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Curative Effect of Earthworm Extract, *Lampito mauritii* (Kinberg) on Inflammation, Oxidation and Blood Profiles in Rats

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ABSTRACT

Information is available on the therapeutic properties of different natural herbal products and formulations and very few studies have been made on the sources from animal origin, especially earthworms. Therefore our aim was to investigate, *in vivo*, the potential of *Lampito mauritii* as anti-inflammatory, anti-oxidative agent and further its positive influence on blood profiles of inflamed rats. Male albino Wistar rats were used for the study. The inflammation of subcutaneous dorsal granuloma pouch was induced by injecting 25 ml of sterile air, followed by injection of 0.5 ml of turpentine oil for 7 days. Rats were treated with earthworm extract (EE) at three different doses (50, 100 and 200 mg/kg body weight) orally. The anti-inflammatory property of EE was studied by weighing the granuloma pouch and the anti-oxidant property of EE was studied by assessing levels of reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and thio barbituric acid reactive substances (TBARS). The haematological studies included differential cell counts and haemoglobin content along with measurements of serum protein albumin, cholesterol and alkaline phosphatase in inflamed rats. The administration of indomethacin (10 mg/kg) or different doses of earthworm extract (50, 100 and 200 mg/kg), reduced inflammation, normalized activity of glutathione peroxidase, catalase, superoxide dismutase, reduced glutathione and thio barbituric acid reactive substances in liver and normalized the blood cell count and haemoglobin content. The serum protein, albumin, cholesterol and alkaline phosphatases (ALP) reached the normal level in inflamed rats after treatment. Significant results were obtained in 200 mg/kg of earthworm extract/kg body weight administered rats. The potential of anti-inflammatory and anti-oxidant nature of earthworm extract can be attributed to the presence of phenolic substances in the earthworm tissue.

Keywords: antiinflammation, antioxidation, earthworm extract, blood profiles, rats
Abbreviations: **ALP**, alkaline phosphatase; **CAT**, catalase; **EE**, earthworm extract; **GPx**, glutathione peroxidase; **GSH**, reduced glutathione; **RBC**, erythrocyte corpuscle; **SOD**, superoxide dismutase; **TBARS**, thiobarbituric acid reactive substances; **WBC**, leukocyte corpuscle

INTRODUCTION

Our pre-historic ancestors have been exploring natural compounds to cure their ills, improve and enrich their own lives. Most of these compounds were derived from plants and animals. Edwards and Bohlen (1996), Cooper *et al.* (2004), Cooper (2005) and Balamurugan *et al*. (2008) have reported the presence of cytolytic, agglutinating, proteolytic, haemolytic, mitogenic, antipyretic, tumor static and antibacterial activities in the earthworm protein and its coelomic fluid. Vohora and Khan (1978) found earthworms to have healing effect on wounds, piles and sore throat. Hori *et al.* (1974) have reported significant anti-pyretic activity in the Japanese earthworms *Lumbricus* spp. and *Perichaeta* spp. Mihara *et al.* (1991) have reported *Lumbricus rubellus* to be potentially very useful in treating thrombosis and in fact, orally administrated earthworm powder was found to be capable of digesting intravascular fibrin clots. Popovi *et al.* (2001) demonstrated anticoagulant and fibirinolytic activity in the blood of the dog with malignant tumors on administration of glycolipoprotein (G-90) from earthworm tissue and their proteolytic enzymes PI and PII. Inflammations such as carageenin induced oedema and cotton pellet granuloma in rats subsided due to the anti-inflammatory activity of earthworm paste and its extracts in different solvents (Ismail 1992). Administrations of natural herbal products were known to enhance and maintain anti-inflammatory, antioxidative, haematological and serum biochemical profiles in animals. Administration of various herbal products and formulation in inflamed rats were shown to normalize inflammation, oxidative stress, haematological and serum biochemical indices (Ray *et al.* 2002; Karumi *et al.* 2003; Speroni *et al.* 2005). Though there are numerous studies on the therapeutic properties of different natural herbal products and formulations, very few studies have been made on the sources from animal origin, especially earthworms. Therefore this paper deals with the medicinal value of Indian earthworm *Lampito mauritii* (Kinberg), in comparison with standard drug, indomethacin on the antiinflammatory, antioxidative, haematological and serum biochemical indices of rats.

MATERIALS AND METHODS

According to the method of Hrzenjak *et al.* (1992), 3 g of gut cleaned earthworm tissue (*L. mauritii*, Kinberg) were homogenized in 40 ml of chloroform-methanol (V/V) solution and left overnight at 4°C. Then, 16 ml of distilled water was added to the homogenate, mixed well and centrifuged (2460 g) for 10 min. From the obtained three clearly visible layers, the upper, water/methanol layer was pipetted out and evaporated. An opalescent fluid (pH 7), was obtained, freeze-dried and kept at 4°C until use.

Received: 11 February, 2009. Accepted: 28 July, 2009.

Original Research Paper

Healthy and pure strain male albino rats (*Rattus norvegicus*), weighing 150-200 g was procured from the Department of Experimental Science, Central Animal House, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar. They were maintained under standard conditions (28 ± 2°C; 55-60% RH) and fed on standard rat diet. Water was given *ad libitum.* The experiments were carried out according to the institutional regulations and national criteria for animal experimentation and also approved by the Institutional Animal Ethics Committee (IAEC).

The rats were divided in to 5 groups and each group had six animals. Of these 5 groups, control received only 2% gum acacia, second group received a standard drug indomethacine (10 mg/kg) and other three experimental groups received EE orally in different doses (50, 100 and 200 mg/kg). Subcutaneous dorsal granuloma pouch was made in ether-anaesthetized rats by injecting 25 ml of air, followed by injection of 0.5 ml of turpentine oil into it and the pouch was weighed and compared with those of the control and standard group (Selye 1953).

The activities of non-enzymatic antioxidant such as reduced glutathione (GSH) and the enzymatic antioxidant such as reduced glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation parameter-thiobarbituric acid reactive substances (TBARS) were assayed according to the methods of Ellman (1959), Rotruck *et al.* (1973), Kakkar *et al.* (1984), Sinha (1972) and Nichans and Samuelson (1968), respectively. The total protein content in the tissue homogenate was measured as per the method of Lowry *et al* (1951).The rats were anesthetized by using inhalational ether. The blood sample was taken by intracardiac puncture. One ml aliquot of blood was drawn from each animal on day 7. The blood was put into heparinized tubes and haematological parameters– red blood corpuscle, (RBC), white blood corpuscle (WBC) neutrophils and haemoglobin content were measured using a Coulter Automated Analyzer. Using standard methods, the serum biochemical parameters- total protein (Lowry *et al.* 1951), albumin, cholesterol (Zlatkis *et al.* 1953) and alkaline phosphatase (ALP) (King and Armstrong 1934) were determined.

Data were statistically evaluated using one-way analysis of variance (ANOVA) (SPSS package) and the values were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

The turpentine induced chronic phase granuloma pouch weight reduced significantly ($P<0.05$) on administration of indomethacin. But administration of EE was found to exhibit better results. Administration of 200 mg/kg EE reduced the granuloma weight and the condition was brought to near normalcy. The resultant effect was observed on administration of 50 and 100 mg/kg, respectively. GSH, GPx, CAT, SOD and TBARS in the liver tissues were restored to near normal level on administration of 200 mg/kg EE. It was found to be comparatively similar to indomethacin administration and other doses of EE (**Table 1**).

Similar to antioxidant properties, administration of 200 mg/kg EE has restored the RBC, WBC and neutrophil populations and haemoglobin to near normal (control) values from the conditions of chronic phase. These results were nearer to treatment with indomethacine and better than other doses of EE (**Table 2**). Chronic phase inflammation caused by turpentine increased the levels of serum protein, albumin, cholesterol and ALP in rats and they were reduced in a dose dependent manner to normal level in rats administrated with EE and rats treated with indomethacine (**Table 3**).

Turpentine oil and cotton pellet-induced granuloma pouch offer models for study of exudative type of inflammation. Ismail *et al.* (1997) found that 1000 mg/kg of root bark powder of *Salacia oblonga* and leaf powder of *Azima tetracantha* to be antiinflammatory by reducing the granuloma and exudates in cotton pellets induced granuloma in the chronic phase of rats. Yegnanarayan *et al.* (1988) found earthworms to have anti-inflammatory properties and found the maximum anti-inflammatory activity at the dosage level

Table 1 Antiinflammatory and antioxidant activities of *L. mauritii* extract in chronic phase inflamed rat. (p<0.05) ANOVA (Analysis of variance- one way).

Treatments	Granuloma pouch weight (g)	GPx (µg GSH consumed/min/mg protein)	GSH (µg/mg protein)	CAT (µmol H_2O_2 consumed /min/mg protein)	SOD (µmol H_2O_2 consumed /min/mg protein)	TBARS (n mol/mg protein)
Normal control		5.6894 ± 0.13	2.4822 ± 0.40	2.9520 ± 0.20	5.2145 ± 0.39	0.9573 ± 0.10
Inflamed control	4.50 ± 0.04	1.8327 ± 0.24	0.6834 ± 0.45	0.9731 ± 0.30	1.3957 ± 0.40	2.4915 ± 0.10
Indomethacin 10 mg/kg	0.12 ± 0.01	4.6745 ± 0.41	2.1851 ± 0.89	2.6592 ± 0.10	5.0067 ± 0.53	0.8897 ± 0.52
EE 50 (mg/kg)	3.19 ± 0.12	2.9849 ± 0.30	1.2368 ± 0.63	1.6892 ± 0.30	2.5387 ± 0.26	2.1043 ± 0.40
EE 100(mg/kg)	2.10 ± 0.21	3.9450 ± 0.39	1.7253 ± 0.12	1.9650 ± 0.20	3.7845 ± 0.40	1.4903 ± 0.53
EE 200(mg/kg)	1.08 ± 0.02	4.3945 ± 0.20	2.0243 ± 0.54	2.3639 ± 0.10	4.9485 ± 0.10	0.7953 ± 0.10
Between groups						
x	7.890	53.595	4.946	16.390	42.504	17.696
y	1.213	7.406	1.533	3.500	6.554	3.604
With in Groups						
x	3.847	1.004	0.501	1.055	1.044	1.043
y	0.108	0.305	0.301	1.505	1.452	1.451
F-value	13.167	12543	991.410	2092.643	2949.273	3857.198

Mean± SE of six observations; x = sum of square; y = mean of square

Table 2 Restoration of haematological parameters by *L. mauritii* extract in chronic phase inflamed rat (p<0.05) ANOVA (Analysis of variance- one way).

Treatments	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Haemoglobin (g/dL)	Neutrophils (10^3/mm^3)
Normal control	6.8317 ± 0.04	6.978 ± 1.48	13.7 ± 0.10	23.243 ± 0.56
Inflamed control	4.2432 ± 0.06	8.272 ± 1.81	05.9 ± 0.13	29.257 ± 0.09
Indomethacin (10 mg/kg)	6.2450 ± 0.01	6.798 ± 1.28	13.1 ± 0.14	24.463 ± 0.05
EE 50 (mg¥kg)	5.4630 ± 0.01	7.620 ± 1.78	08.1 ± 0.13	26.127 ± 0.16
EE 100 (mg¥kg)	5.8700 ± 0.01	7.240 ± 1.68	10.6 ± 0.04	25.743 ± 0.01
EE 200 (mg¥kg)	6.0950 ± 0.01	6.273 ± 1.57	12.7 ± 0.12	24.932 ± 0.23
Between groups				
x	27.243	21465.266	234.614	537.374
y	4.267	5567.345	23.568	73.676
Within groups				
x	1.051	6.5	2.565	6.135
y	0.564	0.463	0.134	1.651
F-value	284.432	278.547	574.947	683.385

Mean ± SE of six observations; x = sum of square; y = mean of square

Table 3 Restoration of serum bio-chemical profiles by *L. mauritii* extract in the chronic phase inflamed rat ($p<0.05$). ANOVA (Analysis of variance- one way).

Treatments	Protein (g/dL)	Albumin (g/dL)	Cholesterol (mg/dL)	Alkaline Phosphatase (U/L)
Normal control	6.4327 ± 0.09	4.1447 ± 0.01	47.2 ± 0.64	255.14 ± 2.45
Inflamed control	8.9493 ± 0.02	2.1617 ± 0.05	54.6 ± 0.27	323.74 ± 1.65
Indomethacin (10 mg/kg)	6.2346 ± 0.01	3.9167 ± 0.01	45.4 ± 0.75	264.23 ± 1.76
EE 50 (mg¥kg)	8.4787 ± 0.01	2.7067 ± 0.01	50.1 ± 0.65	301.14 ± 0.23
EE 100 (mg¥kg)	7.3753 ± 0.03	3.0174 ± 0.01	47.7 ± 0.17	284.37 ± 1.23
EE 200 (mg¥kg)	6.3687 ± 0.03	3.5175 ± 0.01	44.4 ± 0.58	268.83 ± 1.29
Between groups				
x	13.297	15.817	782.389	134253.48
y	1.930	2.864	109.394	19404.529
With in Groups				
x	1.044	1.439	44.583	843.233
y	0.922	0.905	2.196	20.493
F-value	789.497	329.447	98.434	957.389

Mean± SE of six observations; x = sum of square; y = mean of square

of 160 mg/kg of total earthworm paste extracted from petroleum ether than from other extracts like benzene, chloroform and ether. It caused significant reduction in the granuloma pouch weight on cotton pellet induced chronic phase inflammation in rats. Though EE has been shown to have anti-inflammatory property, the most potent species of earthworm, dose and the mechanism of action have not been studied. In the present study EE of *L. mauritii* at the dosage level of 200 mg/kg exhibited significant antiinflammatory activity.

Many antioxidant substances occurring naturally in plant (and animal) were identified as free radical or active oxygen scavengers (Spector 1969). Administration of 75 mg/kg of *Piper betel* leaf extract in streptozotocin induced diabetic rats for 30 days was found to develop significant antioxidant activity (Santhakumari *et al.* 2003). They found increased levels of GSH, GPx, SOD and CAT except lipid peroxidation in the treated animals than those treated with the standard drug, glibenciamide. In the present study the level of antioxidants like GSH, GPx, CAT, SOD and TBARS in the chronic phase liver tissues were restored to near normal level by the administration of 200 mg/kg EE. This dosage was found to be as effective as indomethacin administration and other doses of EE.

Inflammation, in chronic phase, has caused an increase in the GPx and GSH in the liver and they have decreased compared to normal rats. These results agree with those of Bruille and Obled (2000) and Mercier *et al.* (2002) who have reported enhancement of GPx and GSH activities in the liver of chronic inflamed rats. Treatment with indomethacine had brought the level of the enzyme to near normalcy. Administration of EE, respective of the dosage, had restored GPx levels to normal level in liver. Among the various dosages of EE, 200 mg/kg was found to have the best effect. GSH activity, similar to GPx activity, was also restored to near normalcy due to administration of EE.

Kilic *et al.* (2003) reported a reduced WBC and increased RBC and Hb contents due to administration of 50 mg/kg of ciprofloxacin and pefloxacin in rats inflamed with formaldehyde. This was supported by Moura *et al.* (2005) who reported reduced WBC and increased RBC due to administration of 500 mg/kg of the leaf extract of *Ageratum conyzoides* in chronic (formaldehyde-induced arthritis) models of inflamed rats. Falling in line with these observations, it was found that in the present study, 200 mg EE/kg treated rat showed reduced WBC and increased RBC and Haemoglobin that were similar to indomethacin treated rats. Neutrophils play a crucial role in the growth and manifestation of inflammation and they are the major source of free radicals at the site of inflammation. Moura *et al.* (2005) suggested that the formation of free radicals and cytokines from neutrophils cause inflammation. The neutrophils, which were increased during inflammation, were reduced to normal level due to the administration of EE.This clearly demonstrates the antiinflammatory property of EE. It is generally believed that drugs that positively influence the immune system probably possess antiinflammatory activities.

The reduction in the serum protein and cholesterol level and increased level of albumin, due to the administration of EE, falls in line with the suggestion of Arrigoni (1997) who found inhibition of fibroblast population and collagen and mucopolysaccharide synthesis due to antiinflammatory drugs. Phosphatases are the most sensitive enzyme markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. The increase or decrease of these enzyme activities is related to the intensity of cellular damage (Sllie *et al.* 1991). Ismail *et al.* (1997) reported increased level of serum ALP in the cotton pellet induced chronic inflamed rats and this was decreased due to the oral administration of 1000 mg/kg of root bark powder of *S. oblongo* and leaf powder of *A. tetracantha*. In the present study also it was noticed that ALP level was decreased in 200 mg EE/kg treated rats, which were inflamed. Such decreasing activity of lysosomal enzymes suggests the efficacy of EE in protecting lysosomal membrane system during chronic inflammation. Recently Balamurugan *et al.* (2008) reported that the earthworm extract prevents the formation of the reactive oxygen groups, or scavenges these groups, thereby preventing the damage on the hepatic cells, and, on the other hand, modulates the genes responsible for synthesis of antioxidant enzymes, this paper supported to our present study.

Due to the feeding habit of earthworm devouring large amount of litter containing phenols or its derivatives earthworm tissue was shown to contain rich phenolic OH group and the same was found to be responsible for its nematicidal action (Ranganathan 2006). Phenols and its compounds are very important in antiinflammatory activity (Calixto *et al.* 2004) and scavenging of the free radicals due to the presence of hydroxyl groups (Hatano *et al.* 1989). Phenolic groups present in the earthworm tissues (61.1 µg/mg) might act as the principle behind the antiinflammatory and antioxidant property (Balamurugan *et al.* 2009). Further studies are needed to evaluate the actual principle responsible for the antiinflammatory and antioxidant properties of earthworm extract.

CONCLUSION

The anti-inflammatory and anti-oxidant potential of earthworm extract which could be due to the presence of phenolic substances in the earthworm tissue which might scavenge the free radicals, stimulate the activities of anti-oxidative enzymes and normalize the haematological and serum biochemical characteristics.

ACKNOWLEDGEMENTS

We thank the authorities of Annamalai University for facilities and Professor and Head, Department of Pharmacology, Rajah Muthiah Medical College, Annamalai University, for help in the design and discussion of the study.

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