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Preliminary investigation of the anti-inflammatory activity of Harungana madagascariensis leaf extract Lam.Expoir (Hypericaceae)

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The anti-inflammatory activity of *Harungana madagascariensis* (Hypericaceae) leaf was evaluated using formaldehyde-induced acute edema and arthritis respectively, and cotton pellet granuloma in rats. The leaf extract was obtained by cold maceration in methanol:dichloromethane (1:1) for 72 h. The extract was also subjected to acute toxicity test and phytochemical analysis. Acute toxicity test revealed that the extract had an oral LD_{50} > 5000 mg /kg. Results showed that the extract elicited non-significant and non-dose related inhibition of formaldehyde-induced acute edema in rats. The extract also inhibited formaldehyde-induced arthritis, but however failed to inhibit cotton pellet-induced granuloma formation. Phytochemical analysis revealed the presence of alkaloids, saponins, tannins, proteins, reducing sugars, carbohydrates, flavonoids, terpenoid, steroids, acidic compounds, resins, glycosides, fats and oils in the extract. In conclusion, the extract exhibited mild inhibition of acute and chronic edema but has no effect on granuloma formation. The observed effects are likely due to the phytoconstituents of the leaf extract.

Key words: *Harungana madagascariesis,* inflammation, antiinflammation, edema, granuloma.

INTRODUCTION

Harungana madagascariensis (Hypericaceae) is a tropical shrub common in most tropical rainforest margins and stream banks. The plant is native to Central African Republic, Democratic Republic of Congo, Sudan Ethiopia, Lesotho and South Africa. *H. madagascariensis* is a wood plant of the savannah. The morphology of the plant has been described (Adeneye et al., 2008). Its common name is Dragon Blood tree. It is called “Amuje” or “Elepo” by the Yoruba, “Uturu” by the Igbo tribes of Nigeria. Its common name include Dragon Blood tree. It is commonly referred to as ‘‘Amuje’’ or ‘‘Elepo’’ by the Yoruba, ‘‘Uturu’’ by the Igbo tribes of Nigeria, and as ‘‘Bio harangue’’ by the French.

Different parts of the plants are highly valued for the treatments of diverse human diseases (Adeneye et al., 008). The red juice of the stem is used in Sierra Leone to arrest post-partum bleeding and to treat enteritis, scabies and jaundice in Ondo State, Nigeria (Adeneye et al., 2008). It is also used to as a remedy for tapeworm infection and to enhance breast development in young

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female adults. The unopened bud sheaths are eaten with palm oil to treat puerperal infection. Thepowdered bark is used along with *Pentaclethra macrophylla* to treat leprosy (Adeneye et al., 2008). Other uses include as anti-malarial, treatment of liver and kidney diseases. (Adeneye et al., 2008). The young leaves are also used in the treatment of asthma (Orwa et al., 2009).

The aim of the study was to investigate the anti-inflammatory activity of *H. madagascariensis* leaf extract, using formaldehyde induced rat paw edema and cotton pellet induced arthritis in rats.

MATERIALS AND METHODS

Animals

Adult Swiss albino rats (109 to 165 g) and mice (20 to 28 g) of both sexes bred in the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the study. The animals were housed in stainless steel cages under standard conditions and maintained freely on standard pellets and water *ad lititum* and acclimatized for 2 weeks. Animal experiments were in compliance with National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985).

Collection and preparation of the leaves

Fresh leaves of *H. Madagascariensis* were collected from Orba, Udenu Local Government of Enugu State in July. The plant material was identified and authenticated by Mr. Ozioko, a taxonomist at the International Centre for Ethnomedicines and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The leaves were dried under shade for 21 days, and milled using a mechanical grinder.

Preparation of extract

About 2 kg of the milled leaves were extracted by cold maceration at room temperature (25±1°C) with a mixture of methanol and dichloromethane (1:1) with intermittent shaking for 48 h. The marc was rinsed to obtain a clear solvent. The filtrate was concentrated using a rotary evaporator to obtain the extract (HME; 6.22%w/v).

Phytochemical screening

Preliminary phytochemical analysis of the extract was performed using standard methods (Trease and Evans, 1989).

Pharmacological studies

Acute toxicity and lethality (LD_{50}) evaluation of HME

This was done as described by Lorke (1983).

Formaldehyde induced edema in rats

The anti-inflammatory property of HME was investigated using formaldehyde induced edema in rats (Seyle, 1949). Adult Swiss albino rats of both sexes (109 to165) g were randomly placed into 5 groups (n=6) and the volume of water displaced by the right hind limb of each rat in each group was determined at 0 h.

Subsequently, group I received 10 mg/kg piroxicam; group II received 2.5 ml/kg 80% propylene glycol while groups III to IV received 200 , 400 and 800 mg/kg of HME suspended in 80% propylene glycol respectively. One hour later, arthritis was induced by sub plantar injection of 0.1 ml of (2%) formaldehyde into the right hind paw. Four hours later, edema was assessed by measuring the volume of water displaced by the inflamed paw and used to determine the effect of acute inflammation while HME, piroxicam and vehicle were administered daily for ten days. Inflammation was re-induced on the third day. Daily changes in edema was evaluated once daily for those ten days by measuring the volume of water displaced by the inflamed paw. Responses to formaldehyde induced edema were quantified for each group, using the formula:

Percentage (%) inhibition =100 [1-a/b]

Where, a= mean edema for treated group and b= mean edema of control group.

Cotton pellet granuloma test in rats

The effect of HME on granulomatous inflammation was evaluated using the cotton pellet model (Winter and Porter, 1957). Adult Swiss albino rats (104 to 178 g) of both sexes were randomly placed into 5 groups (n = 6). Group I received 10 mg/kg piroxicam, group II received 2.5 ml/kg of 80% propylene glycol and groups III to V received 200, 400, and 800 mg/kg of HME respectively. On day one, the animals were anaesthetized with xylazine (10 mg/kg) and ketamine (50 mg/kg) and the hairs on their axial region depilated, using a sterile razor blade. The depilated skin was then swabbed with methylated spirit. Incisions of about 1cm were made on each side of the axial region at the depilated spots. Sterile autoclaved cotton pellets (30 mg) were aseptically implanted. HME were then administered once daily from day 1 post implantation till day 7. On day 8, the animals were sacrificed using overdose of chloroform anaesthesia. The pellets were carefully dissected out from the surrounding tissues. The pellets were then dried in a hot air oven at 60°C to a constant weight and their respective weights recorded.

Statistical analysis

Data obtained were analyzed using one –way ANOVA is SPSS version 19.0 and subjected to least significance difference (LSD) post-hoc test. Differences between means were accepted to be significant at $P < 0.05$ and results expressed as mean ± standard error of mean (SEM).

RESULTS

Phytochemical constituents of HME

The plant extract contained high quantities of saponins, tannins, proteins, carbohydrates and glycosides. Flavonoids and steroids were in moderate quantities while alkaloids, terpenoids, fats and oils, resins, reducing sugars and acidic compounds were present only in minute quantitiesm (Table 1).

Evaluation of LD_{50}

No death of animal was recorded up to 5000 mg/kg dose

Table 1. Phytoconstituents of HME.

Phytochemical constituent	Relative Presence
Saponins	++++
Glycosides	++++
Tannins	++++
Proteins	+++
Carbohydrate	+++
Flavonoids	++
Resins	+
Fats and Oil	+
Reducing Sugars	+
Terpenoids	+
Alkaloids	+
Steroids	+
Acidity	+

++++ = Abundantly present; +++ = highly present; ++ = moderately present; + = present.

Table 2. Effect of HME on acute formaldehyde induced edema in rats.

Treatment	Dose(mg/kg)	Edema(ml)	Inhibition (%)
Proximal	10	0.20±0.15	47.4
	200	0.30±0.11	21.1
HME	400	0.20±0.20	47.4
	800	0.28±0.08	26.3
Control	2.5 ml/kg	0.38±0.10	

n =6; values of edema are shown as mean ± SEM; HME = *H. madagascariensis* extract.

of the HME extract administered orally. No gross sign of toxicity was also recorded.

Effect of HME on acute formaldehyde induced edema in rats

The extract elicited non-dose related inhibition of formaldehyde-induced edema at 4 h post induction. However, none of the treatment levels gave significant inhibition when compared with control. The order of inhibition was 200 mg/kg<800 mg/kg< 400 mg/kg. The 400 mg/kg dose exhibited comparable inhibition with piroxicam (Table 2).

Effect of HME on formaldehyde induced arthritis in rats.

There was gradual reduction in edema in a non-dose related manner, as the 400 mg/kg dose gave the highest percentage edema inhibition. However, none of the dose levels of HME gave significant inhibition of edema compared with control (Table 3).

Effect of extract on cotton pellet granuloma in rats

The extract did not inhibit granuloma formation compared with control (Table 4).

DISCUSSION

Results obtained from acute toxicity evaluation indicate that the plant extract is safe up to 5000 mg/kg dose by oral administration. However, due to its high saponin content, it may not be safe up to such does if administered by intra-peritoneal rout (i.p), as saponin induces haemolysis. The extract is safe by oral administration due to the presence of micro–organisms that degrade saponins in the gastro-intestinal tract.

Formaldehyde induced inflammation is mediated by pro-inflammatory substances such as histamine, serotonin, bradykinin and prostaglandins (wheeler- Aceto and Cowan, 1991). Its main characteristics are the

Table 3. Effect of HME on Chronic Formaldehyde-induced Arthritis.

Treatment	Dose (mg/kg)	Edema (ml)									
		1	2	3	4	5	6	7	8	9	10
Piroxicam	10	0.15±0.18 (40)	0.13±0.16 (10)	-0.02±0.16 (140)	0.13±0.15 (0.0)	0.02±0.18 (75)	-0.03±0.10 (160)	-0.02±0.10 (129)	0.10±0.11 (-100)	-0.07±0.08 (450)	0.00±0.09 (0.0)
HME	200	0.20±0.6 (20)	0.05±0.08 (67)	-0.07±0.08 (40)	0.08±0.10 (40)	0.08±0.04 (0.0)	-0.07±0.05 (-40)	-0.03±0.5 (58)	0.03±0.5 (40)	-0.07±0.05 (450)	0.05±0.05 (-70)
	400	0.12±0.26 (54)	0.02±0.13 (86.7)	-0.12±0.12 (340)	0.07±0.12 (47)	0.10±0.15 (-25)	-0.02±0.14 (140)	-0.08±0.08 (-14)	-0.05±0.08 (200)	-0.08±0.13 (500)	0.00±0.09(0.0)
	800	0.13±0.10 (48)	0.03±0.10 (80)	-0.07±0.15 (240)	0.08±0.08 (40)	0.10±0.14 (-25)	0.08±0.13 (-60)	0.07±0.10 (0.0)	- 0.08±0.18 (-60)	-0.07±0.08 (-250)	0.03±0.12(0.0)
	2.5 mL/Kg	0.25±0.21	0.15±0.19	0.05±0.12	0.13±0.14	0.08±0.12	0.05±0.10	0.07±0.16	0.05±0.10	0.02±0.12	0.03±0.10

n= 6; values of edema are shown as mean ± SEM. Values in parenthesis represent inhibition (%) of edema

Table 4. Effect of HME on cotton pellet-induced granuloma in rats

Treatment	Dose (mg/kg)	Granuloma weight (mg)	Inhibition (%)
Proximal	10	71±0.10	29.2
HME	200	112.5±9.24	-22.3
	400	109.2±7.00	-18.9
	800	104.0±2.92	-13.0
Control	2.5ml/kg	92.0±4.64	-

n= 6; values shown are Mean ± SEM. HME = *H madagascariensis* extract.

exudation of fluid and plasma proteins and the emigration of leukocytes, predominantly neutrophils and which last for a few minutes to few days. (Robin et al., 1994). The formaldehyde arthritis model has been used to evaluate the effect of anti-inflammatory agents on chronic inflammation where articular changes induced by formaldehyde injection mimic those that occur in rheumatoid arthritis (Akah et al., 2007). Thus the results obtained showed that HME could not effectively disrupt the processes of both acute and chronic rheumatoid inflammation.

The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation (Winter and Porter 1957). A granuloma is a localized aggregate of macrophages (which are activated and transformed into epithelioid cells), usually surrounded by a cuff of lymphocytes and occasionally plasma cells (Burt and Fleming 2008). The weight of the wet cotton pellets correlates with transuded materials and the dry weight of pellet correlates with the amount of granulomatous tissue (macrophages), (Rawat et al., 2011). The result obtained thus indicated that HME did not any significant effect granulomatous inflammation.

Conclusion

The extract inhibited acute and chronic edematous inflammation but had no effect on granulomatous inflammation.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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