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Bioactivity-guided studies on the antiinflammatory activity of extract of aerial parts of *Schwenckia americana* L. (Solanaceae)

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The antiinflammatory activity of the methanol:methylene chloride extract of the aerial parts of *Schwenckia americana* L. (Solanaceae) (SAE) and its mechanisms were evaluated. Bioactivity-guided studies on SAE and its fractions was done using the egg albumin-induced rat paw edema as activity guide. Further screening for anti-inflammatory activity was accomplished using the xylene-induced ear edema and cotton pellet-induced granuloma formation. The putative anti-inflammatory mechanisms were studied using acetic acid-induced vascular permeability, carrageenan-induced neutrophil migration and heat- and hypotonic solution- induced hemolysis. Phytochemical analysis of SAE and fractions was carried out using standard procedures. Results showed that SAE and fractions significantly ($P < 0.05$) inhibited rat pedal edema development with the magnitude of inhibition increasing with fractionation of SAE in the order, fraction A > dichloromethane fraction > SAE. SAE also significantly ($P < 0.05$) inhibited acetic acid-induced vascular permeability and topical edema development in the mouse ear. Granuloma tissue formation and neutrophils count in rats, and heat and hypotonic solution- induced hemolysis of ox erythrocytes were also inhibited. The anti-inflammatory mechanism of *S. americana* may partly be through membrane stabilization and inhibition of vascular permeability, neutrophil migration, cellular infiltration and proliferation. Phytochemical tests revealed the presence of terpenoids and steroids in the most active fraction.

Key words: *Schwenckia americana*, anti-inflammatory, granuloma, ear edema, vascular permeability, neutrophil migration, membrane stabilization.

INTRODUCTION

Schwenckia americana L. (Solanaceae) is a weed native to Central and South America, but has spread to tropical Africa and India and is widely distributed in Nigeria,

where it is variously called 'Dandana', 'Farfetsi' or 'Daudanasor' by Hausa, 'Igbale odan' or 'Aleodan' by Yoruba and 'Ayafe dibia' or 'Aya dibia' by Igbo tribes.

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Among communities in these tribes and beyond, *S. americana* is employed in traditional medicine practice for mitigation of a variety of disorders including sexually transmitted diseases and inflammatory disorders such as rheumatoid arthritis (Iwu, 1993). The powdered plant mixed with fresh fat obtained from cow milk or shea-butter to make a paste is applied topically or its decoction administered orally (Asusheyi et al., 2010). The morphology has been described (Iwu and Anyanwu, 1982; Iwu, 1993).

Generally in West Africa, the aerial parts are widely used to treat diseases in babies and young children. The roots are chewed to treat chest pain and respiratory diseases in children, while the root decoction is given to babies as a purgative. In Yoruba communities (Western Nigeria), the plant is a common ingredient of 'Agbo' (a traditional medicinal concoction) for children in the first and second years of life; it is also boiled and added to their food (Dalziel, 1956). A decoction of the plant is taken by nursing mothers or given to the suckling baby to prevent neonatal diarrhea. A leaf decoction is given to pregnant women when the fetus is suspected to be developing too slowly. It is also taken by nursing mothers to prevent diarrhea in the baby (Iwu, 1993). The root and stem wood are used as chewing stick for cleaning the teeth and oral hygiene (Iwu, 1993). An infusion of the aerial parts is used as a mouthwash to treat mouth infections. The plant sap or a decoction of the whole plant is applied as nose or eye drops to treat headache, sinusitis and conjunctivitis (Iwu, 1993). The pounded whole plant is used as fish poison (Iwu, 1993).

Scientific research has shown that *S. americana* has mild antibacterial (Asusheyi et al., 2010) and antifungal (Jimoh et al., 2011b) activities. Earlier studies on the aqueous extract of the leaves showed mild antibacterial activity against *Proteus mirabilis* and *Staphylococcus aureus*, but no inhibition of *Pseudomonas aeruginosa* and *Escherichia coli* (Hermans et al., 2004; Adamu et al., 2005). The methanol extract of the whole plant demonstrated anti-inflammatory and analgesic effects by inhibiting formalin edema in the rat paw and acetic acid induced writhing, respectively (Oliver- Bever, 1986). The leaves, roots and stems were shown to contain traces of alkaloids and a glycoside, schwenkioside, which has a phenolic aglycone, schwenkiol. A steroid sapogenin was found to be the main constituent of this herb in Nigeria (Oliver-Bever, 1986; Jimoh et al., 2011). The sapogenin has cardiogenic activity and also causes an initial inhibition of toad heart followed by prolonged stimulation. Extracts from various parts of the plant cause hemolysis of red blood cells attributed to saponins (Iwu, 1993). Some closely related plants within the family Solanaceae such as *Solanum nigrum* and *Solanum melongena* have also been found to contain some of these medicinally important phytoconstituents like steroids, terpenoids, flavonoids etc. (Venkatesan et al., 2009; Tiwari et al., 2009).

In furtherance of studies on the anti-inflammatory activity of this plant, we subjected the methanol-methylene chloride leaf extract to biological-activity guided studies using the systemic acute edema of the rat paw as activity-guide with a view of achieving a more detailed evaluation of this property, possibly identify the actual phytoconstituent (s) responsible for its pharmacological activity and putative mechanism(s) of its antiinflammatory action. Hence, the effects of the extract on some mechanisms associated with the inflammatory response were also studied.

MATERIALS AND METHODS

Animals

Adult Swiss albino rats (100 to 150 g) and mice (19 to 22 g) of either sex 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 libitum* and acclimatized for 2 weeks. They were fasted overnight before their use for each experiment. All animal experiments were in compliance with National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985).

Preparation of extract

S. americana was collected between March and July from bushes in Nsukka, Enugu state, Nigeria. The plant was identified and authenticated at the International Centre for Ethnomedicines and Drug Development (InterCEDD) where a voucher specimen was deposited (InterCEDD/066). The aerial parts were cut, cleaned, and dried under the sun for 10 days and pulverized to coarse powder using a milling machine. About 4 kg of the powdered plant material was extracted with a 1:1 mixture of methanol and methylene chloride by cold maceration for 48 h. The plant material was repeatedly washed with fresh solvent mixture until the filtrate became clear. The filtrate was concentrated using a rotary vacuum evaporator under reduced pressure (40 to 50°C) to obtain 170 g of methanol-methylene chloride extract (SAE; 4.25% w/w) which is more suitable and allow for successive partitioning and solvent-guided fractionation compared to the aqueous form in which the herb is normally administered in ethnomedicine.

Solvent-guided fractionation of SAE and bioactivity-guided studies

The SAE (113 g) was subjected to solvent-guided fractionation in a silica gel (70 to 120 mesh size) column (60 cm in length and 7.5 cm in diameter) successively eluted with n-hexane, dichloromethane and methanol in order of increasing polarity. The solvent fractions were collected and concentrated under reduced pressure in a rotary evaporator (40 to 50°C) to obtain 20.95 g of the n-hexane (HF; 18.54% w/w), 10.38 g of the dichloromethane (DCF; 9.18% w/w) and 10.90 g of the methanol (MF; 9.65% w/w) fractions, respectively. The extract and fractions were subjected to bioactivity-guided studies using egg albumin-induced rat pedal edema as activity guide. The DCF elicited the greatest suppression of the development of acute edema of the rat paw and was subsequently separated (8.7 g) in a silica gel column eluted with gradient

mixtures of n-hexane and ethyl acetate. The fractions were collected in volumes of 250 ml and pooled into five broad fractions, A to E, based on the similarity of constituents visualized on silica gel pre-coated thin layer chromatography (TLC) plates developed with ethyl acetate. The fractions were concentrated in a rotary evaporator under reduced pressure to obtain fractions A (1.6 g; 4.34% w/w), B (0.25 g; 0.68% w/w), C (0.26 g; 0.70% w/w), D (0.56 g; 1.52 % w/w) and E (0.47 g; 1.27% w/w), respectively. Fractions A to E were subsequently subjected to bioactivity-guided studies using egg albumin-induced rat pedal edema as the activity guide.

Phytochemical analysis

The *Schwenckia americana* L. (SAE), DCF and fraction A (Fr. A) were subjected to phytochemical analysis for tentative identification of constituents using standard method (Iwu, 1978; Trease and Evans, 1983; Harborne, 1988).

Pharmacological studies

Egg albumin-induced pedal edema in rats

The SAE and its fractions were subjected to activity-guided studies using egg albumin-induced acute edema of the rat paw as activity guide. The assay was carried out in adult Swiss albino rats of both sexes distributed among sixteen treatment groups (n = 5), using the method of Winter et al. (1962). The extract and fractions were suspended in 3% v/v Tween 80 and administered orally to the appropriate group at 200 or 400 mg/kg. The control animals received either acetylsalicylic acid (100 mg/kg) or 3% v/v Tween 80 (2.5 ml/kg). One hour later, acute inflammation was induced by injection of 0.1 ml of fresh egg albumin into the sub plantar region of the right hind paw. The volume of the paw was measured by water displacement before and at 0.5, 1, 2, 3, 4 and 5 h after induction of inflammation. Edema formation was assessed in terms of the difference between the zero time paw volume of the treated paw and the volume at the different time intervals after injection of egg albumin (Okoli et al., 2006). The level of inhibition (%) of edema was calculated using the relation:

$$\text{Inhibition of edema (\%)} = 100 [1 - (a - x / b - y)]$$

Where a = mean paw volume of treated rats after egg albumin injection; x = mean paw volume of treated rats before egg albumin injection; b = mean paw volume of control rats after egg albumin injection and y = mean paw volume of control rats before egg albumin injection.

Topical acute edema of the mouse ear

The effect of SAE on topical acute edema was assessed using xylene-induced ear edema in mice. The two treatment groups (n = 6) received topical application of either SAE (5 mg/ear) or ASA (5 mg/ear) on the anterior surface of the right ear while xylene (0.05 ml) was instantly applied on the posterior surface of the same ear. Control animals received an equivalent volume of the vehicle (3% v/v Tween 80). The left ear was left untreated. Two hours after xylene application, mice were sacrificed and both ears removed. Circular discs were punched out of the ear lobes using a cork borer (6 mm diameter) and weighed. The difference in the weight of discs from the right treated and left untreated ears was calculated and used as a measure of edema (Tubaro et al., 1985; Atta and Alkohafi, 1998). The anti-inflammatory activity was evaluated as percent edema reduction/inhibition in the treated animals relative to control animals using the relation:

$$\text{Edema reduction/inhibition (\%)} = 100 [1 - (R_t - L_t / R_c - L_c)]$$

Where R_t = Mean weight of the right ear plug of treated animals; L_t = mean weight of the left ear plug of treated animals; R_c = mean weight of the right ear plug of control animals and L_c = mean weight of the left ear plug of control animals

Cotton pellet-induced granuloma test

The effect of the extract on proliferative phase of inflammation was evaluated in rats using the cotton pellet granuloma test. The method of Swingle and Shideman (1972) and Penn and Ashford (1963) was used with slight modifications. Cotton pellets (30 mg) sterilized in an autoclave were implanted one on each side of the subcutaneous dead space in the depilated axial region of rats under ketamine anaesthesia. The rodents were placed in four independent treatment groups. The SAE (200 or 400 mg/kg) was administered orally for 7 consecutive days starting from the day of pellet implantation. Control animals received an equivalent volume of vehicle (3% v/v Tween 80) or acetylsalicylic acid (100 mg/kg). On day 8, the animals were decapitated and the pellets carefully removed and made free from extraneous tissues, dried in a hot air oven at 60°C to a constant weight. Granuloma formation was evaluated by the weights of the dry pellets. The level of decrease (%) in the weight of granuloma tissue formed was calculated relative to the control.

Evaluation of anti-inflammatory mechanism(s)

In vivo vascular permeability: The effect of extract on vascular permeability was evaluated in mice using the method of Whittle (1964). Adult albino mice were randomly grouped into four groups (n = 5) to receive oral administrations of SAE (200 or 400 mg/kg), respectively while control animals were given indomethacin (50 mg/kg) or vehicle. One hour later, Evans blue dye in normal saline (0.2 ml of 0.25%) was intravenously administered through the tail vein of the animals. Thirty minutes later the animals received intraperitoneal injection of 0.6% v/v acetic acid (1 ml/100 g). The animals were sacrificed 30 min after acetic acid injection and their peritoneal cavity washed with 3 ml of normal saline into heparinised tubes and centrifuged. The dye content in the supernatant was measured at 610 nm, using a spectrophotometer (Spectrum Lab 21A).

Inflammatory stimulus induced neutrophil migration: The effect of the extract on neutrophil migration induced by inflammatory stimulus was studied according to the method of Ribeiro et al. (1991). Adult Swiss albino rats were randomly grouped into four groups (n = 5) to receive oral administrations of SAE (200 or 400 mg/kg), while control groups received indomethacin (50 mg/kg) or vehicle (2.5 ml/kg), respectively. One hour later, leucocyte migration was induced by intraperitoneal administration of 1 ml of 2.8% (w/v) carrageenan. Four hours later, the animals were sacrificed by overdose of chloroform anaesthesia and their peritoneal cavity opened and washed with 5 ml of phosphate buffer saline containing 0.5 ml of 10% EDTA. Neutrophil counts of the peritoneal washout were determined using improved Neubauer chamber (Tiefe Marienfeld, Germany).

Membrane stabilization: Three independent treatment groups were used for the membrane stabilisation assays - the heat-induced and hypotonic solution-induced hemolysis respectively.

Preparation of erythrocyte suspension: Fresh ox blood (20 ml) was collected from the Slaughter House Abattoir in Nsukka in ethylenediaminetetraacetic acid (EDTA) bottles. About 10 ml was

transferred into heparinized tubes and centrifuged at 3000 rpm for 5 min. The supernatant was decanted and the packed cells washed thrice with 5 ml of normal saline and then reconstituted with 40% (v/v) isotonic buffer solution (pH 7.4) which contained in 1 L of distilled water: $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.26 g), Na_2HPO_4 (1.15 g), NaCl (9 g) (that is, 10 mM sodium phosphate Buffer) (Shinde et al., 1999).

Heat induced hemolysis: The SAE was dissolved in isotonic buffer solution (200 or 400 $\mu\text{g}/\text{ml}$) and about 5 ml was put in four sets (per concentration) of centrifuge tubes. The control however contained 5 ml of distilled water. The erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. A pair of the tubes was incubated at 54°C in a regulated water bath for 20 min and the other pair maintained at 0 to 4°C in a freezer for 20 min. After incubation, the reaction mixture was centrifuged at 1300 g for 3 min and the absorbance of the supernatant measured at 540 nm using a spectrophotometer (Spectrum Lab 21A). The inhibition of hemolysis was calculated using the relation (Shinde et al., 1999):

$$\text{Inhibition of hemolysis (\%)} = 100 [1 - (y - x) / (z - x)]$$

Where x = absorbance of test sample unheated, y = absorbance of test sample heated and z = absorbance of control sample heated.

Hypotonic solution-induced hemolysis: About 5 ml of distilled water (hypotonic solution) mixed with Tween 80 in the ratio 4:1 containing 200 or 400 $\mu\text{g}/\text{ml}$ of SAE was put in 2 pairs (per dose) of centrifuge tubes. The control tubes contained 5 ml of distilled water. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. The mixtures were incubated for 1 h at room temperature (31°C) after which the reaction mixture was centrifuged for 3 min at 1300 g and the absorbance read off at 540 nm using spectrophotometer (Spectrum Lab 21A). The isotonic buffer solution used was composed of 154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4). The level of hemolysis was calculated using the relation:

$$\text{Inhibition of hemolysis (\%)} = 100 (1 - (y - x) / (z - x))$$

Where x = absorbance of test sample in isotonic solution, y = absorbance of test sample in hypotonic solution and z = absorbance of control sample in hypotonic solution.

Statistical analysis

Data obtained was analyzed using one way analysis of variance (ANOVA) and subjected to least significant difference (LSD) post hoc test for multiple comparisons. Differences between means were accepted to be significant at $P < 0.05$ and the results expressed as mean $\pm$ standard error of mean (SEM).

RESULTS

Phytochemical constituents of extract and fractions

Phytochemical analysis showed that SAE gave positive reactions for alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, resins, carbohydrates, glycosides and reducing sugars. The DCF tested positive to alkaloids, flavonoids, steroids, resins and terpenoids; while Fraction A tested positive to resins, steroids and

Table 1. Phytochemical constituents of *S. americana* aerial parts.

Phytochemical Constituent	Relative presence		
	SAE	DCF	Fr.A
Alkaloids	+++	++	-
Carbohydrates	+++	-	-
Flavonoids	+	+++	-
Glycosides	+++	-	-
Reducing sugar	++	-	-
Resins	+++	++	++
Saponins	++	-	-
Steroids	+++	+++	+++
Tannins	++	-	-
Terpenoids	+++	+++	++

SAE = extract; DCF = dichloromethane fraction; Fr.A = fraction A; +++ = Likely present in abundant proportions, ++ = Likely present in moderate concentration, + = Likely present in trace amounts, - = Likely not present.

terpenoids (Table 1).

Effect of SAE and fractions on systemic acute edema

The SAE and its bioactive fractions elicited significant ($P < 0.05$) and progressive inhibition of systemic acute edema of the rat paws to varying extents. The inhibition elicited by SAE was non-dose related, while that of DCF was dose-related.

Fraction A, obtained from the fractionation of DCF, also caused a significant ($P < 0.05$) progressive inhibition of paw edema. A quantification of the global edematous response using the area under the curve (AUC) of the edema-time curve showed the magnitude of inhibition of acute paw edema to be of the order Fraction A > DCF > SAE (Table 2). Therefore, anti-inflammatory activity increased with fractionation.

Effect of extract on topical acute edema of the mouse ear

The SAE significantly ($P < 0.05$) inhibited the development of topical acute edema induced by xylene in the mouse ear (53.3%) while acetylsalicylic acid elicited 40% inhibition (Table 3).

Effect of extract on granuloma formation

The SAE elicited a significant ($P < 0.05$) but non-dose related inhibition of granuloma formation (Table 4). The extent of suppression (39.7%) was comparable to that shown by acetylsalicylic acid (41.2%) (Table 4).

Table 2. Effect of extract and fractions on egg albumin-induced rat paw edema.

Treatment	Dose (mg/kg)	Edema (ml)						AUC (ml/h)
		0.5 h	1 h	2 h	3 h	4 h	5 h	
SAE	200	0.68±0.08* (30.61)	0.76±0.11 (17.39)	0.58±0.08* (28.95)	0.42±0.06* (46.35)	0.42±0.06* (37.16)	0.34±0.06* (56.41)	2.33
	400	0.78±0.12 (20.40)	0.80±0.13 (13.04)	0.62±0.12 (32.61)	0.52±0.12* (35.48)	0.48±0.10* (44.19)	0.44±0.10* (43.59)	2.63
DCF	200	0.82±0.04 (16.33)	0.78±0.07 (15.21)	0.66±0.04* (20.26)	0.56±0.04* (31.13)	0.46±0.05* (46.51)	0.28±0.08* (64.10)	2.01
	400	0.82±0.06 (16.33)	0.74±0.07 (19.57)	0.54±0.07* (33.30)	0.46±0.06* (42.00)	0.36±0.08* (58.14)	0.24±0.10* (69.23)	2.21
Fr.A	400	0.68±0.04* (30.61)	0.60±0.00* (34.78)	0.42±0.02* (46.35)	0.28±0.03* (61.57)	0.16±0.02* (81.40)	0.10±0.04* (87.18)	1.55
ASA	100	0.66±0.12* (32.65)	0.62±0.12* (32.61)	0.52±0.10* (35.48)	0.48±0.08* (39.83)	0.40±0.08* (53.49)	0.22±0.08* (71.79)	2.14
Control	-	0.98±0.06	0.92±0.03	0.92±0.03	0.92±0.03	0.86±0.05	0.78±0.05	4.02

n = 5; *P < 0.05 compared to control (One Way ANOVA; LSD post hoc); value in parenthesis represent inhibition of edema (%) relative to control; SAE = extract; DCF = dichloromethane fraction; Fr. A = fraction A; ASA = acetylsalicylic acid; AUC = area under the curve.

Effect of extract on acetic acid induced *in vivo* vascular permeability

The extract elicited a significant ($P < 0.05$) non-dose related and moderate inhibition of vascular permeability. The effect of SAE (200 mg/kg) was comparable to that of indomethacin (Table 5).

Effect of extract on carrageenan induced neutrophil migration

The SAE reduced the neutrophils count compared to control in a dose related manner (Table 6).

Effect of extract on membrane stabilization

The extract exhibited a significant ($P < 0.05$) inhibition of heat induced hemolysis in a non concentration-related manner, and a very weak and non concentration-related inhibition of hypotonic solution-induced hemolysis (Table 7).

DISCUSSION

Findings from this study have shown that extract of aerial parts of *S. americana* exhibited anti-inflammatory activity in both acute and chronic inflammation in rodent models. The extract also inhibited increase in vascular permeability and neutrophil migration and stabilized the membrane of red cells which are some aspects of the complex events associated with the inflammatory response. Antiinflammatory activity studies showed that single topical or systemic administration of the extract suppressed the development of acute inflammation while repeated administration suppressed the reactions of the proliferative phase of chronic inflammation evidenced by moderate inhibition of granulomatous inflammation. This suggests that the extract contains constituents capable of ameliorating disorders of both acute and chronic inflammation. Also, topical application of the extract may offer relief in disorders of acute or chronic inflammation.

Acute and chronic inflammation are the major

phases of inflammation where complex and interwoven events aimed at healing and containment of insult on tissues amplify basic physiological processes known as the inflammatory response. The inflammatory response chronicles events orchestrated by several mediators ranging from vascular events leading to edema to leukocyte migration to the site of inflammation capable of aggravating the response. Whereas acute inflammation is short-lived, chronic inflammation is prolonged and sustained by persistence of the inflammatory stimulus. Granulomatous inflammation, a distinctive form of chronic inflammation, is a prolonged process involving infiltration and proliferation of mononuclear inflammatory cells such as macrophages, lymphocytes and fibroblasts which are the basic sources of granuloma tissue formation (Mitchell et al., 2006). During granulomatous inflammation, there is often the appearance of nodules of epithelioid macrophages surrounded by a collar of lymphocyte elaborating factors like IFN- γ (Mitchell et al., 2006). There is also vascular proliferation attempted at

Table 3. Effect of extract on xylene-induced topical ear edema.

Treatment	Dose (mg/ear)	Edema (mg)	Inhibition of edema (%)
SAE	5	2.1±0.014*	53.3
ASA	5	2.7±0.013*	40.0
Control	-	4.5±0.64	-

n= 6; *P<0.05 (ANOVA; LSD post hoc test); SAE = extract; ASA = acetylsalicylic acid.

Table 4. Effect of extract on granuloma tissue growth.

Treatment	Dose (mg/kg)	Granuloma tissue weight (mg)	Inhibition (%)
SAE	200	82.0±0.164*	39.7
	400	82.0±0.130*	39.7
ASA	100	80.0±0.141*	41.2
Control	-	136.0±0.498	-

n= 5; *P<0.05 (ANOVA; LSD post hoc test); SAE = extract; ASA = acetylsalicylic acid. Inhibition (%) calculated relative to control.

Table 5. Effect of extract on acetic acid induced vascular permeability.

Treatment	Dose (mg/kg)	Absorbance (610 nm)	Inhibition (%)
SAE	200	0.28±0.076*	31.71
	400	0.36±0.185	11.71
Indomethacin	50	0.27±0.056*	34.21
Control	-	0.47±0.085	-

n= 5; *P<0.05 (ANOVA; LSD post hoc test); SAE = extract; Inhibition (%) calculated relative to control.

Table 6. Effect of extract on carrageenan-induced neutrophil migration.

Treatment	Dose (mg/kg)	Neutrophil count (Cells/mm ³ × 10 ³)	Inhibition (%)
SAE	200	1840.00±810.34*	52.23
	400	2181.63±195.67	43.36
Indomethacin	50	1507.13±400.79*	60.87
Control	-	3851.75±603.46	-

n= 5; * P<0.05 (ANOVA; LSD post hoc test); SAE = extract; Inhibition (%) calculated relative to control.

healing. Hence the cotton pellet-induced granuloma test employed in this study is indicative of proliferative inflammation (Turner, 1965) since the dry weight of the excised cotton pellet correlates well with the amount of granulomatous tissue formed (Thangam and Dhananjayan, 2008). Decrease in the weight of the granuloma tissue in extract treated rats indicates an

effective suppression of the proliferative phase of chronic inflammation and antiproliferative effect which may contribute to the antiinflammatory activity. Thus, the antiinflammatory activity of the extract is likely due to inhibition of some aspects of the reactions of the inflammatory response associated with acute and chronic inflammation.

Table 7. Effect of extract on membrane stabilization.

Treatment	Dose (mg/ml)	Inhibition of heat-induced hemolysis (%)	Inhibition of hypotonic solution-induced hemolysis (%)
SAE	200	75.2*	2.5
	400	74.8*	0.1
Control	-	-	-

n= 4; *P<0.05 (ANOVA; LSD post hoc test); SAE = extract.

Evaluation of the mechanisms likely associated with the antiinflammatory activity of the extract showed that it inhibited vascular permeability and neutrophil migration and stabilized the membrane of red cells. Increase in vascular permeability, a major feature of acute inflammation, is an early response to inflammation. It results from contraction and separation of endothelial cells at their boundaries to expose the basement membrane which is freely permeable to plasma proteins and fluid (Skidgel and Erdos, 2006) and results to leakage of large plasma proteins including immuno-globulins, coagulation factors and cells (predominantly leukocytes) out of the blood vessels (exudation) into the injured tissues with subsequent edema at the site (Burt and Smith, 1996; Cotran et al., 1999). Inhibition of the increase in vascular permeability induced by acetic acid in the mouse peritoneum by the extract may profoundly reduce the magnitude of the inflammatory response by modulating the extent of leakage of plasma proteins, mediators and fluid into tissues with the consequences associated with their actions in the early phase of the inflammatory response.

Studies on cellular migration showed that the extract inhibited neutrophil translocation to site of inflammation induced by carrageenan. Carrageenan-induced leucocyte migration is dependent on the release of chemotactic mediators by resident cells (Souza et al., 1988) and mimics the sequence of events naturally associated with cellular migration. Migration of leucocytes to the affected tissue is a critical step and feature of inflammation and the main cellular phase of acute inflammation. These leucocytes ingest the offending agents/inflammogens, kill microbes, degrade necrotic tissues, immune complexes, cell debris and foreign antigens, in addition to release of lysosomal enzymes, chemical mediators and toxic radicals. These actions are aimed at clearing the inflammation, but may conversely exacerbate and prolong it and cause tissue injury through the actions of the lysosomal enzymes such as bactericidal enzymes and proteases. Thus, in addition to inhibiting the migration of these cells to sites of inflammation, stabilizing their membranes to inhibit lysis and the subsequent release of the cytoplasmic contents would limit the magnitude of the inflammatory response.

In vitro assessment of the effect of the extract on

membrane stabilization showed that it inhibited heat- and hypotonicity-induced lysis of ox red blood cells, indicating membrane stabilization effect. The ability of the extract to stabilize membranes of ox erythrocytes implies that it may also stabilize lysosomal membranes, since both membranes are analogous (Gandhisan et al., 1991). Stabilization of lysosomal membranes serves to limit the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which will cause further tissue inflammation and damage upon extracellular release (Perez and Weissmann, 1981; Chou, 1997). Some non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and acetylsalicylic acid also possess membrane stabilization properties (Muruges et al., 1981; Furst and Munster, 2001) which may contribute to their anti-inflammatory effect. The extract, however, caused a greater inhibition of heat- than hypotonic solution-induced hemolysis, suggesting the ability of the extracts to enhance the stability of heat labile membrane proteins. The reason for this discrepancy is not clear to us.

Bioactivity-guided studies on the extract revealed that the antiinflammatory constituent(s) may reside in the fraction A, the most active fraction obtained from the dichloromethane fraction. Phytochemical tests revealed abundance of terpenoids and steroids in this fraction. These phytochemical constituents may account for the anti-inflammatory activity of the aerial parts of this plant. Several studies have also implicated them in the antiinflammatory activity of some plants for example, cumanin from *Ambrosia psilostachya* (Lastra et al., 2004) as well as ilicic acid and inuviscolide from *Inula viscosa* (Hernandez et al., 2001).

Conclusion

Aerial parts of *S. americana* possess antiinflammatory properties in both acute and chronic inflammation. The antiinflammatory activity may be largely mediated via inhibition of vascular permeability, neutrophil migration and cellular infiltration and proliferation as well as membrane stabilization and attributable to terpenoids and steroids present in the aerial parts.

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