

## EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY COMPOUNDS FROM *STEREOSPERMUM KUNTHIANUM* (BIGNONIACEAE)

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**ABSTRACT:** *Stereospermum kunthianum* (Bignoniaceae) is a woody shrub of the Sudano-Guinea savannah regions of Africa where the plant parts are used to treat various ailments including inflammatory conditions (rheumatoid arthritis) and pain. In continuation of our study of the anti inflammatory activity of this plant, we subjected the compounds previously isolated and characterized to pharmacological evaluation using different models of inflammation.

The analgesic and anti-inflammatory activities of the isolated compounds were studied using the Randall-Selitto and formalin-induced pain tests. At the dose of 20 mg/kg, Stereostin, Stereospermin ( $p < 0.0001$ ) and Stereospermiside ( $p < 0.05$ ) significantly increased the carrageenan-induced pain threshold compared to the distilled water treated animals. Similarly, at the same doses the three compounds significantly ( $p < 0.0001$ ) inhibited both phases of the formalin-induced pain with a more pronounced effect on the second phase than in the first phase. The results obtained show that the compounds from *Stereospermum kunthianum* possess analgesic and antiinflammatory activities. This paper reports for the first time the biological activity of these compounds from *Stereospermum kunthianum* stem bark.

**Keywords:** Analgesic, anti-inflammatory activities, Stereostin, Stereospermin, Stereospermiside, *Stereospermum kunthianum*, compounds.

### INTRODUCTION

*Stereospermum kunthianum* (Cham, Sandrine Petit), family Bignoniaceae is a woody shrub of the Sudano-Guinea savannah regions of Africa and Asia, where the plant parts are used to treat various ailments<sup>1,2</sup>. Among its traditional medical uses is the use in the treatment of inflammatory conditions (including rheumatoid arthritis) and pain. We have recently demonstrated the antidiarrhoeal activity of the aqueous extract of *Stereospermum kunthianum* stem bark in in-vivo experimentally induced diarrhoeal models using mice and rats<sup>3</sup>. Also, the analgesic activity of the aqueous extract of *Stereospermum kunthianum* stem bark has been investigated<sup>4</sup>. The efficacy of the water extract of *Stereospermum kunthianum* in human complement system fixation in-vitro has been reported<sup>5</sup>. Antiplasmodial activity of naphthoquinones and one anthraquinone from the lipophilic extract of the root bark

of *Stereospermum kunthianum* has also been reported<sup>6</sup>. The present study herein, report the analgesic and anti-inflammatory activities of the previously isolated and characterized irridoid and phenylpropanoid glycosides from the stem bark of *Stereospermum kunthianum*.

### EXPERIMENTAL

The compounds Stereospermiside, Stereospermin and Stereostin □ both phenylpropanoid glycosides □ (irridoid glycoside) previously isolated, characterized and elucidated were obtained, pure and crystalline [Falodun et al, 2009].

#### Animals

The experimental protocols and procedures used in this study were approved by the Animals Ethics committee of the University of Benin, Benin City, Nigeria. Wistar rats and Swiss mice of either sex obtained from the Animal House unit of the Department of Pharmacology and

Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals maintained under standard laboratory conditions (12 hours light and dark cycle) had free access to standard chow (Bendel Feeds and Flour mills Plc. Ewu, Nigeria) and drinking water.

#### Effect of compounds on carrageenan-induced pain in rats (Randall-Selitto test).

The method of Randall and Selitto, 1957 and modified of Winter et al.; 1962 was used. Wistar rats (140 – 190 g) of either sex were randomly allocated into groups of at least five animals per group. The animals were fasted over night with free access to water which was only withdrawn during the experiment. The animals were administered orally distilled water (5 ml/kg), indomethacin (10 mg/kg), stereostin (20 mg/kg), stereospermiside (20 mg/kg), or stereospermin (20 mg/kg) isolated from *Stereospermum kunthianum* stem bark. One hour later, 0.1 ml of 1 % w/v carrageenan in normal saline was injected subcutaneously into the plantar surface of the right hind leg of the rat. Three hours later, pressure was applied through a tip to the plantar surface of the rat's foot at a constant rate using the Analgesy meter (Ugo Basile, Apparatus for Biological Research, Milan, Italy). The pain threshold was considered reached when the animal struggles, squeals or attempts to bite. The weight at which this occurred was recorded. The percentage increase in pain threshold was obtained using the following formula:

$$\text{Pain threshold} = \frac{\text{treated mean} - \text{control mean}}{\text{Control mean}} \times 100.$$

#### Effect of compounds on the formalin-induced pain in mice.

The methods of Hunskaar et al; 1986 and Shibata et al; 1989 were used. Mice were randomly allotted to groups of five animals per group. Twenty microlitres (0.02 ml) of 1 % formalin was injected subcutaneously into the right hind paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 minutes immediately after formalin injection (first phase) and the last 15 minutes after formalin injection (second phase), representing the neurogenic respectively. The animals were pretreated with morphine (10 mg/kg, sc), stereostin (20 mg/kg, i.p), stereospermiside (20 mg/kg, i.p), or stereospermin (20 mg/kg, i.p) 30 minutes before being challenged with formalin. Control animals received distilled water (10 ml/kg, i.p). The animals were observed and the time spent licking or biting the injected paw was recorded.

#### Statistical analysis

Data obtained are presented as mean  $\pm$  SEM and/or as simple percentage. Data from distilled water treated

animals were used as negative control. The Student t-test analysis using INSTAT EXE programme was employed in the data analysis and considered significant at  $p < 0.05$ ,  $p < 0.001$  and  $p < 0.0001$ .

## RESULTS AND DISCUSSION

Table 1 shows the results of the effects of the secondary metabolites isolated from *Stereospermum kunthianum* stem bark on carrageenan – induced pain in rats. Stereostin and stereospermin significantly ( $p < 0.0001$ ), while stereospermiside significantly ( $p < 0.05$ ) increased the carrageenan – induced pain threshold with percentage pain inhibition of 73.7 % ,68.9 % and 37.4 % respectively compared to the distilled water treated animals. The Randall – Selitto test is used to measure the anti-inflammatory and analgesic properties of substances Gerhard, 2002. Inflammation increases the sensitivity to pain by reducing the pain threshold and this low pain reaction is elevated by non-narcotic and narcotic analgesics. Prostaglandins and other inflammatory mediators are known to increase the sensitivity to pain. That stereostin, stereospermiside and stereospermin increased the threshold to the carrageenan – induced pain threshold suggest that their antiinflammatory activity may involve interfering with the arachidonic metabolic pathway or the activity of the Arachidonic by products and/or other inflammatory mediators. The effect of the secondary metabolites isolated from *Stereospermum kunthianum* on formalin test are presented in Table 2. Stereostin, stereospermiside and stereospermin significantly ( $p < 0.0001$ ) inhibited both phases of the formalin test with a more intense effect on the second phase than the first phase. Morphine inhibited both phases of the formalin-induced pain with 52.7 % and 100 % inhibition in the first and second phases respectively. The effect of morphine in the first phase was not significantly different from that produced by stereospermiside and stereospermin. Formalin exhibits neurogenic, inflammatory and tonic pain as in clinical pain situations. Drugs which act mainly centrally such as narcotic analgesics inhibit both phases of pain in this model while peripherally acting drugs, such as aspirin or indomethacin only inhibit the late phase Santos et al; 1994. Stereostin, stereospermiside and stereospermin effects on both phases of the formalin pain model confirm that their analgesic activities are via central and peripheral mechanisms. The second phase of the formalin test is essentially the inflammatory phase hence the marked analgesic effect of the isolated compounds in this phase confirm their antiinflammatory activity. With the results of the present study taken together, it is concluded that, *Stereospermum kunthianum* stem bark possesses secondary metabolites with analgesic and antiinflammatory activities which may be mediated via peripheral and central mechanisms. This provides pharmacological credence for its use locally in human medicine to relief pain in the treatment of ailments accompanied by inflammation and pain.

**CONCLUSION**

The compounds showed significant anti inflammatory and analgesic properties at different doses of the

concentration tested. This however, demonstrated the ethno medicinal application of this plant for which it is known for.

**Table 1. Effect of stereostin, stereospermiside and stereospermin on carrageenan – induced pain in rats ( Randall – Selitto test).**

Treatment	Pain threshold (g)	Percentage pain threshold
Distilled water (5 ml/kg)	506.67 ± 32.10	-
Stereostin (20 mg/kg)	880.00 ± 45.09***	73.68
Stereospermiside (20 mg/kg)	696.00 ± 37.03*	37.37
Stereospermin (20 mg/kg)	856.00 ± 26.34***	68.95
Indomethacin (20 mg/kg)	966.67 ± 16.05***	90.79

Values are mean ±SEM of at least five experiments, \*P<0.05, \*\*\*P<0.0001 significantly different from the normal saline treated animals, Student's t-test analysis using INSTAT EXE programme .

**Table 2 : Effect of stereostin, stereospermiside and stereospermin on formalin – induced pain in rats.**

Treatment	Time(sec) spent licking or biting the formalin injected paw			
	First phase 0 – 5 mins.	Inhibition (%)	Second phase 15 – 30 mins.	Inhibition (%)
Distilled water (10 ml/kg)	115.40 ± 3.73	–	134.80 ± 5.03	–
Stereostin (20 mg/kg)	73.75 ± 2.29***	36.1	67.75 ± 2.46***	49.7
Stereospermiside (20 mg/kg)	65.00 ± 2.34***	43.3	52.00 ± 3.22 ***	61.4
Stereospermin (20 mg/kg)	67.20 ± 2.29***	41.8	49.60 ± 1.32***	63.2
Morphine (10 mg/kg)	54.60 ± 2.71***	52.7	0.00 ± 0.00	100

Values are mean ±SEM of at least five experiments, \*\*\*P<0.0001 significantly different from the normal saline treated animals, Student's t-test analysis using INSTAT EXE programme .

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