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Earthworm paste (Lampito mauritii, Kinberg) alters inflammatory, oxidative, haematological and serum biochemical indices of inflamed rat

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Abstract. – **Experiments were conducted to understand the therapeutic properties such as anti-inflammatory, anti-oxidative, haematological and serum biochemical markers of earthworm paste (EP) derived from an indigenous species *Lampito mauritii* (Kinberg), in comparison with the standard anti-inflammatory drug- aspirin, on Wistar albino rat (*Rattus norvegicus*). Administration of earthworm paste of *Lampito mauritii* at the rate of 80 mg/kg into albino rats which were induced of inflammation, was found to reduce inflammation, restore the levels of antioxidants-reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase and thiobarbituric acid reactive substances, normalise the values of erythrocyte, leukocyte, differential levels of neutrophils, lymphocytes, eosinophils, haemoglobin and serum biochemical contents e.g., protein, albumin, glucose, cholesterol, acid and alkaline phosphatase, electrolytes e.g., sodium, potassium and chloride. The anti-inflammatory activity together with antioxidant property of EP seems to be due to the high polyphenolic content of earthworm tissue.**

Key Words:

Earthworm paste, Anti-inflammation, Anti-oxidants, Serum biochemical indices, Rattus norvegicus.

Introduction

From time immemorial earthworms have been used as a therapeutic agent. Recently earthworm protein and its coelomic fluid were reported to have cytolytic, agglutinating, proteolytic, haemolytic, mitogenic, anti-pyritic, tumorstatic and antibacterial activities[1-3]. Vohora and Khan[4] found earthworms to have healing effect on wounds, chronic folds, piles and sore throat. Earthworm's anti-pyretic properties were reportedly tried in China and Japan in reducing fever. Anti-pyretic components were found in the earthworms *Lumbricus spp* and *Perichaeta spp* by Hori et al[5]. Bhatnagar and Palta[6] have reported that earthworms when ingested into our body system increase body heat and are of value in curing neural disorders, bronchitis and tuberculosis and in curing rheumatism. Mihara et al[7] have reported *Lumbricus rubellus* to be potentially very useful in treating thrombosis and in fact, orally administrated earthworm powder was found capable of digesting intravascular fibrin clots. Popovi et al[8] reported the presence of anticoagulant and fibrinolytic activity in the blood of the dog with malignant tumors due to the administration of glycolipoprotein (G-90) from earthworm tissue and their proteolytic enzymes PI and PII. The anti-inflammatory activity of earthworm paste (EP) and its extracts in different solvents were studied in carageenan induced edema and cotton pellet granuloma in rats[9]. It was found that the anti-inflammatory activity of earthworms was similar to that of aspirin on carageenan induced edema[10].

Studies have shown that administration of natural herbal products enhances and maintains anti-inflammatory, antioxidative, haematological and serum biochemical profiles in animals. Administration of various herbal products and formulations in inflamed rats has been shown to normalise inflammation[11-13], oxidative stress[14-18], haematological[19] and serum biochemical indices[20]. These authors reported that the phenolic compounds derived from various plants to exhibit the presence of anti-inflammatory and antiox-

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idative properties. Though pharmacological role of different natural herbal products and formulations has been reported, similar studies have not been made on tissues of animal origin, especially earthworms. Since studies on the medicinal value of indigenous earthworms are limited the present study, first in many aspects, investigates the effect of EP of *Lampito mauritii* (Kinberg), on the anti-inflammatory, anti-oxidative, haematological and serum biochemical indices of rat (*Rattus norvegicus*) in comparison with standard drug-aspirin.

Methods

Preparation of Earthworm Paste

Earthworms, *Lampito mauritii* (Kinberg) were collected from the stock culture, Division of Vermibiotechnology, Department of Zoology, Annamalai University. 500 sexually mature clitellated worms (900 mg/worm) were washed with running tap water and then fed with wet blotting paper for 18-20 hours to clear their gut. The gut-cleared worms were again washed with distilled water. The worms were kept in plastic troughs, covered tightly with polythene cover, and exposed to sunlight for 3 days to kill them. Mucus and coelomic fluid that oozed out digested the dead worms forming a brown coloured paste-earthworm paste (EP).

Selection of Experimental Animals

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 a standard diet of rat and given water *ad libitum.* The experiments were carried out according to the institutional regulations and national criteria for animal experimentation.

In vivo models for anti-inflammatory activity

Carageenan-Induced Rat Paw Edema: For Acute Model Study

Rats of either sex were divided in to 7 groups comprising 6 animals in each. Of these 7 groups, control animals received only 2% gum acacia, second group received a standard drug aspirin (75 mg/kg) and other five experimental groups received EP orally in different doses (20, 40, 80, 160, and 320 mg/kg). Aspirin and EP were suspended in 2% gum acacia and administered orally one hour prior to the sub plantar injection of 1% carageenan (1 ml/100 g/body weight). One hour after the drug administration the paw edema[21] volume was measured by using mercury plethysmograph at 0 and 3 hrs. Mean increase in paw volume was measured and percentage inhibition was calculated.

Granuloma Pouch Method: For a Chronic Model Study

Subcutaneous dorsal granuloma pouch was induced in ether anaesthetized rats by injecting 25 ml of air, followed by injection of 0.5 ml of turpentine oil[22]. All drugs were administered orally one hour prior to turpentine oil injection and continued for seven consecutive days. On day 7, the pouch was weighed and amount of exudates was measured and compared with those of the control and standard group.

Estimation of Antioxidant Parameters

The activities of non-enzymatic antioxidant-reduced glutathione (GSH) and enzymatic antioxidants such as reduced glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) and thiobarbituric acid reactive substances (TBARS) were assayed according to the methods of Ellman[23], Rotruck et al[24], Kakkar et al[25], Sinha[26] and Nichans and Samuelson[27], respectively. The protein content in the tissue homogenate (liver and muscle) was estimated by Lowry et al[28].

Monitoring of Haematological Parameters

The rats were anesthetized by using inhalational ether. The blood sample was taken by intracardiac puncture. 1 ml aliquot of blood was drawn from each animal at day 7. The blood was put into heparinized tubes and haematological parameters – red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and differential counts (DC) – neutrophils, lymphocytes and eosinophils were measured using a Coulter Automated Analyzer.

Serum Biochemical Analysis

Using standard methods, the serum biochemical parameters – total protein and albumin[29], glucose[30], cholesterol[31], alkaline phosphatase

(ALP)[32], acid phosphatase (ACP)[33], and electrolytes – sodium and potassium[34] and chloride[35] were determined.

Determination of Total Phenolic Compounds

Total soluble phenolic compounds in the EP were estimated using the Folin-Ciocalteau reagent according to the method of Slinkard and Singleton[36].

Statistical Analysis

Data were statistically evaluated using one-way analysis of variance (ANOVA) (Statistical Version 0.5). The values were considered significant when $p < 0.05$.

Results

Anti-Inflammatory Status

It is observed in the present study that the carageenan induced acute phase rat hind paw edema volume and the turpentine induced chronic phase granuloma pouch weight and the volume of fluid were reduced significantly due to the administration of aspirin. However, the administration of EP was found to exhibit better results. Administration of 80 mg/kg EP was found to reduce all the above parameters brought to near normalcy, and this was found after administration of 40, 20, 160 and 320 mg/kg, respectively (Table I). The restoration to near normalcy was due to the presence of anti-inflammatory properties of EP (Figure 1) that could affect the synthesis of kinin, prostaglandin, bradykinin, lysozymes synthesis and more particularly both COX-1 and COX-2 as suggested by Banerjee et al[12].

Antioxidant status

The reduced antioxidant indices like GSH, GPx, SOD, CAT and enhanced TBARS in the acute phase liver tissue and GSH and GPx in the chronic phase liver and muscle tissues, were restored to near normal level by the administration of 80 mg/kg EP. It was found to be more effective than aspirin administration and other doses of EP (Figure 2-8).

The acute phase inflamed rat liver was found to exhibit increased levels of TBARS. Treat-

Table I. Effect of earthworm paste on carageenan induced rat hind paw edema and granuloma pouch in rats ($p < 0.05$).

Treatments	Paw edema volume (ml)	Inhibition (%)	Weight of granulation tissue (g)	Inhibition (%)	Volume of exudate (ml)	Inhibition (%)
Inflamed control	1.7517 ± 0.04	–	3.4089 ± 0.04	–	0.3000 ± 0.03	–
Standard drug (Aspirin 75 mg/kg)	0.9667 ± 0.05	45	1.0171 ± 0.34	70	0.2000 ± 0.36	33
Earthworm paste (mg/kg)						
20	0.7617 ± 0.17	56	0.7100 ± 0.16	79	–	–
40	0.7467 ± 0.28	57	0.6315 ± 0.43	81	–	–
80	0.6217 ± 0.07	64	0.5677 ± 0.54	83	–	–
160	0.8300 ± 0.63	52	0.9152 ± 0.18	73	0.1167 ± 0.16	61
320	0.9300 ± 0.81	46	0.9845 ± 0.02	71	0.1333 ± 0.21	55
ANOVA (analysis of variance- one way)						
Between groups						
x	5.053		35.997		0.125	
y	0.842		5.999		0.042	
With in groups						
x	0.071		0.077		0.102	
y	0.002		0.002		0.005	
F-Value	413.284		2738.529		8.169	

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

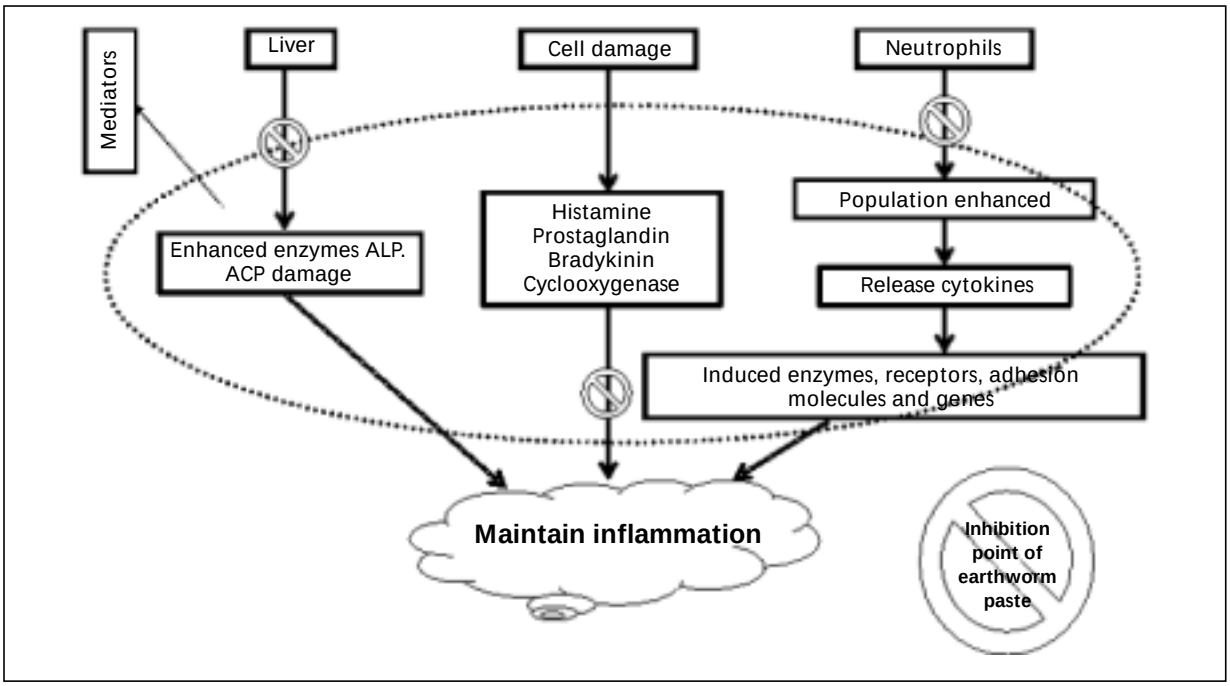

Figure 1. Role of earthworm paste against inflammation.

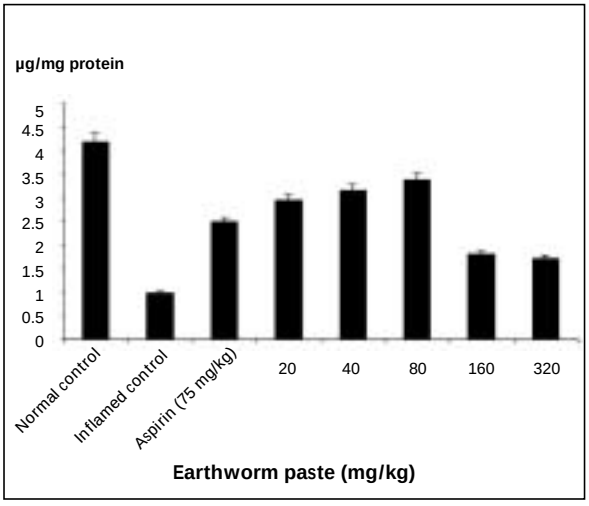

Figure 2. Estimation of anti oxidative enzyme-reduced glutathione in the liver tissue of normal, acute phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

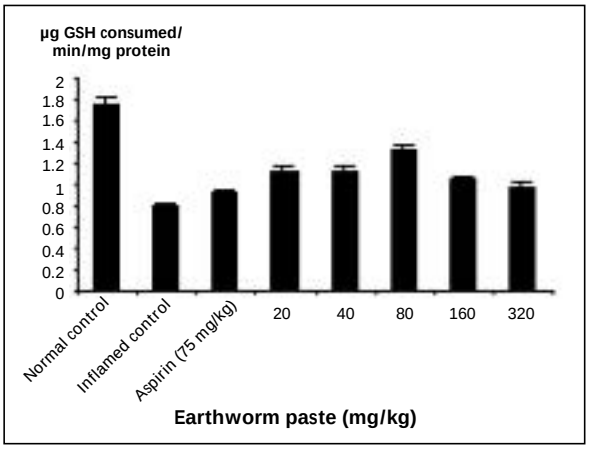

Figure 3. Estimation of anti oxidative enzyme- glutathione peroxidase in the liver tissue of normal, acute phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

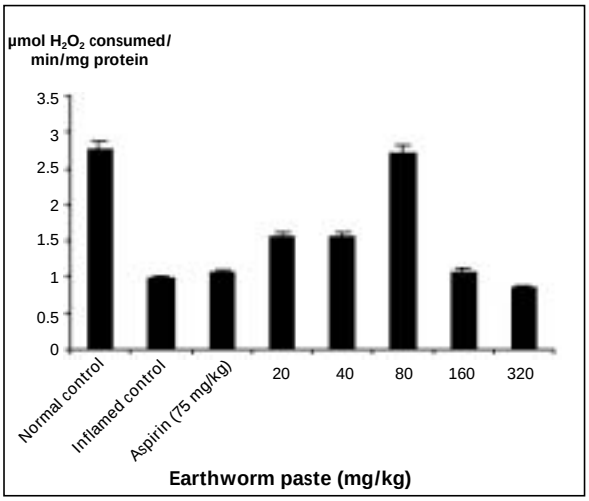

Figure 4. Estimation of anti oxidative enzyme-super oxide dismutase in the liver tissue of normal, acute phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

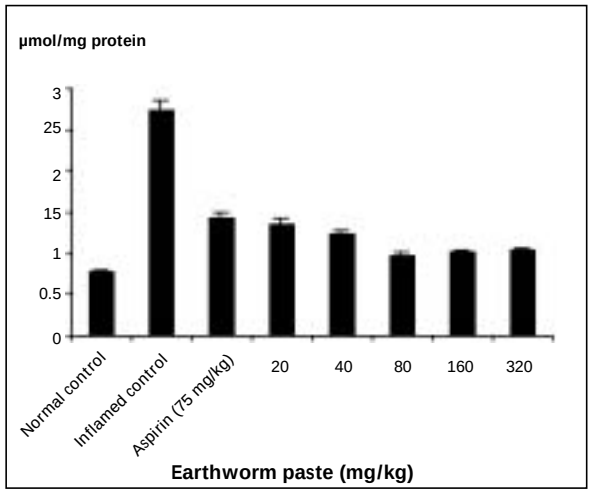

Figure 6. Estimation of thio barbituric acid reactive substances in the liver tissue of normal, acute phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

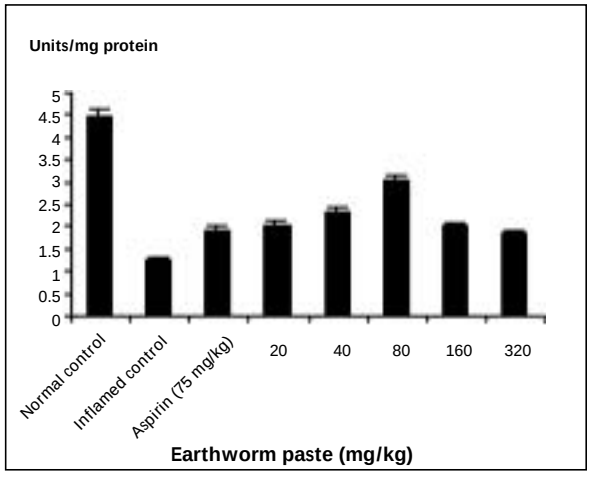

Figure 5. Estimation of anti oxidative enzyme-catalase in the liver tissue of normal, acute phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

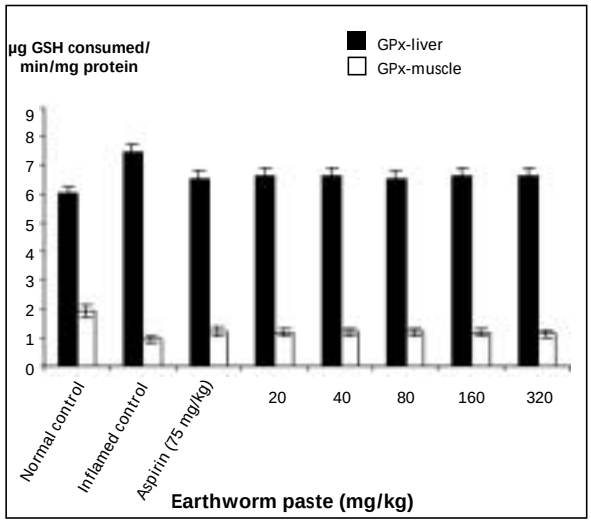

Figure 7. Estimation of glutothione peroxidase in the liver and muscle tissues of normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

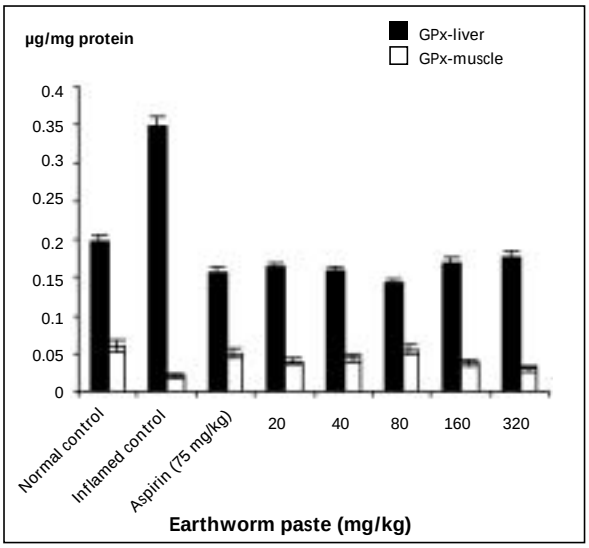

Figure 8. Estimation of reduced glutathione in the liver and muscle tissues of normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

ment with EP protects the cell through the attenuation of lipid peroxidation and decreased the production of free radical derivatives as evident from the decreased levels of TBARS in the liver of acute phase inflamed rats. EP treated animals were found to show the normal GSH and GPx levels in liver of rats than in inflamed

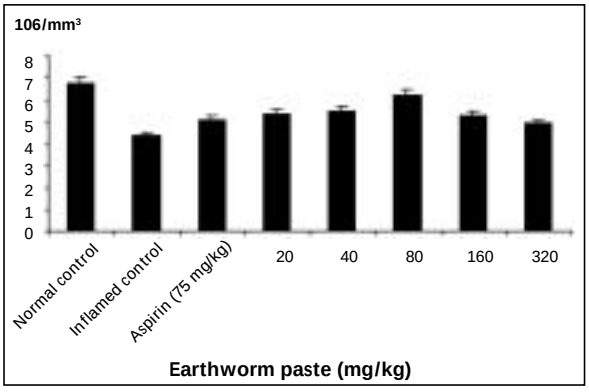

Figure 9. Estimation of RBC in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA)

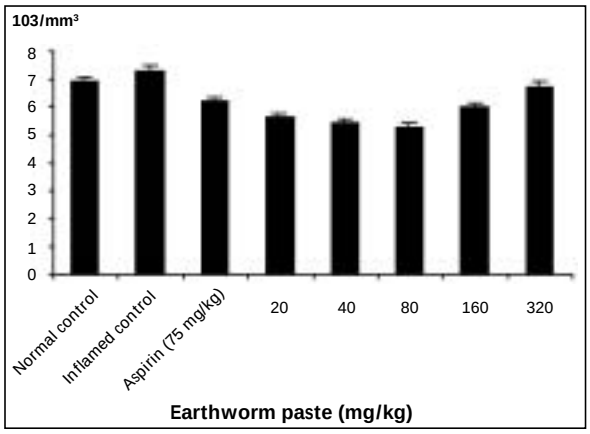

Figure 10. Estimation of WBC in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

rat. These results indicate the potential of the EP's liver calming action and enhancement of the antioxidant and detoxification functions of the liver.

Haematological Status

Similar to antioxidant properties, administration of 80 mg/kg EP has restored the RBC, WBC, Hb and DC to near normal (control) value from the conditions of chronic phase. These results were better than treatment with aspirin and

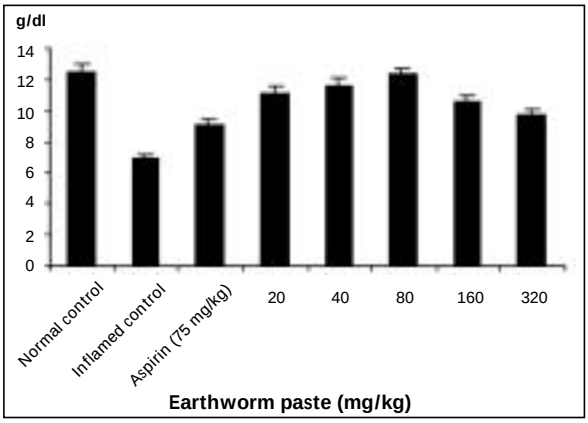

Figure 11. Estimation of haemoglobin in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

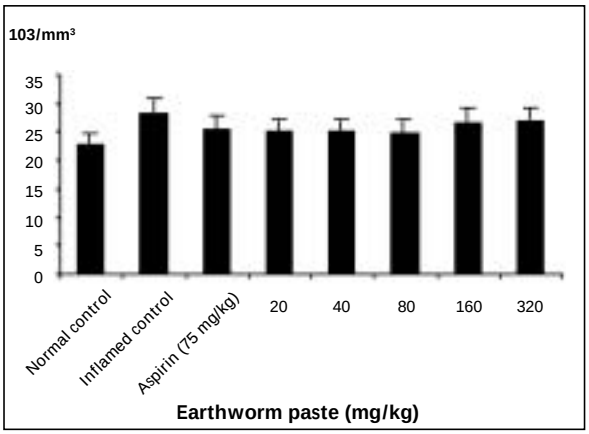

Figure 12. Estimation of neutrophils in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

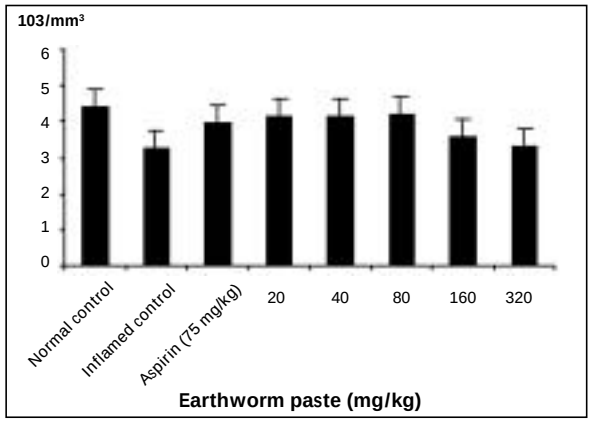

Figure 14. Estimation of eosinophils in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

other doses of EP (Figures 9-14). The haematological changes give an insight to the understanding of the changes during inflammation in rats.

The neutrophil count was found to be increased in the inflamed rats but reduced in the EP and aspirin treated rats. The reduction in the neutrophil count due to application of EP establishes the anti-inflammatory property of EP, which was already shown above by studies on anti-inflammation, anti-oxidant enzymes, and GSH.

Serum Biochemical Status

The turpentine induced chronic phase inflammation increased the level of serum protein (Figure 15) and albumin (Figure 16) in rats and the same was found to be reduced to normal level in rats administrated with 80 mg EP/kg than aspirin and other doses of EP treated rats.

The level of glucose (Figure 17) and cholesterol (Figure 18) were found to have increased in the inflamed rat but it was reduced to the normal levels in the 80 mg EP /kg treated rats than rats

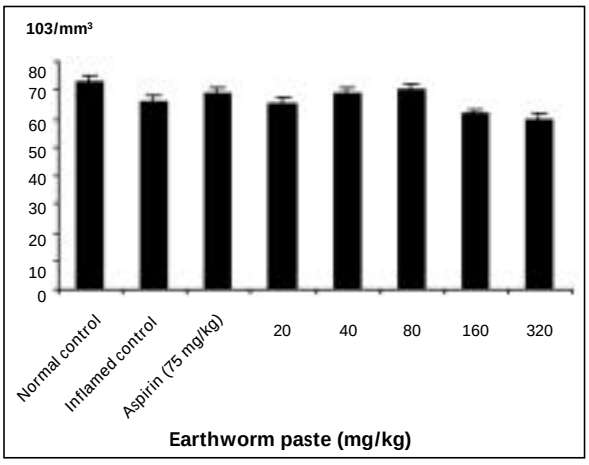

Figure 13. Estimation of lympocytes in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

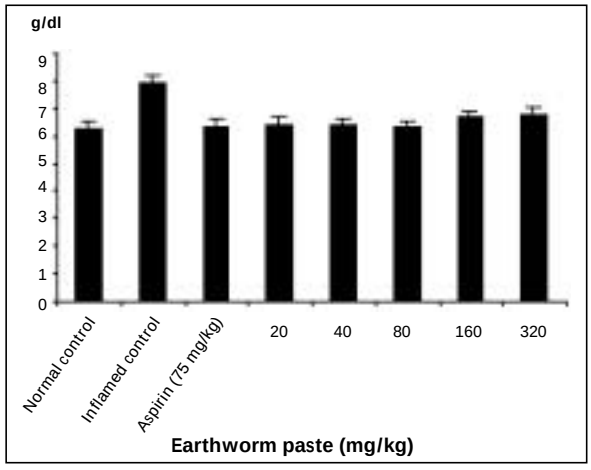

Figure 15. Estimation of serum protein in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

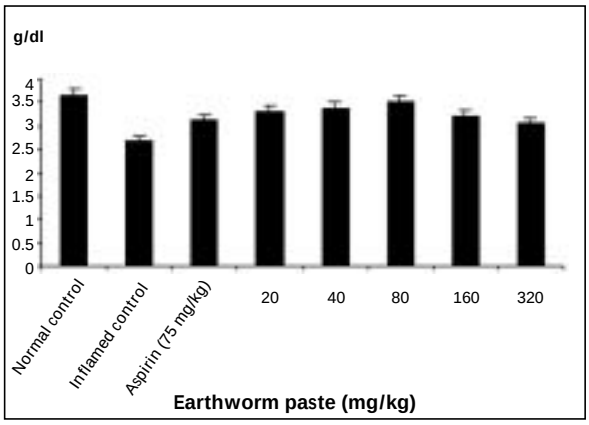

Figure 16. Estimation of serum albumin in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

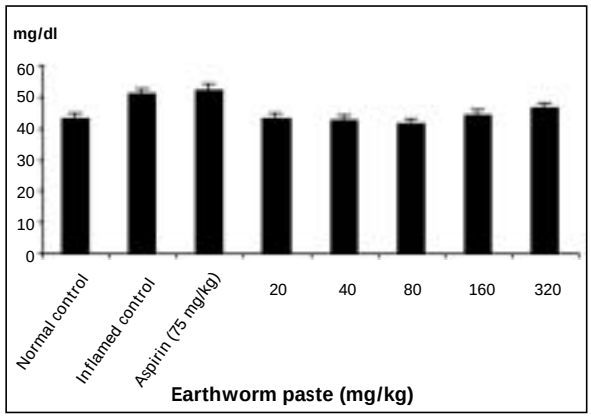

Figure 18. Estimation of serum cholesterol in normal, chronic inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

treated with aspirin and other doses of EP. Similarly, it was found that both serum ACP (Figure 19) and ALP (Figure 20) had increased in the chronic phase inflamed rat. These enzymes level was decreased in treated rats with 80 mg EP/kg than other doses of EP and aspirin treated rats. Such decreased activity of lysosomal enzymes due to EP administration suggests the efficacy of EP in protecting lysosomal membrane system during chronic inflammation. It also indicates that it could repair the function of liver.

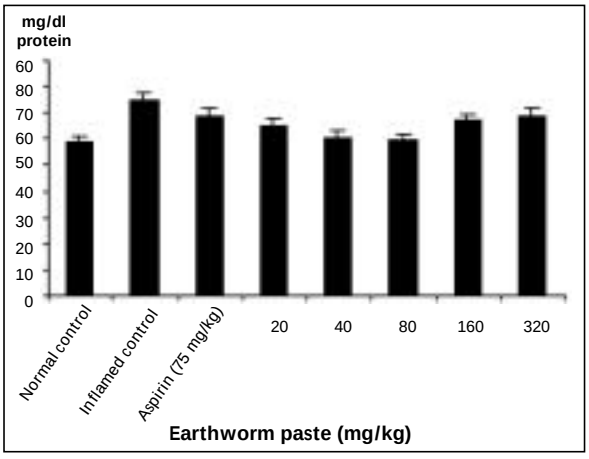

Figure 17. Estimation of serum glucose in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

It was observed that due to the inflammation the electrolyte picture had changed from the normal: Na (Figure 21) and Cl (Figure 22) level were decreased and K (Figure 23) level was enhanced. But treatment with EP (80 mg/kg) was found to retain normal level of Na, Cl and K than treatments with aspirin and other doses of EP in the chronic phase inflamed rat demonstrating the therapeutic properties of EP.

Amount of total Phenolic Compounds in EP

The level of total phenolic compounds of EP as determined by the Folin-Ciocalteau reagent

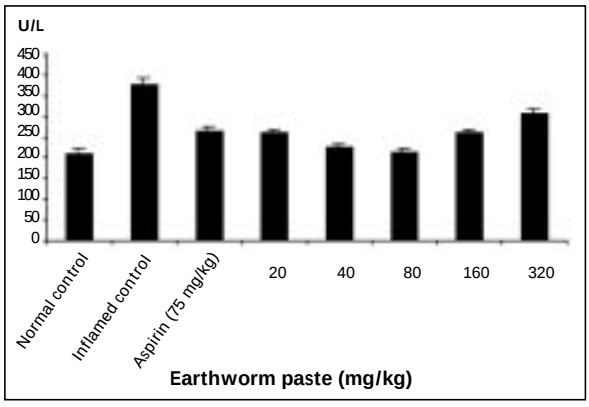

Figure 19. Estimation of serum acid phosphatase in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

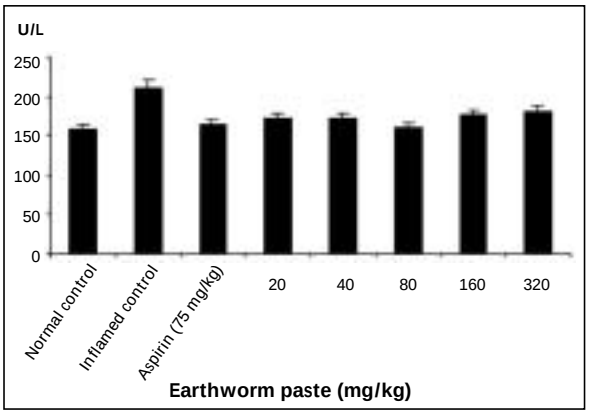

Figure 20. Estimation of serum alkaline phosphatase in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

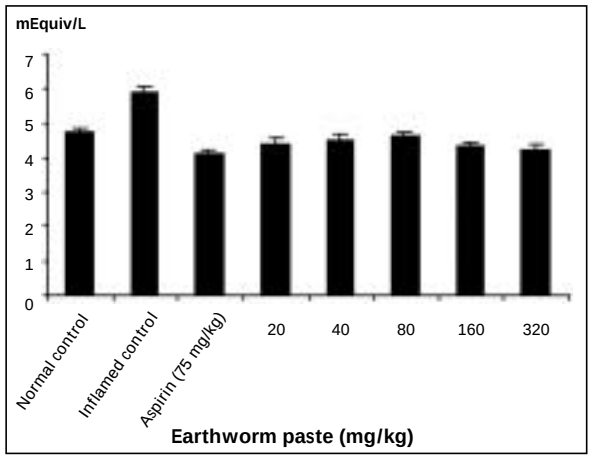

Figure 22. Estimation of serum chloride in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

was 42.2 µg expressed as pyrocatechol equivalents per milligram of EP.

Discussion

Carageenan-induced rat paw edema is commonly used in evaluating the anti-inflammatory agents acting by inhibiting the mediators of acute inflammation and is believed to be biphasic[21]. The first phase is due to the release of histamine, serotonin and kinin in the first hour after the administration of carageenan; a more pronounced second phase is attributed to the release of bradykinin, protease, prostaglandin and lysosome[37]. The later phase of edema is recorded to be sensitive to most of the clinically effective anti-inflammatory agents[38]. The inflammatory granuloma is a typical feature of estab-

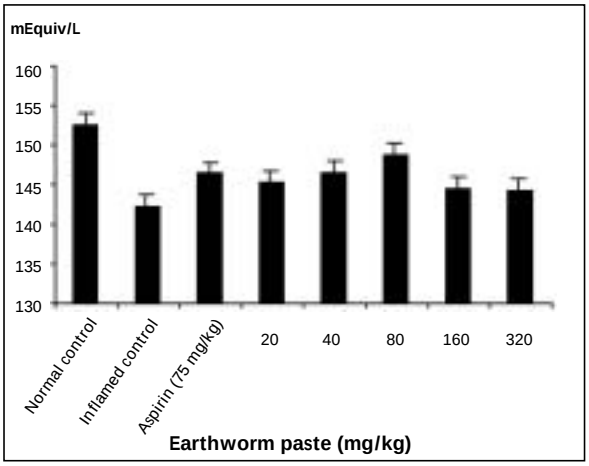

Figure 21. Estimation of serum sodium in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

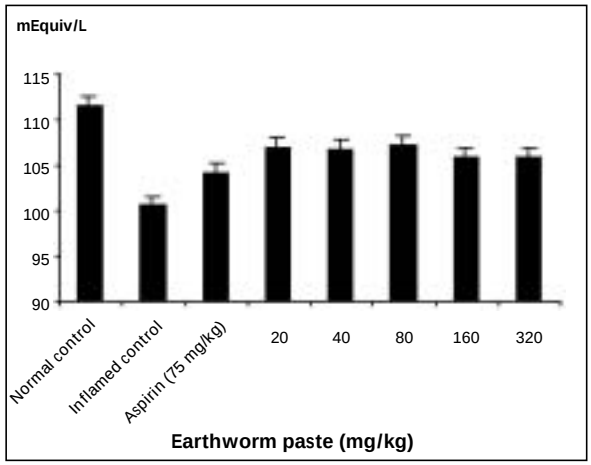

Figure 23. Estimation of serum potassium in normal, chronic phase inflamed, aspirin and different concentration of earthworm paste treated rats: Each value represents mean ± SE, n = 6. The statistical significance was tested between and within groups of each treatment at 0.05 level using one-way analysis of variance (ANOVA).

lished chronic inflammatory reaction[39]. Turpentine oil-induced granuloma pouch offers a model for exudative type of inflammation[12]. Ismail et al[20] found that 1000 mg/kg of root bark powder of *Salacia oblonga* and leaf powder of *Azima tetracantha* to be anti-inflammatory by reducing paw edema volume in the acute phase and reducing the granuloma and exudates in the chronic phase of rats.

Though there are numerous studies on the anti-inflammatory therapeutic property of extracts from variety of plants, very few studies have been made on the sources from animal origin.

Yegnanarayan et al[9] found earthworms to have anti-inflammatory properties and found the maximum anti-inflammatory activity in 160 mg/kg of total EP extracted from petroleum ether than from other solvents like benzene, chloroform and ether. The petroleum ether extract significantly reduced the paw edema volume in the acute phase and significantly reduced granuloma pouch weight on cotton pellet induced chronic phase inflammation in rats. Also Ismail et al[10] found petroleum ether fraction of total EPs of *Lampito mauritii* to have better anti-inflammatory properties on albino rat and they found 160 mg/kg total EP to function similar to that of aspirin in carageenan induced edema. Though EP has been shown to have anti-inflammatory property, the most potent species of worm, dose and the mechanism of action are not clearly understood.

Natural antioxidants protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. Many antioxidant substances occurring naturally in plant (and animal) were identified as free radical or active oxygen scavengers[40]. Santhakumari et al[14] found significant antioxidant activity when 75 mg/kg of *Piper betle* leaf extract was administrated in streptozotocin induced diabetic rats for 30 days. They found increased level of GSH, GPx, SOD and CAT except TBARS in the treated animals than those treated with the standard drug, glibenclamide. In the present study the reduced antioxidant indices like GSH, GPx, SOD, CAT and enhanced TBARS in the acute phase liver tissue and GSH and GPx in the chronic phase liver and muscles tissues were restored to near normal level by the administration of 80 mg/kg EP. It was found to be more effective than aspirin administration and other doses of EP.

Non-enzymic antioxidants such as GSH, vitamin C, E, Tocopherol and Ceruloplasmin play a vital role in protecting the cells against oxidative damage. Tocopherol reduces lipid hydroperoxide generated during the process of peroxidation and protects cell structure against damage. Ceruloplasmin inhibits lipid peroxidation by binding to copper. Among these, GSH plays a vital role in protecting the cells from oxidative damage[41]. It is well known that GSH in blood maintains cellular levels of the active forms of vitamin-C and vitamin-E by neutralizing the free radicals[42]. GSH status is a highly sensitive indicator of cell functionality and viability[43]. In the present study, it was found that inflammation had reduced the level of GSH whereas in rats treated with EP the levels reached near normal due to antioxidant role of EP.

The enzymic antioxidant defense systems are natural protective barriers against lipid peroxidation. SOD, CAT and GPx are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage[44].

Membrane lipids succumb easily to deleterious actions of reactive oxygen species. The highly toxic hydroxyl radical cleaves covalent bonds in proteins and carbohydrates, causes lipid peroxidation, and destroys cell membranes. The measurement of lipid peroxidation is a convenient method of monitoring oxidative damage[45].

Inflammation, in chronic phase, has caused an increase in the GPx in the liver whereas in muscle, it has decreased compared to normal rats. Treatment with aspirin had brought the level of the enzyme to near normalcy in both tissues. But administration of EP, irrespective of the dosage, had restored GPx levels to normal level. Among the various dosages of EP, 80 mg/kg was found to have the best effect. GSH activity was restored to near normalcy due to administration of EP and was slightly lower than aspirin treated one. The chronic phase inflamed muscle showed decreased level of antioxidant than EP treated rat, due to depletion of antioxidants as a result of oxidative stress produced by the inflammation. These results were in agreement with those of Bruille and Obled[46] and Mercier et al[47] who have reported enhancement GPx and GSH activities in the liver of chronic inflamed rat.

Kilic et al[19] reported a reduced WBC and increased RBC and Hb contents due to administration of 50mg/kg of ciprofloxacin and pefloxacin in rats inflamed with formaldehyde. This was sup-

ported by Moura et al[48] who reported reduced WBC and increased RBC and Hb 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, 80 mg EP/kg treated rat showed reduced WBC and increased RBC and Hb than aspirin treated rats. It is generally believed that drugs that positively influence the immune system probably possess anti-inflammatory activities[19].

Neutrophils play a crucial role in the development and manifestation of inflammation and they are the major source of free radicals at the site of inflammation. Neutrophil derived free radical is known to be cause of inflammation[49] and cytokines produced by neutrophils are also responsible for inflammation. The reduction in the population of neutrophils after administration of EP shows its involvement in suppressing inflammation.

Carageenan induced inflammation was found to show a decreased albumin level in rats[50]. In the present study decreased level of serum albumin was found in the inflamed rat and this was found to be normalized in the rats treated with EP. This observation is corroborated by the findings of Ismail et al[20] who found the enhanced serum albumin content to be reduced to normal level after the administration of 1000 mg/kg root bark powder of *Salacia oblongo* and leaf powder of *Azima tetracantha* in inflamed rats. Chronic inflammation is known to stimulate protein metabolism in animals[47]. Administration of 0.45 g/kg of aqueous extract of the flower of *Cassia auriculata* for 30 days, suppressed the elevated blood glucose level, serum and tissue lipid level in streptozotocin induced diabetic rats[51].

ALP and ACP 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[52]. The increase or decrease of these enzyme activities is related to the intensity of cellular damage[53]. The activities of lysosomal enzyme i.e. ACP and ALP in liver were markedly increased during inflammation[54]. Also, these enzymes are the mediators of inflammation[55]. Ismail et al[20] reported increased level of serum ACP and ALP in the cotton pellets induced chronic inflamed rats and this was decreased due to the oral administration of 1000 mg/kg of root bark powder of *Salacia oblongo* and leaf powder of *Azima tetracantha*.

Electrolytes are ionized molecules found throughout the blood, tissues and cells of the body. These molecules are either positive (cations) or negative (anions), conduct an electric current and help to balance pH and acid-base levels in the body[56]. Geidam et al[57] reported that intragastric administration of 0.75 g/kg body weight of *Adansonia digitata* has no significant effect on serum electrolytes such as sodium, potassium and chloride in both alcohol fed and normal rats. Alfaro et al[58] reported the metabolic acid-base disorders induced by inflammatory processes, hydrogen (H^+) homeostasis was maintained, and blood pH remained essentially unchanged in the inflamed rats.

Our present studies have shown the EP to normalize the Na, K and Chloride levels in rats where due to inflammation Na and Chloride were decreased and K level was enhanced. The phenolic compounds were known to contribute directly to the anti-inflammatory activity[59] and anti oxidative activity[60] Phenols are very important in scavenging the free radicals due to the presence of hydroxyl groups[61]. Since the polyphenol content in EP (Table II)[62] is high (42.2 µg/mg) the anti-inflammatory and antioxidative properties of EP may be attributed to it. Further, earthworm feed on organic matter, 2 to 5 times their body weight and after utilizing 10% of the food materials for their growth and reproduction, excretes the mucus coated matter as vermicompost[63]. Recently Ranganathan[64] has shown that the changes in the structure of the humic acid derived from the vermicompost were due to enhanced phenolic OH groups (58%) during the humification process. Further studies are needed to evaluate the actual principal compounds present in the earthworm paste which act as a therapeutic agent[64].

Table II. Biochemical profiles of earthworm paste of *Lampito mauritii*[64].

Total protein (mg/g)	4.002 ± 0.003
Total free amino acids (mg/g)	1.013 ± 0.002
Carbohydrates (mg/g)	4.102 ± 0.124
Glucose (mg/g)	1.752 ± 0.012
Glycogen (mg/g)	1.378 ± 0.004
Total lipids (mg/g)	2.438 ± 0.003
Free fatty acids (mg/100 g)	33.001 ± 0.012
Triglycerides (mg/100 g)	12.011 ± 0.002
Total phenolic compounds (µg/mg)	42.2 ± 0.25

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