniae* extracts on certain plant pathogens were studied [19]. Antitumor activities of earthworm fibrinolytic enzyme on human hepatoma cells were studied [20]. The species selected for study was *Eudrilus eugeniae.* This species is native of Africa and is having good reproduction and maturation capability. In the present investigation, different solvents were used on the basis of increasing polarity such as petroleum ether, 95% ethanol and 0.2 M (pH, 7.0) phosphate buffer to prepare earthworm extracts in order to assess their anti-inflammatory activity.

MATERIALS AND METHODS: All the chemicals and reagents used were from C.D.H and Ranchem. Glasswares used were from Borosil.

Collection of Earthworms: Fully matured earthworms were collected from Jay Bharat Nursery, Ranipokhri, Rishikesh (U. K.) India, and the worms were washed in running tap water in order to remove the sand particles from the surface of earthworms. Then after washed earthworms were soaked in N-saline and solution was exchanged after every time so that the gut of earthworms gets thoroughly cleaned.

Preparation of Earthworm Extracts: The method for preparation of plant extract was modified [21]. About 10 to 20 g of the earthworms were homogenized separately in different solvents used according to decreasing polarity such as that is phosphate buffer (0.2 M, pH 7.0), 95% ethanol and petroleum ether. The homogenized mixtures prepared separately in different solvents were filtered and the filtrates obtained were condensed in water-bath at 35^0C. The crude extracts obtained were diluted in 10% DMSO for evaluation of anti-inflammatory activity.

Determination of Anti-Inflammatory Activity of Earthworm Extracts: Animal model was used for testing the anti-inflammatory activity of earthworm extracts. Healthy male albino rats weighing to 150-200 g were selected for study. The animal was divided into 5 groups of 5 rats each. Pedal edema was produced by injecting 0.1 ml carrageenan in left hand paw. Paw volumes were measured before and after 3 h of the injection to record the degree of inflammation. Three hours following the injection, the first group (control) was offered distilled water orally. The second group was offered Aspirin (160mg kg^{-1} body weight). The 3^{rd}, 4^{th} and 5^{th} body weight groups were offered petroleum ether fraction, 95% ethanolic and phosphate buffer (160 mg/kg body weight) respectively. This dosage of 160 mg/kg has been standardized in previous studies [17-18]. Paw volumes were measured again after 1h and later after 15 h of treatment following anesthetization of the rats.

This experiment was performed in triplicates and the efficacy of different earthworm extract was compared to standard positive anti-inflammatory drug, aspirin.

RESULTS AND DISCUSSION:

Determination of Anti-inflammatory Activity of Earthworm Extracts: For determination of anti-inflammatory activity of earthworm extract, animal model was used and healthy male albino rats weighing 150-250g were selected. The petroleum ether fraction possessed maximum anti-inflammatory activity in carrageenan induced albino rats. The paw volume was determined and was compared with that of aspirin, a standard anti-inflammatory drug. The results indicates that petroleum ether fraction of earthworm extract possessed similar anti-inflammatory activity as that of aspirin (**Table 1**).

Our results were in accordance with the previous studies done [17-18] on this aspect. The petroleum ether fraction amounts to 8.5% of Total Extracted Paste (TEP). NMR and IR spectroscopy analysis of this fraction indicates the presence of steroids. The nature of these steroids is not hereby mentioned.

TABLE 1: DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY OF VARIOUS FRACTIONS OF EARTHWORM EXTRACTS

Experiment	Paw volume ± SD				
	Control	Aspirin	Petroleum ether fraction	Ethanolic fraction	Phosphate buffer fraction (pH,7.0)
Normal	2.75±0.21	2.70± 0.10	2.60± 0.12	2.80±0.10	3.24±0.12
3hafter carrageenan injection	3.80±0.18	3.70±0.39	3.20±0.27	3.75±0.12	3.36±0.20
1 h after treatment	3.60±0.13	3.20±0.25	2.80 ±0.15	3.40±.0.30	3.72±0.30
1.5h after treatment	3.65±0.36	2.50±0.20	2.30 ±0.08	3.68±0.19	3.74±0.11

Carrageenan-induced edema is a model of acute inflammation and the agents responsible for reducing the edema are useful as anti-inflammatory agents. Carrageenan-induced edema is mediated through histamine and serotonin in the first hour. The petroleum ether extract of earthworm showed significant reduction in paw volume similarly to that of aspirin. The studies thus confirm that constituents of earthworm extract solublise in petroleum ether and other non polar solvents can be utilized in preparation of anti-inflammatory drug or can act as potent anti-inflammatory agent. Further studies are needed to refine the technique and to isolate and test the active principles are, however needed.

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