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Anti-inflammatory activity of chromatographic fractions of *Stereospermum kunthianum* Cham Sandrine Petit (Bignoniaceae) stem

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Abstract
The study evaluates the anti-inflammatory activity of the vacuum liquid (A, B and C) and column (L, S and Y) chromatographic fractions of *Stereospermum kunthianum* stem bark. The fractions at the doses of 100, 200 and 400 mg/kg were evaluated using the carrageenan-induced paw oedema model in rats. The control rats received normal saline (5 ml/kg) while indomethacin (10 mg/kg) served as the reference drug. Fractions A, B and C dose-dependently and significantly ($P<0.05$) reduced the paw swelling (oedema) in the treated animals compared to the control animals. The maximum reduction in paw swelling was at the third hour post carrageenan injection. Indomethacin (10 mg/kg) caused a more reduction in the paw swelling compared to the fractions. Similarly, the column chromatographic fractions L, S and Y dose-dependently and significantly ($P<0.05$) caused a reduction in the paw swelling. The results indicate that the fractions may inhibit inflammatory responses mediated by prostaglandins and other inflammatory mediators.

Keywords: Anti-inflammatory activity, Chromatographic fractions, *Stereospermum kunthianum*.

Introduction

Stereospermum kunthianum is reputed for its use in ethnomedicine to treat rheumatoid arthritis and other inflammatory conditions as well as other ailments in most African countries (Keay *et al*., 1989, Gill, 1992). The aqueous stem bark extract of the plant has been evaluated for various pharmacological activities (Ching *et al*., 2008, Ching *et al*., 2009a, b, c). The water extract of its stem bark has been reported to be efficacious in human complement fixation in vitro (Drissa *et al*., 2002). One iridoid and two phenylpropanoid glycosides with inhibitory activity on xanthine oxidase enzymes have been isolated and characterized from *S. kunthianum* stem bark (Falodun *et al*., 2009). The iridoid and the phenylpropanoid glycosides have equally been evaluated for analgesic and anti-inflammatory activities (Ching *et al*., 2009d). Antiplasmodial activity of naphthoquinones and one anthraquinone from the lipophilic extract of its root bark has also been reported (Onegi *et al*., 2002). Two

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lignans identified as (-)-olivil and (+)-cycloolivil have been isolated from *S. kunthianum* stem bark (Ghogomu-Tih *et al*., 1985). The antidiarrhoeal and analgesic activities of chromatographic fractions of the stem bark of *S. kunthianum* have also been reported (Ching *et al*., 2009e, Ching *et al*., 2010).

In continuation of our pharmacological investigation of the chromatographic fractions of its stem bark, the present study herein, report the anti-inflammatory activity of the chromatographic fractions previously obtained from vacuum liquid/column chromatographic (VLC/CC) analyses (Ching *et al*., 2009e).

Experimental

The vacuum liquid chromatographic fractions A, B, C and column chromatographic fractions L, S, Y previously obtained from chromatographic analyses (Ching *et a*l., 2009 e) were evaluated for anti-inflammatory activity using the carrageenan-induced paw oedema in rats.

Animals. The experimental protocols and procedures used in this study were approved by the Animal Ethics committee of the University of Benin, Benin City, Nigeria. Wistar rats 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 were maintained under standard laboratory conditions, natural light and dark cycles, and had free access to standard chow (Bendel Feeds and Flour Mills Plc, Ewu, Nigeria) and tap water for drinking.

Drugs and chemicals. These include carrageenan; indomethacin (Sigma Chemical Co., St .Louis, MO, USA), and sodium chloride (BDH Chemicals Ltd, Poole England)

Effect of VLC fractions A, B and C on carrageenan-induced paw oedema in rats. The method used was described by Winter *et al.*, (1962) and modified by Emmim *et al.*, (1994). Rats were randomly allotted to groups of five animals per group. The animals were fasted over night with free access to water which was only withdrawn during the experiment. Using vernier calipers baseline measurement of the right hind paw diameter of each rat was measured. The animals were orally administered normal saline (5 ml/kg), indomethacin (10 mg/kg), or100, 200, or 400 mg/kg of each of fractions A, B and C. One hour later, carrageenan (0.1 ml of 1% w/v in normal saline) was injected into the plantar aponeurosis of the right hind paw of each rat. Measurement of the diameter of the injected paw was repeated at hourly intervals for a maximum of six hours after carrageenan injection. The paw swelling at each time point was calculated as the difference between the paw diameter at the specific time point and before the injection of carrageenan.

Effects of CC fractions L, S and Y on carrageenan-induced paw oedema in rats. The method used was described by Winter *et al.*, (1962) and modified by Emmim *et al.*, (1994). Rats were randomly allotted to groups of five animals per group. The animals were fasted over night with free access to water which was only withdrawn during the experiment. Using vernier calipers baseline measurement of the right hind paw diameter of each rat was measured. The animals were orally administered normal saline (5 ml/kg), indomethacin (10 mg/kg), or 100, 200, or 400 mg/kg of each of fractions L, S and Y. One hour later, carrageenan (0.1 ml of 1% w/v in normal saline) was injected into the plantar aponeurosis of the right hind paw of each rat. Measurement of the diameter of the injected paw was repeated at hourly intervals for a maximum of six hours after carrageenan injection. The paw swelling at each time point was calculated as the difference between the

paw diameter at the specific time point and before the injection of carrageenan.

Statistical analysis. Data were expressed as mean ± SEM and analyzed using the unpaired Student's t-test. Results were considered significant at $P<0.05$ or better.

Results

As shown in Tables 1, 2 and 3, the vacuum liquid chromatographic fractions (A, B and C respectively) of *S. kunthianum* stem bark dose-dependently and significantly ($P< 0.05$) reduced paw oedema formation in the treated animals compared to the normal saline-treated group at the corresponding time point. At the lower doses (100 – 200 mg/kg) fraction A significantly ($p<0.05$) reduced oedema formation in the carrageenan injected paw compared to the control at the corresponding time point (Table 1). Similarly at the lower doses (100 -200 mg/kg) fractions B and C (Tables 2 and 3 respectively) caused significant ($p<0.05$) reduction in oedema formation in the carrageenan injected paw compared to the control at the corresponding time point. At 400 mg/kg, fraction A (Table 1) caused a significant ($P<0.001$) reduction in oedema formation in the carrageenan injected paw compared to that in the normal saline-treated animals at the corresponding time point. Indomethacin (10 mg/kg), produced a similar effect to the fractions, however, this effect was more pronounced. Comparatively, fractions A (Table 1) and B (Table 2) caused more intense anti-inflammatory effects than fraction C (Table3).

The effects of column chromatographic fractions L, S and Y on carrageenan-induced paw oedema are shown in Tables 4, 5 and 6 respectively. The fractions caused significant ($P<0.05$) and dose-dependent reduction in paw oedema with the most pronounced effects achieved at 3 h post carrageenan administration. All the fractions also exhibited significant reduction of paw oedema at the second hour of carrageenan injection. The antioedematic effect of fractions L and S (Tables 4 and 5 respectively) at the lower doses (100 – 200 mg/kg) were comparatively less than that of fraction Y (Table 6). Fraction Y (Table 6) at all the doses significantly ($p<0.001$) at the third hour post carrageenan administration reduced oedema formation in the carrageenan injected paw compared to the control. Comparatively, fraction Y (Table 6) produced more antioedematic effect than fractions L and S (Tables 4 and 5 respectively).

Table 1: Effect of vacuum liquid chromatographic fraction A on carrageenan-induced paw oedema in rats

Treatment	Percentage paw oedema					
	1hr	2hr	3hr	4hr	5hr	6hr
Normal saline: 5ml/kg	45.73±5.89	56.37±7.20	65.21±4.40	60.92±5.28	58.28±5.44	55.0±4.28
S. kunthianum 100mg/kg	33.88±3.77	41.10±5.25*	46.12±4.17*	43.0±6.36*	37.0±6.36*	37.48±4.53*
S. kunthianum 200mg/kg	34.26±4.80	36.76±5.22*	43.3±4.87*	40.72±6.01*	36.76±5.22*	36.76±5.22*
S. kunthianum 400mg/kg	31.34±3.56	31.44±1.08**	40.94±3.49**	40.44±3.51*	40.0±3.02*	36.96±3.24*
Indomethacin 10mg/kg	25.98±5.75*	32.52±5.64*	30.55±6.47**	30.23±6.77**	29.91±6.91*	29.91±6.91*

Rats were pretreated with the fraction A or indomethacin at the doses indicated one hour before induction of inflammation with carrageenan (0.1 ml of 1% w/v in normal saline) in each rat. Values at each time point are mean ± SEM of percent increase in paw diameter for five experiments. *$P<0.05$, **$P<0.001$ compared to the normal saline-treated animals at the corresponding time point.

Table 2: Effect of vacuum liquid chromatographic fraction B on carrageenan-induced paw oedema in rats

Treatment	Percentage paw oedema					
	1hr	2hr	3hr	4hr	5hr	6hr
Normal saline: 5ml/kg	45.73±5.89	56.37±7.20	65.21±4.40	60.92±5.28	58.28±5.44	55.0±4.28
S. kunthianum 100mg/kg	34.00±3.45	43.52±3.05	43.12±3.12*	43.12±3.12*	41.12±9.17*	34.88±3.07*
S. kunthianum 200mg/kg	35.8±5.94	43.92±5.93	40.24±7.03*	36.9±7.34*	32.9±7.98*	29.76±5.98*
S. kunthianum 400mg/kg	38.26±7.79	42.5±7.13	40.94±7.27*	34.40±8.06*	30.6±8.44*	30.60±8.45*
Indomethacin 10mg/kg	25.98±5.75*	32.52±5.64*	30.55±6.47**	30.23±6.77**	29.91±6.91*	29.91±6.91*

Rats were pretreated with the fraction B or indomethacin at the doses indicated one hour before induction of inflammation with carrageenan (0.1 ml of 1% w/v in normal saline) in each rat. Values at each time point are mean ± SEM of percent increase in paw diameter for five experiments. *$P<0.05$, **$P<0.001$ compared to the normal saline-treated animals at the corresponding time point.

Table 3: Effect of vacuum liquid chromatographic fraction C on carrageenan-induced paw oedema in rats

Treatment	Percentage paw oedema					
	1hr	2hr	3hr	4hr	5hr	6hr
Normal saline 5ml/kg	45.73±5.89	56.37±7.20	65.21±4.40	60.92±5.28	58.28±5.44	55.0±4.28
S. kunthianum 100mg/kg	44.12±3.18	52.62±2.41	50.12±2.19*	49.56±3.61	43.6±3.61	42.78±3413
S. kunthianum 200mg/kg	43.16±7.64	52.72±5.59	48.28±8.63*	44.70±8.62*	44.70±8.62	39.26±7.78
S. kunthianum 400mg/kg	40.94±5.92	50.44±7.14	46.32±8.13*	40.78±9.52*	40.78±8.95*	40.98±6.14
Indomethacin 10mg/kg	25.98±5.75*	32.52±5.64*	30.55±6.47**	30.23±6.77**	29.91±6.91*	29.91±6.91*

Rats were pretreated with the fraction C or indomethacin at the doses indicated one hour before induction of inflammation with carrageenan (0.1 ml of 1% w/v in normal saline) in each rat. Values at each time point are mean ± SEM of percent increase in paw diameter for five experiments. *$P<0.05$, **$P<0.001$ compared to the normal saline-treated animals at the corresponding time point.

Table 4: Effect of column chromatographic fraction L on carrageenan-induced paw oedema in rats

Treatment	Percentage paw oedema					
	1hr	2hr	3hr	4hr	5hr	6hr
Normal saline 5ml/kg	45.73±5.89	56.37±7.20	65.21±4.40	60.92±5.28	58.28±5.44	55.0±4.28
S. kunthianum 100mg/kg	44.12±3.18	52.62±2.41	50.12±2.19*	49.56±3.61	43.6±3.61	42.78±3413
S. kunthianum 200mg/kg	43.16±7.64	52.72±5.59	48.28±8.63*	44.70±8.62*	44.70±8.62	39.26±7.78
S. kunthianum 400mg/kg	40.94±5.92	50.44±7.14	46.32±8.13*	40.78±9.52*	40.78±8.95*	40.98±6.14
Indomethacin 10mg/kg	25.98±5.75*	32.52±5.64*	30.55±6.47**	30.23±6.77**	29.91±6.91*	29.91±6.91*

Rats were pretreated either with fraction L or indomethacin at the doses indicated one hour before induction of inflammation with carrageenan (0.1 ml of 1% w/v in normal saline) in each rat. Values at each time point are mean ± SEM of percent increase in paw diameter for five experiments. *$P<0.05$, **$P<0.001$ compared to the normal saline-treated animals at the corresponding time point.

Table 5: Effect of column chromatographic fraction S on carrageenan-induced paw oedema in rats

Treatment	Percentage paw oedema					
	1hr	2hr	3hr	4hr	5hr	6hr
Normal saline: 5ml/kg	58.33±12.3	80.83±5.65	86.25±5.65	72.92±5.05	56.67±6.01	52.92±6.14
S. kunthianum 100mg/kg	53.00±6.63	67.50±3.06	62.50±5.58*	58.50±4.71	50.00±3.95	45.00±3.06
S. kunthianum 200mg/kg	46.27±6.30	55.72±7.33*	54.61±7.42*	50.88±7.32*	48.55±7.62	49.88±7.25
S. kunthianum 400mg/kg	33.98±4.75	46.48±5.27*	35.98±3.56**	33.98±4.75**	29.26±3.43**	25.72±4.14**
Indomethacin 10mg/kg	25.98±5.75*	32.52±5.64**	30.55±6.47**	30.23±6.77**	29.91±6.91*	26.97±5.76*

Rats were pretreated either with fraction S or indomethacin at the doses indicated one hour before induction of inflammation with carrageenan (0.1 ml of 1% w/v in normal saline) in each rat. Values at each time point are mean ± SEM of percent increase in paw diameter for five experiments. *$P<0.05$, **$P<0.001$ compared to the normal saline-treated animals at the corresponding time point.

Table 6: Effect of column chromatographic fraction Y on carrageenan-induced paw oedema in rats

Treatment	Percentage paw oedema					
	1hr	2hr	3hr	4hr	5hr	6hr
Normal saline 5ml/kg	58.33±12.3	80.83±5.65	86.25±5.65	72.92±5.05	56.67±6.01	52.92±6.14
S. kunthianum 100mg/kg	56.12±4.83	58.62±4.69*	56.12±4.83**	52.30±4.66*	50.38±4.27	50.16±5.36
S. kunthianum 200mg/kg	51.38±2.98	53.12±4.51**	54.16±6.96**	49.16±4.64**	49.16±4.64	49.16±6.09
S. kunthianum 400mg/kg	50.00±0.00	51.00±2.80**	53.50±2.45**	47.00±0.00**	42.25±4.99	40.00±6.11
Indomethacin 10mg/kg	25.98±5.75*	32.52±5.64**	30.55±6.47**	30.23±6.77**	29.91±6.91*	26.97±5.76*

Rats were pretreated either with fraction Y or indomethacin at the doses indicated one hour before induction of inflammation with carrageenan (0.1 ml of 1% w/v in normal saline) in each rat. Values at each time point are mean ± SEM of percent increase in paw diameter for five experiments. *$P<0.05$, **$P<0.001$ compared to the normal saline-treated animals at the corresponding time point.

Discussion

The vacuum liquid chromatographic fractions dose-dependently and significantly reduced paw oedema formation in the treated animals compared to the normal saline-treated animals at the corresponding time point. Fractions A and B caused more intense anti-inflammatory activities than fraction C suggesting that the active constituents in the fractions possess anti-inflammatory activity to varying degrees. Their anti-inflammatory effects peaked at the third hour post carrageenan challenge which corresponded with the inflammatory phase in which prostaglandin is the major mediator.

The column chromatographic fractions similarly dose-dependently and significantly caused reduction in paw oedema in the treated rats. The anti-inflammatory effects of the column chromatographic fractions were significant at the second hour post - carrageenan injection and peaked at the third hour post-carrageenan injection. These periods correspond to the inflammatory phases in which serotonin, histamine and prostaglandins are the major mediators. This study establishes the anti-inflammatory activity of the chromatographic fractions of *S. kunthianum* stem bark in the carrageenan-induced paw oedema model, which represent different phases of inflammation.

Carrageenan-induced oedema is a model of acute inflammation used in the study of non-steroidal anti-inflammatory drugs (Vinegar *et al.*, 1969). The model is suitable for evaluating the anti-inflammatory effect of natural products and is believed to be triphasic. The first phase which occurs between 0 – 1.5 h is believed to involve the release of serotonin and histamine while the second phase which occurs between 1.5 – 2.5 h which releases kinins and the third phase (2.5 – 6 h) has been attributed to prostaglandins release (Vinegar *et al.*, 1969). That the fractions produced marked anti-inflammatory effect 3h post-carrageenan injection suggests that their anti-inflammatory activity may involve the inhibition of prostaglandin synthesis and other cyclooxygenase products since the carrageenan inflammatory model basically reflects the action of prostaglandins (Flower *et al.*, 1972). That the column chromatographic fractions (L, S and Y) produced significant anti-inflammatory effects at the second hour post carrageenan injection suggests that their anti-inflammatory activities may involve the inhibition of histamine or serotonin which characterizes this inflammatory phase.

Conclusion

It is therefore likely that the mechanisms of actions of the fractions involve interfering with serotonin, histamine and prostaglandin activities in the inflammatory process. The anti-inflammatory effects of the fractions corroborate that observed with the aqueous extract (Ching *et al.*, 2009 b). However, the effects of the fractions were more pronounced. These results further support the ethnomedicinal use of the *S. kunthianum* stem bark in the treatment of inflammatory conditions.

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