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Original Article

Evaluating Anti-Inflammatory activity of aqueous root extract of *Strophanthus hispidus* DC. (Apocynaceae)

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Summary: The present study explored the anti-inflammatory potential of aqueous root extract of *Strophanthus hispidus* (SPH) DC (Apocynaceae) in rodents, using standard laboratory models. Doses of 50, 100, 500 and 1000 mg/kg of aqueous SPH were administered orally in carrageenan-induced rat hind paw oedema, xylene-induced ear oedema in mice, and formalin-induced mice hind paw oedema (sub-acute 6 days), using indomethacin (10 mg/kg), dexamethasone 1 mg/kg and acetylsalicylic acid (Aspirin), 100 mg/kg) respectively as standard drugs. The study further explored the effect of the herbal drug on some inflammatory mediators-histamine, serotonin and prostaglandin, using only the highest dose of SPH. Results obtained showed that the extract exerted a dose-dependent and significant ($p<0.05$) anti-inflammatory activity, which compared favourably with the positive control. Significant inhibitions of mediators were also recorded; however, the least inhibition (42.8 %) was produced in the serotonin model. Phytochemical analysis indicated the presence of flavonoids, cardiac glycosides, tannins, and anthraquinones. It is also noteworthy that zinc, copper, manganese, lead, and chromium were the elemental constituents in the aqueous extract of SPH, some of which have been reported to possess anti-inflammatory property. While 2 g/kg of SPH administered orally did not produce any mortality, the median lethal dose by i.p route was 39.81 mg/kg, and it is thought that the lead contribute to the toxicity recorded. The pH of the herbal drug was 6.7. Our findings substantiate the local use of SPH in the treatment of acute and sub-acute inflammatory conditions, while it also suggests some possible pathways for its anti-inflammatory activity. Lastly, since the herbal drug is liable to producing toxic effects, it must be used with caution.

Industrial relevance: Herbal remedies continue to serve as an important source of conventional therapies for diverse disease conditions, including inflammatory reactions. These reported benefits are attributed to the phytoconstituents in the natural medicaments. The present study will aid the industry to produce new anti-inflammatory drugs, which apart from lacking the adverse effects often recorded with the orthodox therapies, are more affordable and accessible to users.

Key words: Oedema; Inflammation; *Strophanthus hispidus*; rodents.

INTRODUCTION

Nature has offered a complete store-house of remdies for all ailments of mankind by providing drugs from herbs, whole plants and algae (Ravi et al., 2009; Kokate et al., 2002), most of which are of moderate toxicity relative to western medicines. Medicinal plants are used by 80 % of the world population, and it has become imperative to investigate the acclaimed ones for their possible therapeutic benefits, especially nowadays, when treatment of many serious diseases still faces diverse challenges (Adedapo et al., 2008), for example, chronic inflammatory diseases remain one of the world's major health problems (Li et al., 2003).

Inflammation is the response of living tissues to injury and it involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Perianayagam et al., 2006). Due to its implication in virtually all human and animal diseases, inflammation has become the focus of

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global scientific research, more so, since the currently used anti-inflammatory agents both steroidal and non-steroidal are prone to evoking serious adverse reactions (Dharmasiri et al., 2003; Park et al., 2004).

The plant SPH, popularly known as brown strophanthus, and hairy strophanthus in West Africa, including Nigeria, is indigenous to Africa (Beentje, 1982). A deciduous shrub of 5 m tall and up to 100 cm wide, having its stem bark dark grey in colour, with few lenticels, has been reported to have diverse medicinal uses; for example, in the Savannah Zone of West Africa, the latex and seeds of *Strophanthus hispidus* are used as arrow poison, while decoctions of root, stem bark or leaf are used externally to treat skin diseases, leprosy, ulcers, malaria, dysentery and gonorrhoea (Burkill, 1985). In Nigeria and Ghana, the root decoction is ingested to treat rheumatic diseases, while in Togo; the root bark macerate is employed for treating oedema.

The above-stated folkloric uses formed the basis for the present study, which aimed at exploring SPH root extract activity in both acute and sub-acute inflammatory reactions, as well as its possible interaction with some inflammatory mediators.

MATERIALS AND METHODS

Animals: Healthy Swiss mice (8-12 weeks) and Sprague Dawley rats (3-4 months) used in this study were obtained from National Agency for Food and Drugs Administration and Control (NAFDAC), Yaba, and maintained under standard laboratory conditions, as approved by the United States National Institute of Health (NIH) guide for Care and Use of laboratory animals and recommendation of IASP (Zimmerman, 1983). The animals, acclimatized for one week were fed on rodent diet (Livestock Feeds PLC, Ibadan, Oyo-State, Nigeria) and had free access to drinking water. However, they were fasted for at least 12 h prior to experimentation and all experiments were conducted between 9.00 am and 6.00 pm.

Plant Extraction: Fresh roots of *Strophanthus hispidus* were purchased from an herb seller at Mushin market, in Lagos, Nigeria. Identification and authentication were carried out by Mr. T.K. Odewo of the Department of Botany and Microbiology, University of Lagos, Lagos-Nigeria, with voucher number LUH 2618.

The fresh root of SPH was chopped into tiny bits and dried at room temperature for two weeks to obtain a constant weight. A known weight was boiled in a measured volume of distilled water on a hot plate for 1 h; and thereafter macerated for 72 h before filtering through sterile cotton wool. The filtrate was thereafter evaporated to dryness in an oven at 40° C. Percentage yield (w/w) of 5.48 % was obtained and pH measured at 100 mg/ml as 6.7.

Acute Toxicity Study: Groups of mice randomly selected were separately administered intraperitoneally (i.p) and orally with doses of the extract 10-200 mg/kg and 500-2000 mg/kg respectively. Behavioural parameters and mortality were monitored closely for the initial 2h and thereafter for 24 h. Delayed toxicity was also studied for the next 14 days and all observations were recorded. Lethal dose in fifty per-cent of the total population (LD_{50}) was interpolated using Miller and Tainter method (1944).

Anti-inflammatory studies: ***Carrageenan-induced rat paw oedema:*** Assessment was conducted as reported by Winter et al., (1962) and Agbaje et al., (2008). Overnight fasted adult rats were randomized into six groups (n=5) and treated as follows, 1 h prior oedema induction:

Group I: Saline 10 ml/kg
Group II: SPH 50 mg/kg
Group III: SPH 100 mg/kg
Group IV: SPH 500 mg/kg
Group V: SPH 1000 mg/kg
Group VI: IND (indomethacin) 10 mg/kg

Carrageenan (0.1 ml, 1 % w/v in saline) was injected into the subplantar tissue of the right hind paw to induce oedema. The linear paw circumference was measured (Bamgbose and Noamesi, 1981, Agbaje et al., 2008). Percentage (%) oedema inhibition was thereafter calculated according to Gupta et al. (2005) and Sawadogo et al. (2006) as

$$\% \text{ inhibition} = \frac{D_0 - D_t}{D_0} \times 100$$

D_0 = Mean paw diameter of Control group at a given time
D_t = Mean paw diameter of treated (extract or standard) group at the same time.

Xylene-induced ear oedema: Method of Junping et al., (2005) was adopted. Overnight fasted mice were divided into five groups (n = 5) and groups 1-4 were treated with distilled water and SPH in doses of 50, 100 and 500 mg/kg. Group 5, positive control, received 1.0 mg/kg dexamethasone (DEX). One hour afterwards, 30 µL xylene was applied to the inner surface of the right ear to induce oedema. The animals were anaesthetized with ether 1 h later, and both ears were removed and sectioned circularly, using a cork borer with diameter of 7 mm. The sections were weighed and percentage inhibition of ear oedema was calculated relative to the left ear that was without xylene. ***Serotonin-induced rat paw oedema:*** Two groups of fasted rats were randomly administered orally 10 ml/kg distilled water and 1000 mg/kg SPH. One hour afterwards, both were injected with

0.1 ml serotonin (10^{-3} mg/ml) into the sub-plantar tissue of the right hand paw (Amann et al., 1995; Agbaje et al., 2008). The linear paw was measured at 0 min as before and thereafter every 30 min for 3 h. Percentage oedema inhibition was also calculated. ***Histamine-induced rat paw oedema:*** The same procedure described above was repeated except that histamine (10^{-3} mg/ml) was used in place of serotonin. ***Formalin-induced mice paw oedema:*** Fasted mice were randomly divided into five groups of five animals each and separately given distilled water and the three doses of SPH as described before, while group 5, the positive control received 100 mg/kg aspirin. Inflammation was induced in all the animals by sub-plantar injection of 20 µL of freshly prepared 2% formalin in the right hind paw (Turner, 1965). Paw thickness was measured 1 h prior to and also after formalin injection. The drug treatments were continued for 6 consecutive days and paw oedema measured 1 h ~~of~~ after drug treatment each day. Percentage inhibitions were determined. ***Castor oil-induced diarrhoea:*** This method (Awounters et al., 1978) was to elucidate the involvement of prostaglandins in the anti-inflammatory activity of SPH. Aspirin 100 mg/kg, distilled water 10 ml/kg and SPH 1000 mg/kg were separately given through oral intubation to groups of mice followed by 20 ml/kg castor oil 1 h thereafter. Animals were examined for presence or absence of characteristic diarrhoeal droppings, onset of diarrhoea and number of wet stools, semi-solid and solid stools on a white paper on the floor of their cages every hour for 4 h. Absence of diarrhoeal droppings as well as delay in onset of diarrhoea, were recorded as a positive result, indicating possible inhibition of prostaglandin biosynthesis.

Phytochemical screening: Presence of various secondary metabolites was investigated, using simple chemical tests outlined by Odebiyi and Sofowora (1978).

Trace Elements Determination: The extract was prepared using standard procedure meant for Atomic Absorption Spectroscopy (AAS) (Smith and Hieftye, 1983).

Statistical Analysis: Data were recorded as mean ± standard error of mean (S.E.M), while statistical significance between groups were done using students' t-test at significant level $p<0.05$.

RESULTS

Acute Toxicity Test: No mortality was observed from oral feeding of up to 2 g/kg SPH, whereas, i.p dosing of 10, 35, 70 and 200 mg/kg recorded respective 0 %, 40 %, 80 % and 100 % deaths in the animals. LD_{50} was interpolated as 39.81 mg/kg (Table 1). Among the behavioural changes observed were abdominal writhes, increased respiratory rate, reduced motor activity and sedation. The latter effect was most prolonged.

Table 1. Acute toxicity of *Strophanthus hispidus* using i.p. route

SPH dose mg/kg	Log dose	No. Of deaths	% Response	Pro bit
10	1	0/5	0	0
35	1.54	2/5	40	4.74
70	1.84	4/5	80	5.84
200	2.3	5/5	100	8.72

LD_{50} = 39.81 mg/kg

Carrageenan-induced oedema: A significant ($p<0.05$) and dose-dependent inhibition of oedema was observed in all the treated animals (Figure 1). However, while the herbal drug effect peaked at 30 min post-induction, followed by a gradual reduction in efficacy, indomethacin featured a higher efficacy with peak effect at 90 min, followed by a sharp decline in activity.

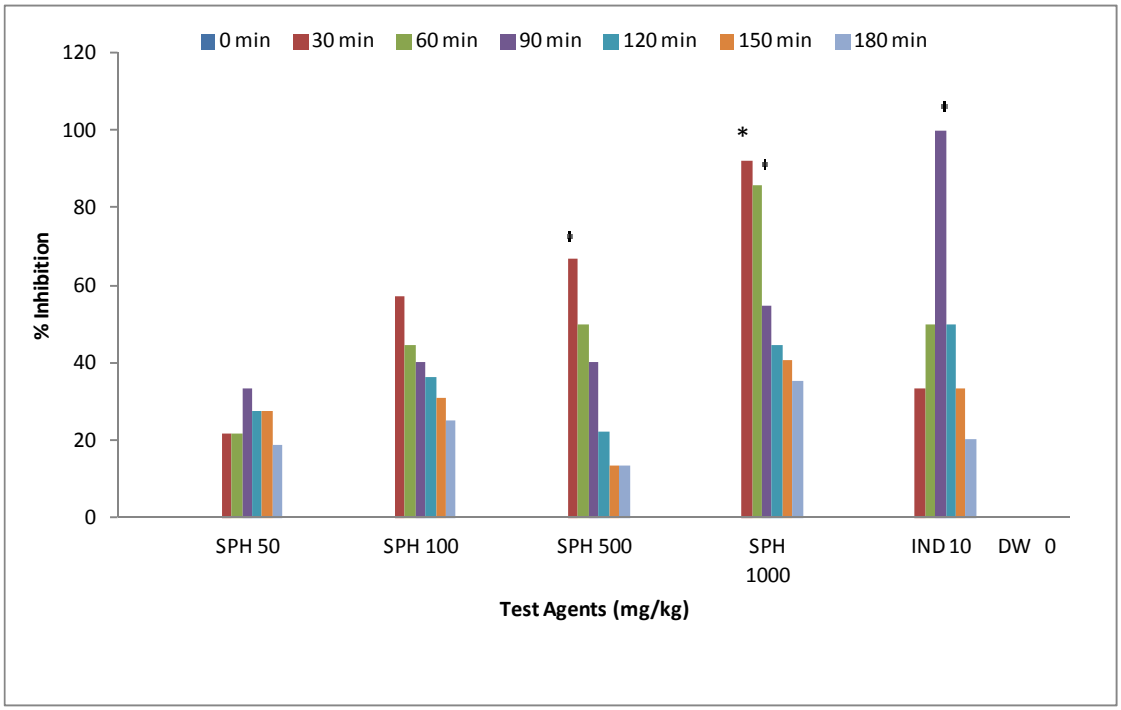

Figure 1: Effects of test agents on Carrageenan-induced Rat paw oedema0
*p<0.05 when compared with control

Xylene-induced oedema: A similar trend of activity as recorded in the carrageenan model ensued and it compared favourably with the effect of the standard drug (Figure 2).

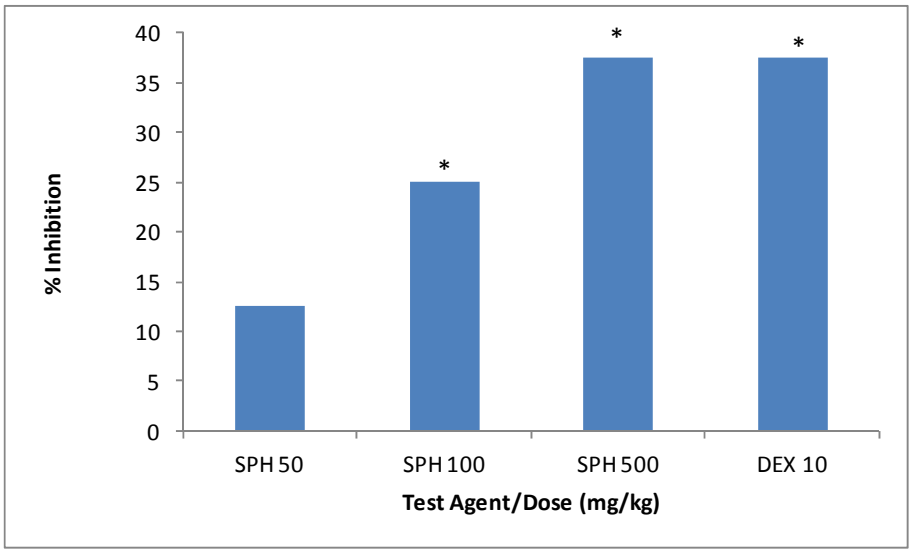

Figure 2: Xylene-induced ear oedema in mice
*p<0.05 when compared with control

Formalin-induced oedema: An appreciable significant (p<0.05) daily inhibition of formalin activity, similar to the standard drug (Figure 3) was observed.

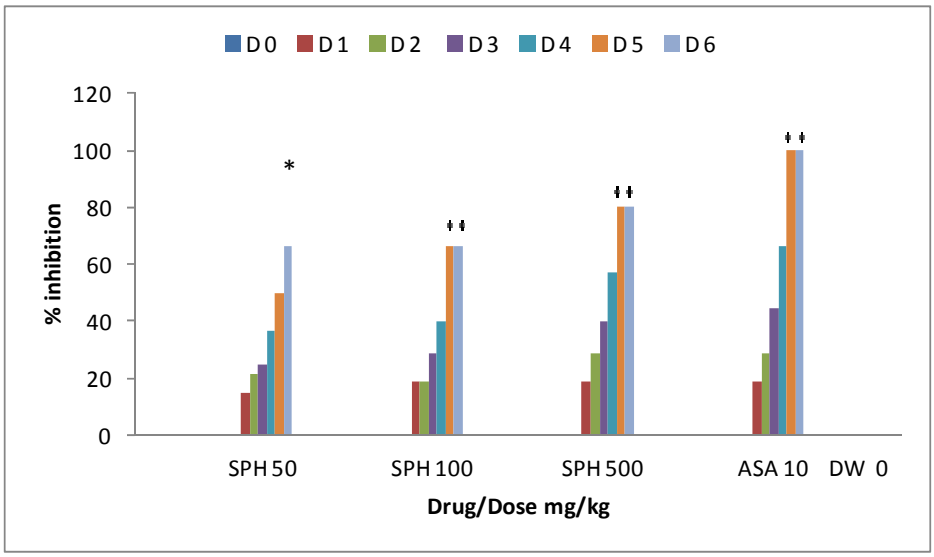

Figure 3: Effect of Test Agents on formalin-induced mice paw oedema; * p<0.05 compared with control

Serotonin-induced oedema: The highest activity (% inhibition of 42.86) was observed at 150 min (Figure 4).

Histamine-induced oedema: Extract produced a more pronounced and a better sustained inhibition (87.75 %) of histamine (Figure 4).

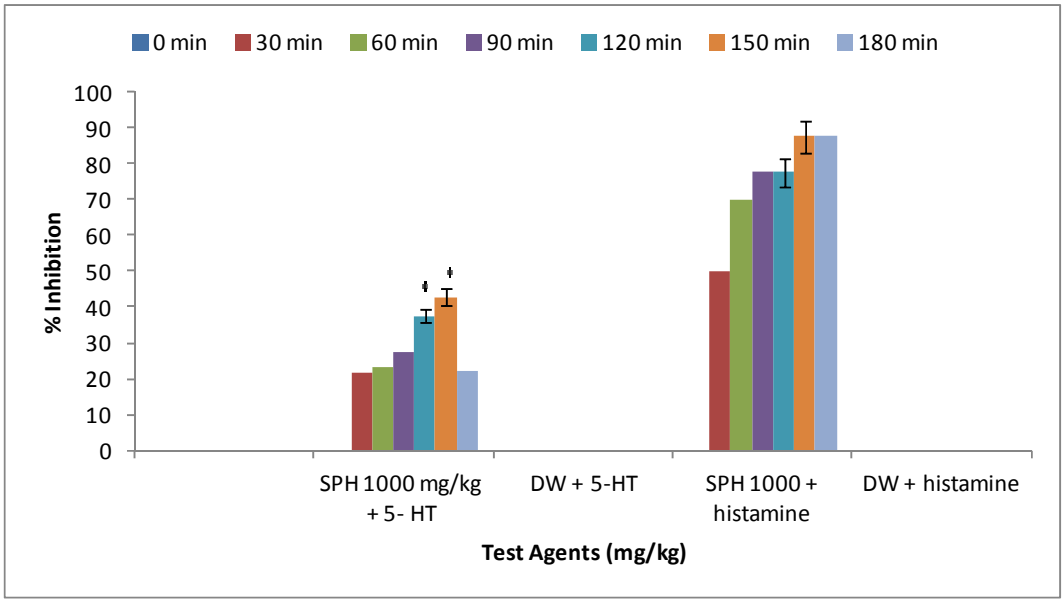

Figure 4: Effect of SPH on serotonin (5-HT) and histamine-induced oedema
*p<0.05 compared with control

Castor oil-induced diarrhoea: The extract (1000 mg/kg) showed a significant inhibition of prostaglandin synthesis as a result of delayed onset of diarrhoea and decreased number of wet stools coupled with increased number of solid stool (Table 2).

Table 2. Effect of SPH on Castor oil-induced Diarrhoea

Test Agent/Dose (mg/kg)	Onset of Diarrhoea (min)	Number of wet stool	Weight of wet stool (g)	Number of semi-solid stool	Weight of semi-solid stool (g)	number of solid stool	Weight of solid stool (g)
ASA 100	*92.0±4.64	*4.00±0.71	*0.46±0.21	*1.00±0.00	*0.02±0.01	*3.40±0.51	0.07±0.04
SPH	*80.0±3.54	*4.80±1.02	*0.80±0.23	*1.00±0.00	0.31±0.01	*3.20±0.37	0.03±0.42
DW	20.2±0.66	10.0±0.71	6.09±0.62	6.20±0.58	0.12±0.03	0.80±0.58	0.01±0.03

Parameters expressed as ± SEM; *p<0.05 compared with control. ASA = Acetylsalicylic acid

Phytochemical Analysis: Flavonoids, tannins, anthraquinones, and cardiac glycosides were identified in SPH.

Trace Metal Determination: Manganese, zinc, copper, lead and chromium were identified in the herbal preparation (Table 3).

Table 3: Concentrations of trace elements present in SPH

Trace Elements	Concentration (mg/L)
Zinc	0.028
Copper	0.093
Manganese	1.212
Chromium	-0.234
Lead	0.102

DISCUSSION

The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent (Winter et al., 1962). Carrageenan-induced oedema has been commonly used as an experimental model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the surrounding damaged tissues. On the other hand, the late phase is sustained by prostaglandins and mediated by bradykinin and leukotrienes, produced by tissue macrophages (Gupta et al., 2006; Ravi et al., 2009; Unnisa and Parven, 2011).

The effect of indomethacin (10 mg/kg) on carrageenan-induced paw oedema was most pronounced 90 min after injecting the phlogistic agent, while the extract showed highest activity at 30 min after drug administration. The anti oedematogenic effect on paw oedema induced by carrageenan is indicative of SPH involvement and benefits in acute inflammation. The latter could be substantiated by the inhibition of oedema induced by the different mediators employed in the study, the activity of which was most pronounced in the histamine model, which recorded 87.5 % inhibition, while serotonin model gave 42.86 %; both recorded at 150 min. The ability of the herbal drug to inhibit mediator-induced oedema suggests its antihistamine and antiserotonin properties, both mediators being potent vasodilators and also agents which increase vascular permeability. Inhibition of mediators could have been at the level of synthesis, release or receptor interaction (Vasudevan et al., 2007), and further studies are required to elucidate which of these levels.

Evaluating the activity of SPH on prostaglandin, the mediator of phase two response in carrageenan-induced inflammation; the herbal decoction delayed onset of diarrhoea in castor oil model, since all the parameters indicative of diarrhoea were inhibited (Table 2).

Xylene-induced ear oedema model is partially associated with substance P, which is an undecapeptide that is widely distributed in the central and peripheral nervous system and it functions as a neurotransmitter or a neuromodulator in a variety of physiological processes (Junping et al., 2005). Release of substance P from the sensory neurons causes vasodilatation and plasma extravasations, suggesting its role in neurogenic inflammation, thus causing the swelling of ear in mice. The latter test distinguishes non-steroidal anti-inflammatory drugs (NSAIDs) from steroidal anti-inflammatory agents.

Aqueous root extract of SPH in doses of 50 and 100 mg/kg showed a mild but significant ($p<0.05$) dose dependent inhibition of inflammation, when compared with distilled water (control) group, however, the largest dose of 500 mg/kg, compared well with 1.0 mg dexamethasone.

The formalin model suggests anti-arthritic effect of SPH, which produced 80 % inhibition of formalin on days 5 and 6. Activity of formalin is biphasic, consisting of an early neurogenic component followed by a later tissue-mediated response. In the first phase, there is a release of histamine, serotonin and kinin, while the second phase is related to the release of prostaglandins (Gupta et al., 2006; Turner et al., 1971). Peak inhibition of formalin on days 5 and 6 by the extract suggests inhibition of both phases.

Flavonoids, tannins, and glycosides present in SPH have been reported to possess anti-inflammatory property (Ahmadiani et al., 2000; Bittar et al., 2000; Kim et al., 2004; Agbaje et al., 2008). Also found present in the herbal preparation are lead (Pb), zinc, copper, manganese and chromium. Lead has been reported to be toxic in a wide variety of organs in both human and experimental animals (WHO, 1999), therefore, toxicity of SPH as observed in this study with its low LD_{50} (39.81 mg/kg) i.p. could be attributed to presence of Pb.

Anti-inflammatory activity of the extract could also be due to the contribution of the trace elements present. Copper and zinc supplementation have been reported to produce anti-inflammatory effect due to their ability to form complexes that serve as selective antioxidants (Di-Silvestro and Marten, 1990). Manganese on the other hand, has been established as a popular remedy for strains, sprains and inflammation, due to its ability to increase the level of superoxide dismutase (SOD), thus increasing antioxidant activity. Patients with rheumatoid arthritis or other inflammatory conditions have an increased need for manganese (Michael et al., 1996).

From the fore-going, the aqueous root extract of SPH has been established to possess anti-inflammatory property, which compared well with both conventional NSAIDs and steroidal anti-inflammatory drugs, in both acute and sub-acute inflammatory models used in this study. Furthermore, the scope of the present investigation has established the involvement of SPH in prostaglandin synthesis as well as its interaction with serotonin and histamine, which are inflammatory mediators.

CONCLUSION

The herbal plant, with a pH of 6.7 may serve as a suitable substitute to NSAIDs, if the toxic constituents, especially, lead could be precipitated or removed.

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