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Full Length Research Paper

Anti-inflammatory and anti-oxidant effects of *Sterculia tragacantha* fractions in mice

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Six fractions obtained from the methanol leaf extract of *Sterculia tragacantha* were screened for anti-inflammatory and anti-oxidant activities. Treatment of mice with 10 and 90 mg/kg fractions 3, 4, 5, 6 and 7 significantly inhibited carrageenan induced paw edema in mice. The higher doses (90 mg/kg) showed superior anti-inflammatory activity compared to 10 mg/kg. The percentage edema inhibitions of 90 mg/kg fractions 5, 6 and 7 at 4 h were 75, 80 and 75%, respectively. Daily administration of fractions 3, 4, 5, 6 and 7 significantly suppressed formaldehyde induced paw edema by day three and five post edema induction. Fractions 3, 4, 5, 6 and 7 inhibited granuloma formation significantly. The anti-inflammatory effects of fractions 5, 6 and 7 on granuloma formation were better than that of dexamethasone. The fractions showed concentration dependent 1,1-diphenyl-2-hydrazyl (DPPH) scavenging activity. At 400 µg/ml, the anti-oxidant activity of fraction 5 (75.6%) and 6 (73.6%) were comparable to that of ascorbic acid (79.1%). In conclusion, this study has shown that the fractions of *S. tragacantha* possessed anti-inflammatory and anti-oxidant activities, thus, providing further proof that the leaves of *S. tragacantha* contains an active compound with potent anti-inflammatory activity.

Key words: Fractions, edema, granuloma, anti-oxidant, *S. tragacantha*.

INTRODUCTION

Inflammation is a biologic process initiated by noxious stimuli such as chemical injury, trauma or surgery (O'Byrne and Dalgleish, 2001). This biologic response protects the host and heals damaged tissues after an infection or tissue damage (Nathan, 2002; Narendhirakannan et al., 2007). The initial inflammatory response is usually acute and may or may not evolve into chronic inflammation (Walsh and Pearson, 2001).

Acute inflammation is rapid in onset and of short duration (Lee and Jeong, 2002; Iwalewa et al., 2007). Its cardinal signs of heat, hyperemia, edema and pain are brought about by cutaneous vasodilation, fluid exudation and neutrophil migration into the damaged tissues (Robbins et al., 1994). Chemical mediators of acute inflammation include prostaglandins, histamine, serotonin and bradykinins (Snow, 1981). Chronic inflammation on the contrary is characterized by exudation, macrocytic infiltration, fibroblast proliferation and granuloma formation (Walsh and Pearson, 2001; Dunne, 1990; Jackson et al., 1997). Acute and chronic inflammatory disorders account for most of the painful medical problems encountered all over the world (Mehmet, 2002). Numerous orthodox anti-inflammatory drugs including the non-steroidal anti-inflammatory drugs are used to treat inflammatory diseases (Rang et al., 2003). However, prolonged use of these agents may cause gastric ulceration, kidney disorder and bone marrow suppression (Mehmet, 2002). Thus, the use of ethno medicines in the management of acute and chronic inflammatory disease appears safer (Dharmasiri et al., 2003).

The leaves, bark, shoots and seeds of *Sterculia tragacantha* Lindl are used to prepare ethno medicines for the treatment inflammatory disorders such as edema,

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Abbreviation: DPPH, 1, 1-Diphenyl-2-hydrazyl.

arthritis, rheumatism, and whitlow (Iwu, 1993). Recently, we reported that the methanol leaf extract of *S. tragacantha* showed anti-nociceptive, anti-inflammatory and anti-oxidant activities (Udegbunam et al., 2011). Phytochemical screening of this extract revealed the presence of carbohydrates, starch, glycosides, alkaloids, flavonoids, terpenes, tannins and saponins (Udegbunam et al., 2011).

This study was carried out to separate the components of the crude extract of *S. tragacantha* and to test the fractions obtained for anti-inflammatory and anti-oxidant activities. The result of this study will provide further scientific evidence supporting the use of this plant in the management of inflammatory diseases.

MATERIALS AND METHODS

Plant collection and identification

Fresh leaves of *S. tragacantha* were collected in September, 2009 from Nsukka area. They were authenticated by Mr. A.O. Ozioko, a taxonomist with the International Centre for Ethnomedicine and Drug Development, Nsukka. The voucher specimens (INTERCEED, 819) were deposited in their herbarium for reference purposes.

Extraction and fractionation

The fresh leaves of *S. tragacantha* were air dried and then pulverized. The powdered plant materials were cold macerated in 80% v/v methanol for 48 h. The extract obtained after filtration was concentrated using a vacuum rotary evaporator (yield: 11.1%). The methanol extract (10 g) was subjected to column chromatography to separate its components. Briefly, the extract was first adsorbed unto silica gel (70 to 230 mesh, 60 A) and the slurry introduced into a glass column. Elution was done successively using n-hexane, chloroform, ethyl acetate and methanol as follows: hexane (200 ml); hexane-chloroform (320:80 ml); hexane-chloroform-ethyl acetate (90:180:30 ml); chloroform-ethyl acetate (400:100 ml); ethyl acetate-methanol (180:120 ml); ethyl acetate-methanol (80:120 ml); ethyl acetate-methanol (40:160 ml) and methanol (400 ml). The elutes (10 ml) were collected into test tubes and spotted on precoated silica gel GF_{254} aluminium thin layer chromatography (TLC) plates. Spots were allowed to dry and the TLC plates inserted into a TLC tank containing chloroform, ethyl acetate and methanol (7.5:5:2.5 ml). On ascension of the solvent system up to two-third of the plates, plates were removed and allowed to dry. Subsequently, the plates were sprayed with a mixture of vanillin and sulphuric acid and then dried. These plates were viewed under a ultraviolet (UV) lamp. Fractions with similar bands were pooled and dried to obtain fractions 2, 3, 4, 5, 6 and 7 which were used in this study.

Phytochemical analysis of fractions

The fractions were tested for the presence of alkaloids, flavonoids, tannins, terpenes and saponins (Harborne, 1984).

Carrageenan-induced paw oedema test

The effect of the extract on acute inflammation was evaluated using the carrageenan-induced paw edema test (Winter et al., 1962). Six groups (n=5) of mice were treated intra peritoneally (i.p) with 10 mg/kg of fractions (F_2, F_3, F_4, F_5, F_6 and F_7) while another six groups of mice were treated i.p with 90 mg/kg of the aforementioned fractions. The mice in the control group were treated with distilled water (1 ml/kg, i.p). Indomethacin (10 mg/kg, i.p) was used as standard. Acute inflammation was induced 30 min post treatments by sub plantar injection of 0.02 ml of 1% carrageenan. The paw thickness of each mouse was measured using a Vernier calliper before edema induction and at 1 and 4 h post edema induction. Edema and percentage edema inhibition were calculated as described by Zhang et al. (2008).

Formaldehyde induced paw edema test

Sub acute inflammation was induced in eight groups (n = 5) of mice by sub plantar injection of 2.5% formaldehyde (Musa et al., 2007). Mice were injected i.p with 90 mg/kg F_2, F_3, F_4, F_5, F_6 and F_7 30 min prior to formaldehyde injection and subsequently daily for a period of four days. The control group were injected daily with distilled water (1 ml/kg). Dexamethasone (3 mg/kg) was used as standard. Each paw size was measured on day 0 and then on days 2 and 5.

Cotton-pellet induced granuloma test

The effect of the fractions on chronic inflammation was investigated by cotton pellet granuloma test (Niemegeers et al., 1975). A pair of ventral longitudinal incision were made on the right and left axillae of mice under pentobarbitone (35 mg/kg) anaesthesia. Two sterile cotton pellets, weighing 20 mg each, were implanted through the incision. Mice were injected i.p daily with 90 mg/kg F_2, F_3, F_4, F_5, F_6 and F_7 after cotton implantation for seven days. Mice in the control group were injected i.p with distilled water. Dexamethasone (3 mg/kg) was used as standard. On day eight, mice were euthanized with chloroform and the cotton pellet with adhering granulomatous tissue were dissected out, dried for 24 h at 60°C and weighed. The granuloma weight was calculated as described by Udegbunam et al. (2011).

1, 1-Diphenyl-2-hydrazyl (DPPH) assay

The DPPH free radical scavenging activity of the fractions at 10, 50, 100, 200 and 400 µg/ml were evaluated as described by Mensor et al. (2001). Ascorbic acid was used as reference standard. Absorbance at 517 nm was taken after 30 min incubation in the dark at room temperature. The concentrations were prepared in triplicates. Percentage anti-oxidant activity was calculated as described by Hsu (2006).

Data analysis

The mean edema size and granuloma weights of the treated groups and those of the control were compared using one way analysis of variance in the SPSS version 16.0. Duncan multiple range test was used to separate variant mean at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical analysis

As shown in Table 1, all fractions contained alkaloids and flavonoids while three fractions (F_5, F_6 and F_7) contained saponins.

Table 1. *Sterculia tragacantha* fractions and their phytoconstituents.

Fraction	Phytoconstituent				
	Alkaloid	Tannin	Flavonoid	Terpene	Saponin
F2	+	-	+	-	-
F3	+	-	+	-	-
F4	+	-	+	-	-
F5	++	-	++	-	+
F6	+	-	++	-	+
F7	+	-	++	-	+

+, Present; ++, highly present; -, absent.

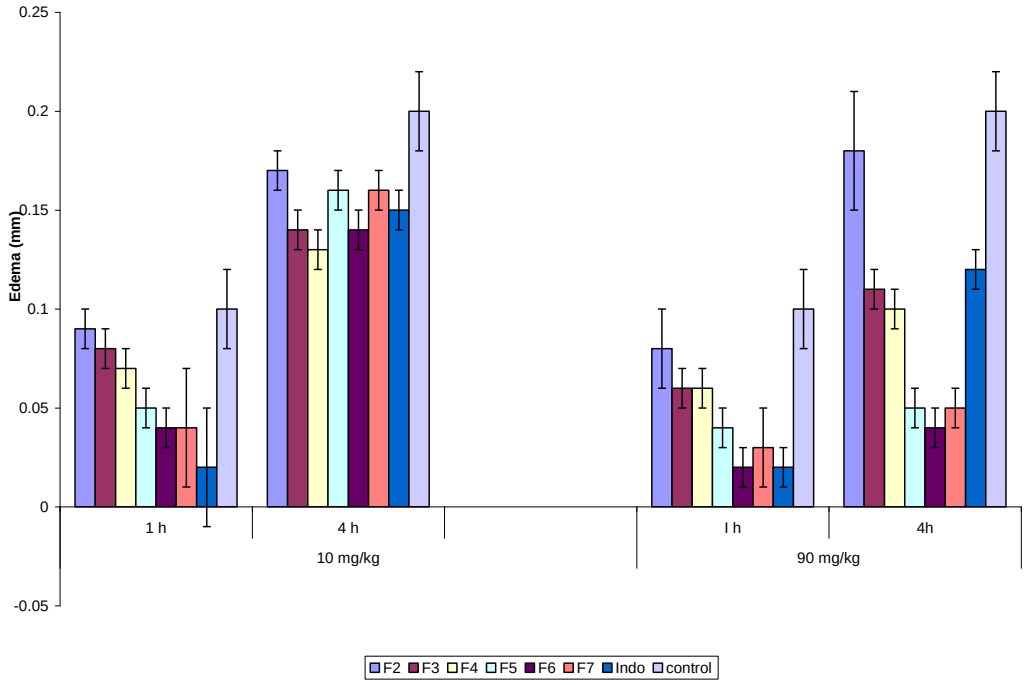

Figure 1. Paw edema (mm) in mice treated with fractions of *S. tragacantha*.

Carrageenan induced paw edema

In this test, 10 and 90 mg/kg F_3, F_4, F_5, F_6 and F_7 showed significant ($p < 0.05$) anti-inflammatory activity (Figure 1). The higher doses (90 mg/kg) showed superior anti-inflammatory activity compared to 10 mg/kg. The percentage edema inhibitions of 90 mg/kg F_5, F_6 and F_7 at 4 h were 75, 80 and 75% respectively (Table 2). Indomethacin showed edema inhibition of 40%.

Formaldehyde induced paw edema

Daily administration of F_3, F_4, F_5, F_6 and F_7 suppressed formaldehyde induced paw edema by day three and five post edema induction (Table 3). The effects of F_5, F_6 and F_7 were comparable to that of dexamethasone.

Cotton pellet granuloma

Five fractions (F_3, F_4, F_5, F_6 and F_7) significantly ($p < 0.05$) inhibited granuloma formation (Table 4). The inhibitory effect of F_5 (67%), F_6 (71%) and F_7 (62%) on granuloma formation were better than that of dexamethasone (57%).

DPPH free radical scavenging activity

The fractions showed concentration dependent DPPH scavenging activities (Table 5). At 400 µg/ml, the anti-oxidant activity of F_5 (75.6%) and F_6 (73.6%) were

Table 2. Percentage inhibition of carrageenan-induced paw edema by fractions.

Treatment	Percentage (%) edema inhibition			
	10 mg/kg		90 mg/kg	
	1 h	4 h	1 h	4 h
F_2	10	15	20	10
F_3	20	30	40	35
F_4	30	35	40	35
F_5	50	20	50	75
F_6	60	30	80	80
F_7	60	20	70	75
Indo	80	25	80	40

Indo, Indomethacine.

Table 3. Effect of fractions on formaldehyde -induced paw edema.

Treatment	Paw thickness (mm)			
	Day 0	Day 1	Day 3	Day 5
F_2	0.2 ± 0.01	0.3 ± 0.02	0.32 ± 0.03^{ab}	0.33 ± 0.01^{a}
F_3	0.19±0.02	0.3 ± 0.01	0.29 ± 0.00^{c}	0.28 ± 0.00^{c}
F_4	0.2 ± 0.01	0.3 ± 0.01	0.29 ± 0.01^{bc}	0.26 ± 0.01^{bc}
F_5	0.2 ± 0.01	0.29 ± 0.02	0.27 ± 0.02^{cd}	0.25 ± 0.03^{cd}
F_6	0.2 ± 0.01	0.3 ± 0.00	0.25 ± 0.00^{d}	0.24 ± 0.01^{d}
F_7	0.2 ± 0.01	0.3 ± 0.00	0.25 ± 0.01^{d}	0.24 ± 0.02^{d}
Dexa	0.21±0.01	0.3 ± 0.02	0.25 ± 0.01^{d}	0.23 ± 0.01^{d}
Control	0.2 ± 0.01	0.31 ± 0.01	0.33 ± 0.01^{a}	0.35 ± 0.00^{a}

Dexa, Dexamethasone; different superscripts in a column show significant difference ($p<0.05$).

Table 4. Effect of fractions on granuloma formation in mice.

Treatment	Granuloma wt (g)	% inhibition
F_2	0.18 ± 0.04^{c}	14
F_3	0.09 ± 0.05^{b}	57
F_4	0.10 ± 0.02^{b}	52
F_5	0.07 ± 0.03^{ab}	67
F_6	0.06 ± 0.04^{a}	71
F_7	0.08 ± 0.02^{ab}	62
Dexamethasone	0.09 ± 0.02^{ab}	57
Control	0.21 ± 0.01^{c}	-

Different superscripts in a column show significant difference ($p<0.05$).

comparable to that of ascorbic acid (79.1%).

The results of this study show that five fractions of *S. tragacantha* (F_{3-7}) exhibited inhibitory effect on inflammation induced by carrageenan and formaldehyde. Carrageenan and formaldehyde induced paw edema tests are routinely used to screen plants for anti-inflammatory properties (Lawal et al., 2010; Sini et al., 2010; Sofidiya et al., 2010). Subcutaneous injection of these inflamogens into the paw of mice leads to the release of algogens such as histamine, serotonin, bradykinin, substance P and prostaglandins (Wheeler-Aceto and Cowan, 1991; Lalenti et al., 1992; Sumen et al., 2001). The inhibition of both carrageenan and formaldehyde induced paw edema by (F_{3-7}) was an indication that these fractions suppressed the release of chemical mediators of inflammation. These findings further validate our earlier report that *S. tragacantha* extract possessed anti-inflammatory activities

Table 5. Percentage DPPH free radical scavenging activity of fractions.

Treatment	Concentration (µg/ml)				
	10	50	100	200	400
Ascorbic acid	73.6	73.8	74.6	77.2	79.1
F2	39.8	59.2	59.5	59.7	61.7
F3	32.1	43.7	47.6	57.4	69.4
F4	15.6	30.6	59.9	63.9	65.1
F5	32.1	43.8	47.5	57.4	75.6
F6	46.8	66.9	68.2	71.5	73.6
F7	49.2	50.2	53.8	60.4	65.6

(Udegbunam et al., 2011).

The cotton-pellet induced granuloma test is used to evaluate the effect of test drugs on proliferative component of chronic inflammation (Swingle and Shiderman, 1972; Niemegeers et al., 1975). The F_3 to F_7 showed moderate to potent inhibitory effect on granuloma tissue formation, thus indicating that they suppressed fibroblast proliferation and collagen synthesis needed for granuloma formation (Swingle and Shiderman, 1972). Similar studies have shown that extracts and fractions of medicinal plants suppressed the proliferative phase of chronic inflammation (Gupta et al., 2007; Garg and Paliwal, 2011).

Fractionation of the extract of *S. tragacantha* by column chromatography yielded six fractions (F_2 to F_7). F_2 showed insignificant anti-inflammatory activity, F_3 to F_4 showed moderate activity while F_5 to F_7 showed the most potent effect on inflammation induced by carrageenan, formaldehyde and cotton pellet. F_5 to F_7 also showed better DPPH scavenging activities compared to F_{2-4}. These results suggest that the chemical components of *S. tragacantha* responsible for its anti-inflammatory and anti-oxidant activities reside predominantly in F_5, F_6 and F_7. Phytochemical screening of the fractions of *S. tragacantha* showed that F_5, F_6 and F_7 contained higher concentration of flavonoids compared to the other three fractions. Although, F_5 to F_7 were not further fractionated to enable characterization and identification of the actual active component responsible for the observed anti-inflammatory and anti-oxidant activities, we attribute these activities to the presence of flavonoids in the leaves of *S. tragacantha* (Alcaraz and Ferrandiz, 1987; Ferrandiz and Alcaraz, 1991; Hanasaki et al., 1994; Kerry and Abbey, 1997; Middleton, 1998).

In conclusion, this study has shown that the fractions of *S. tragacantha* possessed moderate to profound anti-inflammatory activity, thus, providing further proof that the leaves of *S. tragacantha* contains an active compound with potent anti-inflammatory activity.

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