

Analgesic, Anti-Inflammatory and Antipyretic Potential of the Stem Bark Extract of *Stachytarpheta indica*

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Abstract: Aim of study: *Stachytarpheta indica* is used as herbal remedy for pains in Nigeria. Methods: To validate this claim, the analgesic, anti-inflammatory and antipyretic effects of the stem bark extract were examined in mice and rats by using acetic acid-induced writhing, tail immersion, xylene and egg albumin-induced paw oedema. While yeast and amphetamine induced pyrexia were used to study antipyretic activity in rats. LD₅₀ of the stem bark extract was carried out to determine its safety. Results: The methanol stem bark extract of *Stachytarpheta indica* showed significant (p<0.05) dose dependent effect in the parameters assayed. The activity of the extract was comparable to acetylsalicylic acid and morphine respectively. The acute toxicity test was greater than 5000 mg/kg. Conclusions: It may be possible that the methanol stem bark extract of *Stachytarpheta indica* contains biologically active substances with potential value for the treatment of painful and feverish conditions.

Keywords: Herbal medicine, inflammation, nociception, pyrexia, rats, *Stachytarpheta indica*

INTRODUCTION

Many plants synthesize substances that are useful to the maintenance of human health. Before synthetic drugs were produced, man was completely dependent on medicinal plants for curing diseases (Singh *et al.*, 2008). Natural medicines obtained from plants are being increasingly used to treat different diseases. A wide variety of herbal remedies have been used traditionally for treatment of diseases in Nigeria. (Akuodor *et al.*, 2011). The World Health Organization (WHO) advocates the inclusion of herbal medicine in primary health care because of their great potentials. Constituents in natural products have shown many biological and pharmacological activities, which includes anti-inflammatory and antiviral effects (Hakiman and Maziah, 2009). The investigation into medicinal plants which are used as pain relievers should be viewed as a fruitful search for new analgesic drugs (Elisabetskey *et al.*, 1995).

Stachytarpheta indica Vahl (Verbenaceae) is commonly known as snake weed. A well branched herb, 2-3 ft high with very long narrow spikes, flowers deep blue with white centre. The plant is known by various names in different parts of Nigeria, such as 'Tsarkiyar kuse' (Hausa), 'Iru amure' (Yoruba)

(Burkill, 1998). The plant has been used locally in the management of asthma, headache, alopecia, bronchitis, bruises, constipation, diarrhoea, skin sore, dysentery, dysmenorrheal, fever, inflammation, liver disease, poisoning, tumor, venereal diseases, cataract, sedative and rheumatism (Ayensu, 1978). In Northern Nigeria, a decoction of the leaves is given for dysentery in humans and for similar conditions in horses (Burkill, 1998).

The aim of this study therefore was to authenticate the potential of the methanol stem bark extract of *Stachytarpheta indica* by evaluating its analgesic, anti-inflammatory and antipyretic activities in experimental animal models.

MATERIALS AND METHODS

Plant collection: The stem bark of *Stachytarpheta indica* was collected from plants growing within the campus of University of Calabar, Nigeria. The plant was authenticated by Mr. Frank Akpejoye, Department of Botany, University of Calabar, where a voucher specimen (No.133) is maintained. The international plant name index is Verbenaceae *Stachytarpheta indica* Vahl. Enum.pl.1:206.1804. The stem bark was cut into smaller pieces and dried at room temperature for 7

days. *S. indica* stem bark was later ground to dry powder using a mortar and pestle.

Extraction: (500 g) of the dry stem bark powder was extracted in methanol by cold maceration for 48 h. The methanol extract was concentrated to dryness in vacuum at 40°C. The yield was (12% w/w). The dry extract was stored in a refrigerator at 4°C until use for the experiment.

Pharmacological tests:

Animals: Adult albino rats (160-200 g) and mice (18-25 g) of both male and female were obtained from the animal house, Department of Pharmacology, College of Medical Sciences, University of Calabar, Calabar, Nigeria. The animals were kept in cages and allowed free access to standard pellets and water.

Phytochemical screening: The methanol stem bark extract of *S. indica* was subjected to qualitative phytochemical screening according to standard methods (Sofowora, 1993; Evans, 2005).

Acute toxicity test: The acute toxicity test of the methanol extract was determined in albino mice following OECD guidelines (2001) and Ghosh (2005).

Acetic acid-induced writhing test: Analgesic activity of methanol stem bark extract of *S. indica* against acetic acid-induced writhing was carried out following the procedure of Singh and Majumda (1995) and Akuodor *et al.* (2011). The adult albino mice used for this study were randomized into 5 groups of 6 mice in each cage. They were fasted for 24 h but were allowed free access to water. Group 1 which served as control received distilled water 20 mL/kg p.o.), while groups 2-4 received 50,100 and 200 mg/kg p.o. of the stem bark extract. Group 5 received 150 mg/kg of acetylsalicylic acid (ASA). Thirty minutes post drug administration, each mouse was injected intraperitoneally with acetic acid 0.7% at a dose of (20 mL/kg) to create pain sensation. Each mouse was later placed in a transparent observation box. The number of abdominal constrictions for each mouse was counted for 30 min, commencing 5 min after injection of acetic acid.

Tail immersion test: This was based on the method described by Jansen and Jagenau (1959) with slight modification. The mice selected for this study were grouped into 5 groups of 6 mice in each cage. The mice were fasted for 24 h but were allowed access to water. Group 1 which served as control received distilled water (20 mL/kg p.o.), while group 2-4 received 50,100 and 200 mg/kg of the methanol stem bark extracted orally. Group 5 received 10 mg/kg of morphine subcutaneously. Thirty minutes post drug administration, each mouse was restrained in a horizontal cylinder leaving the tail hanging freely in a

water bath maintained at 52±1°C and the time taken for the animal to withdraw its tail out of the water was recorded. The latency was evaluated at 30, 60, 90 and 120 min. The initial reading was taken immediately before administration of test samples.

Xylene-induced ear oedema in mice: Adult mice of both male and female selected into 5 groups of 6 mice in each cage were used for the study. Group 1 (control) was administered distilled water (20 mL/kg). *S. indica* stem bark extract (50, 100 and 200 mg/kg p.o.) was given to groups 2, 3 and 4, while dexamethasone (4 mg/kg) was administered to group 5. One hour post drug administration, oedema was induced in each mouse by applying a drop of xylene at the inner surface of the right ear. Three hours afterwards, the animals were sacrificed under light anaesthesia and both ears were cut off to equal size and weight. Inflammation was taken as mean difference between the right and left ear for each group (Jumping *et al.*, 2005).

Egg-albumin-induced inflammation in rats: Adult albino rats of both male and female fasted for 24 h and selected into 5 groups of 6 rats per cage were used for the study. Distilled water (20 mL/kg) given to group 1. The stem bark extract (50,100 and 200 mg/kg) and 150 mg/kg of Acetyl Salicylic Acid (ASA) were administered to the group 2, 3, 4 and 5, respectively. All were administered orally. One hour after, inflammation was induced in rats by injection of 0.1 mL of fresh egg-albumin into the subplantar of the right hind paw (Winter *et al.*, 1962; Akah and Nwabie, 1994; Agbeje *et al.*, 2008). The paw volumes were measured at 0, 20, 40, 60, 80, 100 and 120 min, respectively by using plethysmometer.

Yeast-induced pyrexia: The method described by Al-Ghamdi (2001) and Mukherjee *et al.* (2002) was adopted with slight modification. Rats for this experiment were divided into 5 groups of 6 rats each and their basal rectal temperature measured using clinical thermometer (Boots, Birmingham England). They were subcutaneously injected with 20 mL/kg of 15% yeast (Danbaoli, china) suspended in 0.5% methylcellulose solution to induce pyrexia. Rats not showing a minimum increase of 0.5°C in temperature after 24 h of yeast injection were removed.

Thereafter, distilled water (20 mL/kg) which served as control was administered to group 1 while the methanol stem bark extract of *S. indica* was administered at doses of 50, 100 and 200 mg/kg to group 2, 3 and 4 of the test animals respectively. The standard drug, aspirin (150 mg/kg) was given to group 5. All administered orally. Their rectal temperature was again recorded at 1 h interval, after drug administration.

D-Amphetamine-induced Pyrexia: The method of Berkan *et al.* (1991) was adopted with slight

modification. Rats used for this study were divided into 5 groups of 6 rats in each group and fasted for 24 h. Their basal rectal temperature was recorded prior to the induction of pyrexia by intraperitoneal injection of d-amphetamine, 10 mg/kg. Thirty minutes after d-amphetamine administration and confirmation of hyperthermia in the experimental animals, group 1 received distilled water (20 mL/kg p.o.) The extract group (2, 3 and 4) received 50, 100 and 200 mg/kg p.o. respectively. The standard drug, aspirin (150 mg/kg p.o.) was administered to group 5. The rectal temperature was thereafter recorded at one hour interval.

Statistical analysis: Results were expressed as mean±S.E.M. Difference between means of treated and control groups was considered significant at p<0.05.

RESULTS

Phytochemical screening of the extract revealed the presence of saponins, flavonoids, steroids, terpenoids, alkaloids and carbohydrates. These constituents have been reported to possess important biological activities (Hollander-Hadacek, 2002; Ghoghari and Rajani, 2006; Panda and Kar, 2007).

Acute toxicity test: There was no mortality observed in mice after oral administration of the extract. Hence, the oral LD₅₀ was greater than 5000 mg/kg. The experimental doses used were within safe margin.

Acetic acid-inducing writhing in mice: The extract (50-200 mg/kg) dose-dependently reduced abdominal

Table 1: Effect of *S. indica* stem bark extract on acetic acid induced writhing in mice

Treatment	Dose (mg/kg)	Writhing	% Inhibition
Distilled water	0.2 mL/kg	18.50±1.28	-
<i>S. indica</i>	50	9.67±1.45	47.73*
	100	6.33±0.67	65.78*
	200	6.67±1.12	78.95*
Aspirin	150	4.17±0.87	77.46*

Data are expressed as mean±SEM (n = 6) *significantly different from control at p<0.05

contractions in mice. The reduction was significant (p<0.05) when compared with control (Table 1). The effect of the extract was comparable to that of the standard drug, aspirin (150 mg/kg).

Tail immersion: The extract significantly (p<0.05) protected the animals from the thermal stimuli. Morphine produced a greater percentage of protection compared to the extract (Table 2).

Xylene-induced ear oedema: The extract at various doses administered, significantly (p<0.05) reduced the xylene-induced ear oedema in mice. The effect of the extract was comparable to the standard drug, Dexamethasone (4 mg/kg) (Table 3).

Egg albumin-induced oedema: The methanol extract significantly (p<0.05) exhibited dose-dependent anti-inflammatory activity in egg albumin-induced paw oedema when compared to control (Table 4). The effect of the extract was comparable to aspirin (150 mg/kg).

Yeast-induced pyrexia: The extract significantly (p<0.05) decreased the temperature of rats in a dose-

Table 2: Effect of *S. indica* stem bark extract on Tail Immersion in 52±1 °C hot water (mice)

Treatment	Dose (mg/kg)	After treatment				
		Pretreatment 0 min	30 min	60 min	90 min	120 min
Distilled water	0.2 mL/kg	7.50±1.60	8.0±0.45	9.33±1.61	10.17±1.80	13.33±1.22
<i>S. indica</i>	50	7.50±0.56	10.17± 0.54	12.33±0.10	13.00±0.82	14.67±0.80*
	100	7.83±0.48	12.00±0.77	13.17±0.83	14.50±1.28	15.83±1.05*
Morphine	200	8.00.0 ±73	13.38±0.88	15.50±0.72	16.17±1.62	17.00±0.63*
	10	7.83±1.64	17.67±1.33	19.50±1.71	21.00±1.24	23.50±1.09*

Data are expressed as Mean±SEM (n = 6) *significantly different from control at p<0.05

Table 3: Effect of *S. indica* stem bark extract on xylene-induced ear oedema in mice

Treatment	Dose (mg/kg)	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight (g)	% inhibition
Distilled water	0.2	0.080±0.00	0.045±0.00	0.035±0.01	-
<i>S. indica</i>	50	0.063±0.00	0.042±0.00	0.021±0.00	40*
	100	0.054±0.00	0.043±0.00	0.011±0.00	69*
	200	0.043±0.00	0.036±0.00	0.007±0.00	80*
Dexamethasone	4	0.044±0.00	0.037±0.00	0.007±0.00	80*

Data are expressed as mean±SEM (n = 6) *significantly different from control at p<0.05

Table 4: Effect of *S. indica* stem bark extract on egg albumin- induced paw oedema in rats

Treatment	Dose (mg/kg)	Paw oedema volume (ml) versus Time (min.)						
		0 min.	20 min.	40 min.	60 min.	80 min.	100 min.	120 min.
Distilled water	0.2ml/kg	1.22±0.04	1.65±0.03	1.75±0.03	1.83±0.02	1.89±0.03	1.97±0.03	2.08±0.04
<i>S. indica</i>	50	1.18±0.04	1.70±0.04	1.59±0.03	1.35±0.04	1.45±0.03	1.42±0.05	1.36±0.06*
	100	1.16±0.05	1.60±0.05	1.55±0.06	1.47±0.04	1.40±0.04	1.33±0.04	1.27±0.05*
	200	1.19±0.03	1.75±0.04	1.63±0.04	1.53±0.03	1.39±0.04	1.29±0.03	1.20±0.03*
Aspirin	150	1.16±0.05	1.55±0.04	1.47±0.04	1.41±0.02	1.35±0.03	1.27±0.03	1.18±0.03*

Data are expressed as mean ±SEM (n = 6) *significantly different from control at p<0.05

Table 5: Effect of *S. indica* stem bark extract on yeast-induced pyrexia in rat Rectal Temperature

Treatment	Dose (mg/kg)	0 h	24 h	1 h	2 h	3 h	4 h	5 h
Distilled water	0.2 mL/kg	35.68±0.15	36.42±0.17	36.75±0.16	36.95±0.16	37.45±0.16	37.62±0.16	37.81±0.16
<i>S.indica</i>	50	35.97±0.30	37.12±0.17	36.99±0.16	36.85±0.16	36.75±0.18	36.18±0.21	35.98±0.20*
<i>S.indica</i>	100	35.60±0.08	36.76±0.14	36.60±0.12	36.55±0.14	36.38±0.09	35.94±0.07	35.61±0.08*
<i>S.indica</i>	200	35.75±0.11	36.95±0.13	36.72±0.14	36.38±0.12	36.17±0.13	35.74±0.12	35.47±0.02*
Aspirin	150	35.5±0.08	36.54±0.14	36.37±0.13	36.20±0.09	35.85±0.10	35.69±0.11	35.45±0.04*

Data are expressed as mean±SEM (n = 6) *significantly different from control at p<0.05

Table 6: Effect of *S. indica* stem bark extract on amphetamine-induced pyrexia in rat Rectal Temperature

Treatment	Dose (mg/kg)	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Distilled water	0.2 mL/kg	36.56±0.19	36.56±0.15	36.67±0.15	36.76±0.15	36.83±0.16	36.99±0.16	37.30±0.18
<i>S.indica</i>	50	35.66±0.06	36.88±0.02	36.49±0.04	36.35±0.02	36.21±0.02	35.94±0.04	35.69±0.06*
<i>S.indica</i>	100	35.56±0.06	36.73±0.06	36.45±0.06	36.28±0.05	36.08±0.04	35.80±0.04	35.57±0.06*
<i>S.indica</i>	200	35.53±0.03	36.71±0.03	36.44±0.07	36.25±0.05	36.06±0.03	35.76±0.05	35.50±0.02*
Aspirin	150	35.60±0.06	36.69±0.01	36.36±0.06	36.21±0.07	36.04±0.02	35.64±0.02	35.43±0.03*

Data are expressed as mean±SEM (n = 6) *significantly different from control at p<0.05

dependent manner compared to the control (Table 5). The reduction was comparable to that of the standard drug, aspirin (150 mg/kg).

Amphetamine-induced pyrexia: The stem bark showed significant (p<0.05) and dose-dependent reduction of pyrexia when compared to control (Table 6). The effect of the extract was comparable to the standard drug, aspirin (150 mg/kg).

DISCUSSION

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer and vascular diseases. In various traditional medical systems, a number of natural products are used to relieve the symptoms of pain and inflammation (Akuodor *et al.*, 2011). The methanol stem bark extract of *S. indica* exhibited significant antinociceptive activity in different animal models of pain.

In living animal tissues, inflammatory processes involve the release of several mediators which include prostaglandins, histamine and cytokines and substances that regulate adhesion of molecules and cell migration, activation and degeneration (Hollameter *et al.*, 2003; Ganesh *et al.*, 2008).

Acetic acid-induced abdominal constrictions in mice as well as tail immersion tests were used to evaluate peripheral and central analgesic effects of the extract. Acetic acid method is simple, reliable and affords rapid examination of peripheral type of analgesic action. It was found that the stem bark extract of *S. indica* inhibited the acetic acid- induced writhing response. The analgesic action of the extract can be attributed to the blockade of release of the endogenous mediators of pain, the prostaglandins. It is therefore possible that *S. indica* stem bark extract possesses some inhibitory action on the cyclooxygenase pathway which is actually involved in the biosynthesis of prostaglandin.

Tail immersion test further confirm the analgesic action of the extract. This goes further to suggest a central mechanism of action for the stem bark extract. It has been reported that centrally acting analgesic drugs

elevate the pain threshold of mice towards heat and pressure (Singh and Majumbar, 1995).

Xylene is known to cause irritation in mouse ear which leads to accumulation of fluid and oedema, characteristic of acute inflammatory response. Suppression of this response may indicate antiphlogistic effect (Atta and Alkohafi, 1998). The stem bark extract exhibited a significant inhibition of ear oedema caused by xylene. This activity suggests the inhibition of phospholipase A₂ which is involved in the pathophysiology of inflammation due to xylene (Lin *et al.*, 1992). The anti-inflammatory activity of the stem extract which was comparable to the activity of dexamethasone may be due to the presence of flavonoids, saponins, tannins, steroids, terpenoids and alkaloids.

S. Indica stem bark extract also caused marked inhibition of egg-albumin induced hind paw oedema in rats. Albumin induced oedema has a biphasic response and the early phase is mediated through the release of histamine, serotonin and kinins. The late phase is related to the release of prostaglandins which is mediated by bradykinin, leucotrienes and polymorphonuclear produced by tissue macrophages. The anti-inflammatory activity shown by the extract was quite similar to that exhibited by the group treated with standard drug. These results indicate that the extract acts on both early and late phases of inflammation. The mechanism of anti-inflammatory activity of *S. indica* could be speculated to be through the inhibition of the one or more of the released inflammatory mediators. *S. indica* stem bark extract contains some active principles with potential value in treatment of pain and inflammation. Moreso, this study has established that *S. indica* has a non steroidal like anti-inflammatory activity which may be similar to its mechanism of antipyretic effect.

Antipyretic effect is a characteristic of drugs or compounds which have inhibitory activity on prostaglandin biosynthesis. The Non-Steroidal Anti-Inflammatory Drugs (NSAID) such as acetylsalicylic acid exhibits their antipyretic action mainly by inhibiting Prostaglandin E (PGE) production in the hypothalamus (Rang *et al.*, 2003). Consequently,

elevated plasma prostaglandin level as observed in fever is suppressed.

The methanol stem bark extract of *S. indica* demonstrated effective activity as evident in the inhibition of temperature rise in the yeast and amphetamine models of pyrexia. The antipyretic action of the extract may possibly be through inhibition of PGE production leading to suppression of elevated plasma level especially since the extract possesses analgesic and anti-inflammatory activities.

The therapeutic importance of traditional remedies is most often attributed to a combination of its active constituents. Several flavonoids found in medicinal plants have shown to possess significant analgesic anti-inflammatory effects (Duke, 1992; Mutalik *et al.*, 2003). The analgesic and anti-inflammatory effects observed may be attributed to flavonoids present in the plant. These findings revealed its potential for the development of putative herbal analgesic remedies. Further investigations are ongoing to isolate and characterize the specific active components of the plant extract responsible for the observed pharmacological actions.

CONCLUSION

In conclusion, this study clearly indicates that *Stachytarpheta indica* stem bark extract has remarkable analgesic, anti-inflammatory and antipyretic activities. The ability of methanol stem bark extract of *S. indica* to suppress abdominal writhing, elevate pain threshold, suppress both egg albumin and xylene-induced inflammation shows the extract possesses analgesic and anti-inflammatory activities, acting through both central and peripheral pathways.

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