INHIBITION OF VASCULAR RESPONSE IN INFLAMMATION BY CRUDE AQUEOUS EXTRACT OF THE ROOT BARK OF *ZANTHOXYLUM XANTHOXYLOIDES*

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SUMMARY

Background: The root bark extract of *Zanthoxylum xanthozyloides* is used in folklore medicine in Ghana and Nigeria to treat inflammation. A previous pharmacological study confirmed the anti-inflammatory activity of the extract.

Objective: To study the effect of the extract on vascular response in inflammation.

Method: The extract was obtained by Soxhlet extraction and rotatory evaporation, followed by freeze-drying. Groups of rats (with carrageenin-induced paw inflammation) and mice (with xylene-induced pinna inflammation) were, respectively, assigned randomly to treatment groups. The animals were given three different treatments orally: 0.9% saline (control), the extract (400mg/kg and 800mg/kg for mice; 1000mg/kg, 2000 mg/kg, and 4000mg/kg for rats), and indomethacin (5mg/kg and 10mg/kg for mice; 10mg/kg, 20mg/kg, and 40mg/kg for rats). In another set of experiment, each treatment group received phenylephrine subcutaneously (30µg/kg for rats and 20µg/kg for mice) in addition to the specified treatment aforementioned. In both sets of experiments, each group of rats was rotated through the entire treatment groups such that each animal served as control as well as received all the treatments. Analysis of variance was used as the statistical test.

Results: The extract and indomethacin both caused dose-dependent reduction in the carrageenin-induced increase in paw volume in rats and also reduced xylene-induced increase in blood flow in mice pinna arteries. Phenylephrine enhanced the decrease in capillary permeability and vasodilatation caused by low dose extract but not that caused by high dose extract or both low and high dose indomethacin.

Conclusion: The extract reduced vasodilatation and decreased capillary permeability in inflammation.

Keywords: Root bark extract, *Zanthoxylum xanthoxyloides*, capillary permeability, vasodilatation, inflammation.

INTRODUCTION

Zanthoxylum xanthoxyloides (a member of the family Rutaceae) is a stout tree that grows wild in coastal grassland, closed forest, and in the Guinea savannah. The root bark extract is used in folklore medicine in Ghana and Nigeria to treat various conditions, notably, inflammation.[1]

The initial step of the inflammatory process is vascular response which comprises vasodilatation and increased capillary and venular permeability as well as endothelial expression of cell adhesion molecules. The vascular response culminates in excess accumulation of fluid in the tissue (oedema) while the vasodilatation is expressed outwardly as an area of reddening (erythema).

Previous study of anti-inflammatory activity of isolated and purified alkaloids of the root bark of *Z. xanthoxyloides* employed plethysmometry to measure volume changes in carrageenin-induced inflammation in rat paw, using decrease in paw volume as an index of the anti-inflammatory activity.[2] Decrease in paw volume could be due to either decreased blood flow into the capillaries consequent to vasoconstriction or decreased capillary permeability or to both mechanisms. The plethysmometry method alone, however, cannot discriminate such mechanisms.

A rational approach to assessing the anti-inflammatory activity of the extract at the vascular level, therefore, entails studying the effect of the extract on oedema and erythema, using experimental animal models that test for oedema and erythema. Accordingly, the mouse pinna inflammation (for erythema) [3] and carrageenin-induced inflammation in rat paw (for oedema) [4] were used as test models to elicit the effect of the root bark extract on vascular response in inflammation. All the doses of the extract used were within the dose range (on the dose axis) corresponding to the linear portion of the log dose-response curve.

MATERIALS AND METHODS

Collection and extraction of the root bark

The roots of *Z. xanthoxyloides* (identified and confirmed in the Department of Botany, University of Ghana, Legon) were collected from a forest at Akatsi, Volta Region, in the month of August and solar-dried for 1 day. The root barks were removed, washed, and dried in hot oven (55^{O}C) for five days. The dried root barks were pulverized to powder. Aliquot of the powder, 300g, was extracted in water, 3L, in Soxhlet apparatus[5]. The extraction was allowed to continue until a point where no more brown colouration was imparted to the water. This was used as an index for completion of extraction. The clear brown extract was concentrated 10-fold in a rotatory evaporator (RE-100 (Bibby Sterilin, Newport, U.K.). The viscous brown fluid was freeze-dried in Edward Modulyo freeze-dryer (Edwards High Vacuum, Crawley, U.K.). The freeze-dried powder was stored at -18^{o}C until when needed. Reconstituted freeze-dried powder in 0.9% saline is referred to as "the extract" in this text.

Carrageenin- induced inflammation

Thirty-five Wistar rats (150-200g) of both sexes (18 males and 17 females) were randomly assigned to 7 treatment groups of 5 rats each. The volumes of the right hind paw of each rat (Vo) were measured by water displacement in a plethysmometer (Ugo Basile, Comerio, Italy). The rats were then treated as follows: one group received 0.9% saline on weight basis (control); three groups received three dose levels of indomethacin, 10mg/kg, 20mg/kg and 40mg/kg respectively; and the remaining three groups received three dose levels of the extract, 1000mg/kg, 2000mg/kg and 4000mg/kg respectively. The doses were given orally. One hour after treatment, 0.1 ml of 1% (w/v) carrageenin in 0.9% saline was injected into the subplantar surface of the right hind paw of each rat to induce inflammation. The paw volume of each rat (Vt) was measured again at 1, 2, 3, 4 and 5hr after the injection of the inflammatory agent. Each group of rats was rotated through the entire seven treatment groups (randomized complete block design), and paw volumes measured in each rotation. Thus each rat served as a control as well as received the other six treatments. The hind paws used for induction of inflammation were alternated during the rotations, and the animals were rested for one week after every two rotations at 3-day interval.

Another set of 25 Wistar rats (150-200g) of both sexes (12 males and 13 females) was randomly assigned to 5 treatment groups of 5 rats each. Paw volume of each rat was measured. One group was given 0.9% saline on weight basis (control); two groups received two dose levels of indomethacin, 10mg/kg and 40mg/kg respectively; and the remaining two groups, two dose levels of the extract, 1000mg/kg and 4000mg/kg respectively. Each treatment group was also given phenylephrine, 30µg/kg, subcutaneously.

Mouse pinna inflammation

Twenty-five Balb/C mice (25-35g) of both sexes (13 males and 12 females) were randomly assigned to 5 treatment groups of 5 mice each. One group received 0.9% saline on weight basis (control); two groups were given two dose levels of indomethacin, 5mg/kg and 10mg/kg, respectively; and the remaining two groups, two dose levels of the extract, 400mg/kg and 800mg/kg respectively. All doses were given orally. One hour after receiving treatment, each mouse was restrained in a narrow holder and 0.1 ml of Evan's blue dye injected through the tail vein. The dye was allowed to equilibrate in the circulation for 30min to 1hr. Under ether anaesthesia, a drop of xylene was inoculated into the right pinna by pricking it with a 26G hypodermic needle through the drop. Fifteen minutes later, the animals were euthanised and their ears severed. The diameter of the blue patch at the site of inoculation was measured with a pair of dividers and the area calculated.

Another set of 25 Balb/C mice (25-35g) of both sexes (13 males and 12 females) was randomly assigned to 5 treatment groups of five mice each. One group received 0.9% saline on weight basis (control); two groups, 400mg/kg and 800mg/kg of the extract respectively; and the remaining two groups, 5mg/kg and 10mg/kg of indomethacin respectively. Each treatment group also received phenylephrine, 20µg/kg, subcutaneously.

Data analysis

The average paw volume for each treated rat group in the Carrageenin- induced inflammation experiment was determined and compared with that obtained for each group before treatment. Percentage increase in paw volume (P %) was derived, using the formula:

$$P\ \% = \{(Vt - Vo)/Vo\} \times 100$$

Where Vt = the average paw volume at a given time, t, and Vo = the average paw volume at zero time.

Percent reduction in paw volume (I %), at a given time, caused by a treatment was computed from the following equation:

$$I\% = \{1 - (Pt/Po)\} \times 100$$

Where Pt = mean percentage increase in paw volume of drug or the extract treated rats at time, t.
Po = mean percentage increase in paw volume of control rats at the specified time.

The mean blue patch area of each treated group in the pinna inflammation experiment was calculated and

compared with that of the control group. The mean percent reduction in the area, I%, was calculated for each group, using the equation:

$$I\% = \{1 - (A/Ao)\} \times 100$$

Where A = mean area of blue patch in drug or extract treated group. Ao = mean area of blue patch in control group.

Statistical analysis of the data was performed using analysis of variance to test the difference in the means of groups. The difference between the average mean paw volume (ml) of the treated rats and those of the control at the specified time intervals was expressed as percent reduction of the control. In the figures, each point represents the mean percentage reduction in paw volume of 35 rats. Differences at $p<0.05$ were considered statistically significant.

RESULTS

In animals given saline (control), 0.1 ml of 1% (w/v) carrageenin in 0.9% saline increased paw volume significantly ($p<0.05$). Increased paw volume was noticeable at 1hr, peaked at 3hr and thereafter reduced relatively. Percentage increase in paw volume was 65% at 1hr, 73% at 3hr and 68% at 5hr after injection of carrageenin. The extract (Figure 1) and indomethacin (Figure 2) significantly reduced the carrageenin-induced increase in paw volume. The percentage reduction differed significantly among groups of rats ($p<0.05$) given different dose levels of the extract and indomethacin.

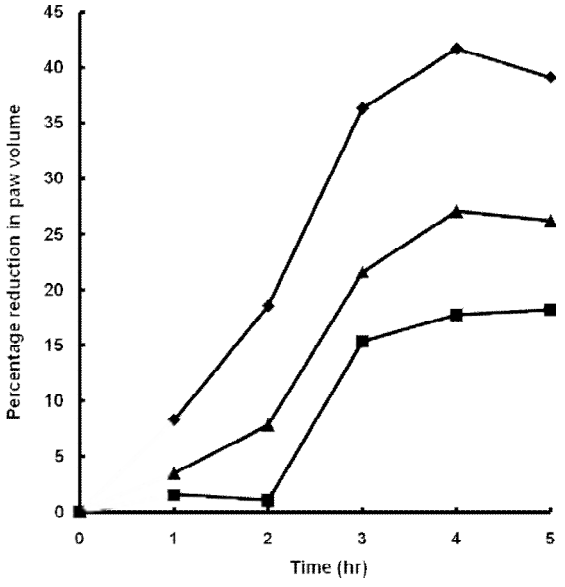

Figure 1 Percentage reduction of oedema in the right hind paw of rats by the extract (■–■, 1000mg/kg; ▲–▲, 2000mg/kg; and ♦–♦, 4000mg/kg).

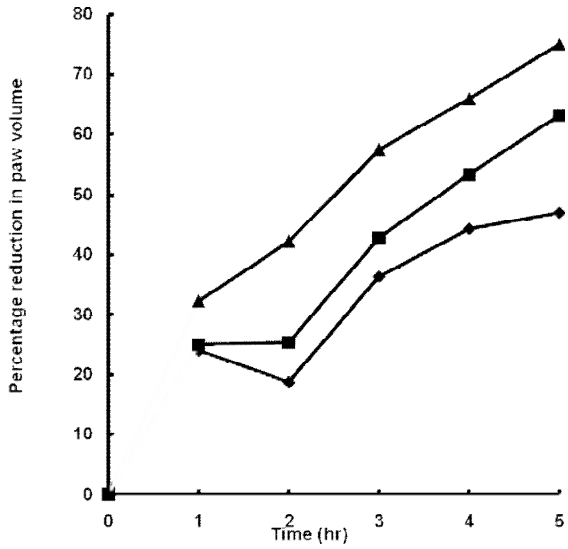

Figure 2: Percentage reduction of oedema in the right hind paw of rats by indomethacin (♦–♦, 10mg/kg; ■–■, 20 mg/kg; ▲–▲, 40 mg/kg).

The extract caused significant ($p<0.05$) dose-dependent reduction in the carrageenin-induced increase in paw volume, with maximum percentage reduction observed 4hr after injection of the inflammatory agent (Figure 1). The maximum percentage reduction in paw volume caused by 1000, 2000, and 4000mg/kg extract was 18%, 27% and 42% respectively. Indomethacin, like the extract, dose-dependently reduced the carrageenin-induced increase in paw volume. The maximum percentage reduction in paw volume observed at 10mg/kg was 47% (Figure 2). However, the percentage reduction did not reach a maximum at the higher dose levels. Compared to the extract, indomethacin caused a significantly greater reduction in paw volume ($p<0.05$) at the three dose levels at all the specified time intervals except at 2hr after injecting the inflammatory agent when there was no significant difference ($p=0.693$) between 10 mg/kg indomethacin and 4000 mg/kg extract; each causing 19% reduction in paw volume.

Phenylephrine enhanced significantly ($p=0.047$) the reduction in paw volume caused by low dose (1000mg/kg) of the extract (Table 1) from 20% to 39% but had no significant effect ($p=0.918$) on that caused by the large dose (4000mg/kg).

Phenylephrine, also, did not have any significant effect ($p>0.05$) on the percentage reduction in paw volume caused by low (10mg/kg) or high (40mg/kg) dose of indomethacin (Table 1).

Table 1 Effect of the extract and indomethacin on carrageenin-induced rat paw oedema (paw volume) in the presence (+) and absence (-) of phenylephrine.

Treatment	Mean Volume (ml) ± SEM		Percentage reduction in paw volume		P-value
	(-) Phenylephrine	(+) Phenylephrine	(-) Phenylephrine	(+) Phenylephrine	
Control	2.72 ± 0.25				
Extract, 1000mg/kg	2.12 ± 0.32	1.67 ± 0.31	20.3	38.6	0.047
Extract, 4000mg/kg	1.61 ± 0.21	1.54 ± 0.18	41.0	43.2	0.923
Indomethacin, 10mg/kg	1.47 ± 0.17	1.43 ± 0.13	45.7	47.3	0.941
Indomethacin, 40mg/kg	0.64 ± 0.09	0.74 ± 0.10	76.4	72.9	0.864

Table 2: Effect of the extract and indomethacin on pinna blood flow in xylene-induced mouse pinna inflammation in the presence (+) and absence (-) of phenylephrine.

Treatment	Mean area (mm^2) ± SEM		Percentage reduction in blood flow		P-value
	(-) Phenylephrine	(+) Phenylephrine	(-) Phenylephrine	(+) Phenylephrine	
Control	76.02 ± 5.95				
Extract, 400 mg/kg	33.10 ± 5.24	10.46 ± 2.23	56.5	86.8	0.042
Extract, 800 mg/kg	19.14 ± 2.20	6.69 ± 1.71	74.8	91.2	0.196
Indomethacin, 5 mg/kg	11.80 ± 2.31	6.56 ± 3.51	84.5	91.4	0.582
Indomethacin,10 mg/kg	6.90 ± 2.33	3.10 ± 1.83	90.9	96.0	0.560

Although the percentage increase in paw volume/time profile was identical for the extract and indomethacin the slope of the curves was steeper for indomethacin than the extract. At 5hr after administration of carrageenin, indomethacin retained a significantly greater activity ($p<0.05$) than the extract. The profiles of the percentage reduction/time curves of indomethacin and the extract were different (Figures 1 and 2). The curves for the extract at all dose levels and 10mg/kg indomethacin consist of three portions: initial gentle slope, middle steep slope and a plateau whilst the curves for indomethacin at 20mg/kg and 40mg/kg, comprise two contiguous linear portions: one with a gentle slope and the other, a steep slope. Indomethacin retained a significantly greater activity ($p<0.05$) than the extract 5hr after their administration.

Xylene-induced inflammation in the pinna of mice was fully elicited in the saline treated group but was significantly reduced ($p < 0.05$) by both indomethacin and the extract at all dose levels tested (Table 2). The extract, like indomethacin, dose-dependently reduced ($p<0.05$) the area of blue patch observed in the pinna.

Compared to the extract, indomethacin reduced the area of the blue patch more significantly ($p<0.05$): 400 mg/kg and 800 mg/kg extract reduced the area by 57% and 75% respectively whilst indomethacin 5mg/kg and 10mg/kg caused 85% and 91% reduction respectively. In the presence of phenylephrine, the extract, 400mg/kg and 800mg/kg, reduced the blue patch area by 87% and 91% respectively (Table 2) whilst indomethacin, 5mg/kg and 10mg/kg, caused 91% and 96% reduction respectively. Thus phenylephrine enhanced the reduction in the blue patch area caused by 400mg/kg extract more significantly ($p<0.05$) than that caused by both he low and high dose indomethacin.

DISCUSSION

The vascular response to the crude extract of *Z. xanthoxyloides* in inflammation was studied in two established laboratory models, namely, carrageenin-induced rat paw oedema and xylene-induced mouse pinna inflammation. The data show that both the extract and indomethacin dose-dependently reduced carrageenin-induced increase in the paw volume of rats, and also reduced xylene-induced increase in pinna blood flow in mice. The decrease in blue patch area in the mouse pinna inflammation model indicates that the extract or indomethacin reduced blood flow consequent to reduction of vasodilatation.

This is supported by the observation that phenylephrine, a vasoconstrictor, significantly ($p<0.05$) enhanced the reduction of the blue patch area caused by the extract. Although phenylephrine also enhanced the reduction of the blue patch area caused by indomethacin, the percentage enhancement was not significant at $p<0.05$.This is because indomethacin might have reduced vasodilatation completely and, therefore, phenylephrine could not constrict the vessels to a significant extent. Consequently, blood flow and, hence, blue patch area was not significantly reduced by phenylephrine.

The extract, likewise indomethacin, reduced oedema formation which could be due to either decreased capillary permeability or reduced blood flow into capillaries as a result of reduced vasodilatation or to both mechanisms. Phenylephrine was used to discriminate these possible mechanisms underlying the reduction of oedema formation by the extract and indomethacin. In the presence of the extract or indomethacin, phenylephrine was expected to prevent the development of oedema if the reduction of oedema formation caused by the extract or indomethacin was solely due to reduced blood flow to the capillaries. In the present study, phenylephrine did not prevent oedema formation but enhanced the percentage reduction in oedema formation caused by low dose (1000mg/kg) of the extract. The percentage reduction in oedema formation caused by large dose extract (4000mg/kg), low dose indomethacin (10mg/kg) or large dose indomethacin (40mg/kg) was, however, not significantly changed by phenylephrine. The difference in the effect of phenylephrine in the presence of low dose extract, on one hand, and in the other regimens, on the other hand, could be due to the difference in the degree of vasodilatation. Low dose extract did not reduce vasodilatation completely and, therefore, phenylephrine was able to constrict the arteries to further reduce blood flow and, hence, enhanced the reduction in oedema formation. In the other regimens, reduction of vasodilatation was complete and, therefore, phenylephrine could not constrict the arterioles to enhance reduction in blood flow to the capillaries. The data suggest that the decrease in paw volume or oedema caused by the extract or indomethacin was not solely due to reduction in blood flow into the capillaries but, also, partly to decreased capillary permeability.

The profile of the activity (reduction in oedema)/time curve would seem to suggest that oedema-reducing activity of the extract may involve more than one process and may be related to the sequential release of chemical mediators.[6] Thus the three portions of the curve may each represent interaction of the extract with different chemical mediators at different times. Alternatively, the lower and middle portions of the curve may each represent interaction with different chemical mediators whilst the upper portion of the curve represents wearing-off of the extract effect. Discrimination of these interactions would require time-dependent measurements of the various chemical mediators in the presence and absence of their respective blockers. This would provide an insight to the molecular mechanisms underlying the vascular response to the extract in inflammation.

CONCLUSION

The study of vascular response to the extract of the root bark of *Z. xanthoxyloides* in inflammation, using carrageenin-induced paw oedema and xylene-induced mouse pinna inflammation has revealed that the extract as well as indomethacin decreased both capillary permeability and vasodilatation in inflammation. Since the extract shared similar vascular response property with indomethacin, a standard NSAID, it could be inferred that decrease in both capillary permeability and vasodilatation may contribute to the anti-inflammatory activity of the extract. Further study involving temporal measurements of biomarkers for inflammation would be required to identify the molecular mechanisms underlying the vascular response to the extract in inflammation.

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REFERENCES

1. Ghana Herbal Pharmacopoeia, 1992; pp56-58, Advent Press, Accra.
2. Oriowo, M.A. Anti-inflammatory activity of Piperonyl-4-acrylic isobutyl amide, an extractive-from Z. xanthoxyloides, *Planta Medica*, 1982; 44:54-6
3. Brown, D.M and Robson, R.D. Effect of anti-inflammatory agents on capillary permeability and oedema formation. *Nature,* 1964; 202:812-813
4. Winter C. A., Risley E.A and Nuss G.W. Carrageenin-induced oedema in the hind paw of rats as an anti-inflammatory drug. *Proc. Soc. Exptl. Biol. Med* 1962; 111:544-7
5. Vogel, A.L. *A textbook of practical organic chemistry including qualitatitive organic analysis, fifth edition,* 1989; pp 164-5, Longman Group.
6. DiRosa M., Girould J.P. and Willoughby, D.A. Study of the mediators of the acute inflammatory response induced in rats in different sites by carrageenin and turpentine, *J. Path.*, 1971; 104: 15 – 29