IN VIVO INHIBITION OF PROSTAGLANDIN E_2 PRODUCTION BY CRUDE AQUEOUS EXTRACT OF THE ROOT BARK OF *ZANTHOXYLUM XANTHOXYLOIDES.*

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SUMMARY

Background: Crude aqueous extract of the root bark of *Zanthoxylum xanthoxyloide*s is used in folklore medicine for its anti-inflammatory activity. Although it shares the analgesic and anti-inflammatory property of non-steroidal anti-inflammatory drugs (NSAIDs), its mechanism of action has not been well elucidated.

Objective: To ascertain whether the extract decreases carrageenin-induced increase in plasma prostaglandin E_2 (PGE_2) concentration with the view to shed light on the mechanism of action.

Methods: The extract was obtained by Soxhlet extraction and rotatory evaporation, followed by freeze-drying. Forty Wistar rats (150g - 200g) were assigned to 8 groups of 5 rats each. The rats were given four different treatments orally: 0.9% saline (two groups of control); two groups received indomethacin, 20mg/kg and 40mg/kg respectively; another two groups received the extract, 2000mg/kg and 4000mg/kg respectively; and the remaining two groups, 50mg/kg and 100mg/kg nimesulide respectively. Inflammation was induced with carrageenin in one of the two groups of control. Enzyme-linked immunospecific assay was used to measure plasma PGE_2 concentration in the control and treated groups of rats. Analysis of variance was used as the statistical test. Differences in means at $p<0.05$ were considered significant.

Results: Carrageenin increased plasma PGE_2 concentration which was reduced by the extract, indomethacin and nimesulide. High dose extract and indomethacin reduced plasma PGE_2 concentration to a comparable extent which was much greater than that of the reduction caused by nimesulide.

Conclusion: It was concluded that the extract might act by non-selective inhibition of cyclooxygenase 1 and 2 to decrease plasma PGE_2 concentration.

Key Words: *Zanthoxylum xanthoxyloides*, root bark extract, inhibition, plasma PGE_2 concentration.

INTRODUCTION

Crude aqueous extract of the root bark of *Zanthoxylum xanthoxyloides* is used in folklore medicine for its anti-inflammatory activity.[1] It is now well established by pharmacological study that the extract has anti-inflammatory activity.[2,3] Chemical analysis of the extract has not revealed any steroid constituent[4], and the isolated and purified active principle has been identified as fagaramide.[3] The extract, as an anti-inflammatory agent, can therefore be categorized as non-steroidal anti-inflammatory drug (NSAID).

NSAIDs produce their anti-inflammatory activity by: 1) inhibiting the release or biosynthesis of chemical mediators[5,6]; 2) inhibiting migration of inflammatory cells[7,8]; and 3) inhibiting expression of cell adhesion molecules.[9,10] All NSAIDs share the first mechanism. Some NSAIDs exhibit the second and/or the third mechanism in addition to the first. Indomethacin, a prototype of NSAIDs, exhibits all the three mechanisms.

Inhibition of prostaglandin (PG) biosynthesis is now recognized as the major mechanism underlying the analgesic, antipyretic, and anti-inflammatory property of NSAIDs. It is this same mechanism that confers adverse effects or therapeutic disasters on NSAIDs. Among the prostaglandins, PGE_2 has been established as a chemical mediator of inflammation as it is produced most abundantly in inflammation in contrast to its low production under physiological conditions.[11] NSAIDs decrease production of prostaglandin consequent to inhibition of cyclooxygenase (COX).[12]

Although the extract shares the analgesic[2] and anti-inflammatory[2,3] property of indomethacin, its mechanism of action has not been well elucidated. *In vitro*, the extract inhibits prostaglandin synthetase activity to reduce PGE_2 production.[3] The study was, therefore, undertaken to verify whether the extract could inhibit PGE_2 production *in vivo*.

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 were solar-dried for 1 day. Voucher specimen (Collection No: GC 47717) of the plant was deposited at Ghana Herbarium. The root barks were removed, washed, and dried in hot oven (55^OC) for five days. The dried root barks were pulverized and 300mg aliquots of the powder extracted in 3L of water in Soxhlet apparatus[13]. 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 complete extraction. The clear brown extract was concentrated 10-fold in a rotatory evaporator (Bibby Sterilin rotatory evaporator RE - 100). The viscous brown fluid was freeze-dried in Edward Modulyo freeze-drier (Edwards High Vacuum). The freeze-dried powder was stored at -18^oC until when needed. Reconstituted freeze-dried powder in 0.9% saline is referred to as "the extract" in this text.

Drug administration and blood collection

Forty Wistar rats of both sexes (150g - 200g) were randomly assigned to 8 groups (treatment cells) of five rats each (cohort). Two groups received normal saline (control); two groups were given two doses of indomethacin, 20mg/kg and 40mg/kg respectively; another two groups were given nimesulide at two dose levels, 50mg/kg and 100mg/kg respectively; and the remaining two groups, given the extract at two dose levels, 2000mg/kg and 4000mg/kg respectively. One percent (w/v) carrageenin in 0.9% saline, 0.1ml, was used to induce inflammation in the right hind paw of one group of control rats (control 2) and the treated groups, one hour after treatment. The carrageenin-treated control group (control 2) served as positive control. One hour after the injection of carrageenin, the rats were anaesthetized with ether, and dissected to expose the heart. Blood samples were collected separately from each animal by cardiac puncture and immediately put into tubes containing EDTA. The blood samples were centrifuged at 10,000rpm for 15min, and the plasma decanted and stored at -4^oC.

Measurement of plasma PGE_2 concentration

Enzyme-linked immunospecific assay (ELISA)[14] was used to measure plasma PGE_2 concentration. Optical density (O.D) was read for solution in wells containing: 1) serially diluted concentration (0 – 320pg) of standard prostaglandin (provided in the kit) bound to both antibody (specific binding) and non-antibody sites (non-specific binding) on the well wall. Zero concentration of standard prostaglandin is referred to as zero standard; 2) plasma PGE_2 (sample) bound to both antibody (specific binding) and non-antibody sites (non-specific binding) on the well wall; and 3) prostaglandin PGE_2 bound to only non-antibody site (non-specific binding, NSB).

The amount of PGE_2 bound (specific binding) to the wall (B_w) was expressed as a percentage of the total PGE_2 in the well (B_T), using the formula:

$\%B_W/B_T$ = {(standard or sample OD - NSB OD) / (zero standard OD - NSB OD)} x 100

A calibration curve was constructed by plotting the $\%B_W/B_T$ of the standards as a function of the log concentrations. The concentration of plasma PGE_2 was determined by extrapolation from the calibration curve.

The percent inhibition of PG synthesis (I%) was calculated, using the formula: I% = {1 – (conc. S/ conc. C)} x 100 where conc S = concentration of plasma PGE_2 in drug or extract treated rats and conc C = concentration of plasma PGE_2 in control rats

Statistical analysis

Analysis of variance was used to compare the means of plasma PGE_2 concentration of the different treatment groups. Post hoc analysis (after-test ANOVA) was employed to compare the means of paired observations (inter-groups and intra-group). Inhibition was expressed as percent of control. Differences in means at $p<0.05$ were considered significant.

RESULTS

A plot of percentage absorbance ($\%B_W/B_T$) against log concentration of standard PGE_2 (calibration curve) was linear with 0.9056 coefficient of regression. Carrageenin-treatment significantly ($p<0.05$) increased plasma PGE_2 concentration as indicated by the higher concentration in control group 2 than in control group 1 (Table 1).

The concentration of plasma PGE_2 in the extract, indomethacin or nimesulide treatment groups was lower than that of the two control groups. Indomethacin, nimesulide, and the extract significantly ($p<0.05$) reduced plasma PGE_2 concentration at both dose levels (Table 1 and Fig. 1).

Low dose (2000mg/kg) and high dose (4000mg/kg) extract caused 35% and 84% reduction in plasma PGE_2 concentration respectively. The percentage reduction (84%) caused by high dose extract was comparable to the 84% and 96% reduction in plasma PGE_2 concentration caused by low dose (20mg/kg) and high dose (40mg/kg) indomethacin respectively. Unlike in the case of the extract, doubling the dose of indomethacin

did not greatly enhance the decrease in plasma PGE_2 concentration.

Table 1: Percentage reduction in plasma PGE_2 concentration (pg/ml) by different dose levels of the extract, indomethacin and nimesulide.

Treatment	Mean PGE_2 Concentration ± SEM (pg/ml)	Percentage Inhibition (%) [p-value]
Control 1 (no carrageenin)	1329.0 ± 128.3	
Control 2 (+ carrageenin)	1737.6 ± 248.2	
Extract, 2000 mg/kg	1121.1 ± 93.0	35.48 [0.0019]
Extract, 4000 mg/kg	282.2 ± 47.0	83.76 [<0.0001]
Indomethacin, 20 mg/kg	273.6 ± 34.2	84.25 [<0.0001]
Indomethacin, 40 mg/kg	71.4 ± 14.3	95.89 [<0.0001]
Nimesulide, 50 mg/kg	1328.0 ± 120.7	23.53 [0.0203]
Nimesulide, 100 mg/kg	818.0 ± 91.8	52.92 [0.0001]

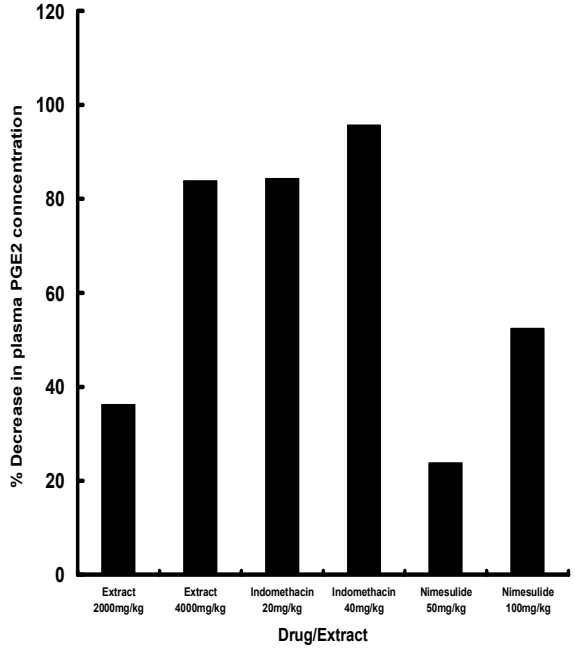

Figure 1: Effect of extract (2000 mg/kg and 4000 mg/kg), indomethacin (20 mg/kg and 40 mg/kg), and nimesulide (50mg/kg and 100mg/kg) on plasma PGE_2 concentration. Figure shows the % decrease in plasma PGE_2 concentration caused by the extract, indomethacin and nimesulide.

Nimesulide, even at a large dose (100mg/kg), did not decrease plasma PGE_2 concentration to an extent as great as that of the decrease caused by high dose extract or both high and low dose indomethacin.

Post-hoc analysis showed that the extract, indomethacin or nimesulide caused significant dose-dependent ($p<0.05$) percentage reduction in plasma PGE_2 concentration (Table 1). The percentage reduction in plasma PGE_2 concentration also differed significantly ($p<0.05$) among the groups of rats receiving different dose levels of the extract, indomethacin and nimesulide. Compared to the extract, indomethacin caused a significantly greater percentage reduction ($p<0.05$) in plasma PGE_2 concentration at the two dose levels. However, there was no significant difference ($p = 0.7800$) between the percentage reduction in plasma PGE_2 concentration caused by 4000mg/kg extract and 20mg/kg indomethacin.

DISCUSSION

The data show that plasma PGE_2 concentration in rats increased following carrageenin-induced inflammation but decreased when the inflammation was challenged with varying doses of the extract, indomethacin or nimesulide. Although indomethacin and other NSAIDs have been shown to decrease plasma PGE_2 concentration[7], no such finding has been reported for the extract.

PGE_2 is produced in small quantity under normal physiological conditions but in large quantity during inflammation.[11] The substantial increase in PGE_2 in inflammation is attributable to expression of COX-2[15]. Since indomethacin (a non-selective inhibitor of COX-1 and COX-2) and nimesulide (a selective COX-2 inhibitor) both decreased carrageenin-induced increase in the plasma PGE_2 concentration suggests that the higher plasma PGE_2 concentration in carrageenin treated rats (control 2) as compared to that in non-carrageenin treated rats (control 1) is due to expression of COX-2 (Table 1). A selective COX-2 inhibitor, such as nimesulide, would therefore be expected to reduce inflammation or carrageenin-induced increase in plasma PGE_2 concentration to a lesser extent than non-selective inhibitor of COX-1 and COX-2 as, indeed, was observed: low doses of indomethacin (20mg/kg) and nimesulide (50mg/kg) caused 84% and 24% reduction in carrageenin-induced increase in the plasma PGE_2 concentration respectively. The corresponding values at high dose were 96% and 53% for indomethacin (40mg/kg) and nimesulide (100mg/kg) respectively (Table 1 and Fig 1). The relatively low percentage reduction in the plasma PGE_2 concentration caused by nimesulide suggests that nimesulide reduced only the component of increased plasma PGE_2 concentration due to the expression of COX-2.

However, considering the percentage increase in the plasma PGE_2 concentration (31%), caused by carrageenin treatment, it would seem large dose nimesulide (100mg/kg) caused a greater reduction (53%) in the plasma PGE_2 concentration than could be accounted by the inhibition of only COX-2. This could be due to the fact that either nimesulide loses its selectivity of action at high doses or that COX-1 and COX-2 coexist in the non-inflammatory state with COX-2 occurring in minute or small quantity.

The extract, at large dose, reduced the carrageenin-induced increase in the plasma PGE_2 concentration to an extent comparable to that of indomethacin but greater than that caused by nimesulide. In this regard, the extract acted in a manner similar to indomethacin and, therefore, it is suggested that the extract might inhibit both COX-1 and COX-2. Inhibition of the COX isomers, however, may not be the sole basis for the reduction in the carrageenin-induced increase in the plasma PGE_2 concentration as fagaramide, an isolated and purified compound of the extract, inhibits prostaglandin synthetase activity in vitro.[3]

PGE_2 has been implicated in a majority of inflammatory reactions including pain, increased capillary permeability, vascular dilatation and recruitment of inflammatory cells; and the inhibition of its biosynthesis by anti-inflammatory agent causes the reversal of the inflammatory reaction. The ability of the extract to inhibit biosynthesis of PGE_2 may underlie, to a large extent, its anti-inflammatory activity, viz., suppression of pain (analgesia), reversal of vasodilatation, decreased capillary permeability and inhibition of migration of inflammatory cells.

CONCLUSION

Carrageenin-induced increase in plasma PGE_2 concentration was reduced by the extract, indomethacin and nimesulide. High dose extract and both low and high dose indomethacin decreased the plasma PGE_2 concentration to a comparable extent which was much greater than the reduction caused by nimesulide. It would seem, therefore, that the extract and indomethacin share similar mechanism of action, that is, inhibition of both COX-1 and COX-2 with consequent reduction in plasma PGE_2 concentration. The decrease in plasma PGE_2 concentration can account for the anti-inflammatory activity of the extract.

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REFERENCE

1. Ghana Herbal Pharmacopoeia 1992; pp 56-58, Advent Press, Accra
2. Prempeh A.B.A. and Mensah-Attipoe. Analgesic activity of crude aqueous extract of the root bark of Zanthoxylum xanthoxyloides (unpublished).
3. Oriowo, M. A. Anti-inflammatory activity of piperonyl-4-acrylic isobutyl amide, an extractive from Zanthoxylum zanthoxyloides, *Planta Medica,* 1982; 44:54-56.
4. Enyeihi, V. D. *PhD. Thesis,* 1975; University of Ibadan, Nigeria.
5. Almeida, A.P., Bayer, B.M.. Horakova, Z. and Beaven, M.A. Influence of Indomethacin and other anti-inflammatory drugs on mobilization and production of neutrophils; studies with carragenan-induced inflammation in rats. *J. Pharmacol. Exp. Ther*., 1980; 214:74-75
6. Ferreira, S. H and Vane, J.R. New aspects of the mode of action of non- steroidal anti-inflammatory drugs, *Ann Rev. Pharmacol.,* 1974; 14:57-71.
7. Blackham, A. and Owen, R.T. Prostaglandin synthetase inhibitors and leukocyte emigration, *J. Pharm. Pharmacol.,* 1975; 27:201-202
8. Phelps, P. and McCarty, D. J. Suppressive effect of indomethacin on crystal-induced inflammation in canine joints and on neutrophilic motility in vitro. *J. Pharmacol. Exp. Ther.,* 1971; 158: 546-553
9. Diaz-Gonzalez, F. and Sanchez-Madrid, F. Inhibition of leukocyte adhesion: an alternative mechanism of action for anti-inflammatory drugs. *Immunol. Today*, 1998; 19:169-172.
10. Endemann G., Abe Y., Bryant C. M., Feng Y., Smith C. W. and Liu D. Y. Novel anti-inflammatory compounds induce shedding of L-selectin and block primary capture of neutrophils under flow conditions. *J. Immunol.* 1997; 158:4879-4885.
11. Girloy D.W., Colville-Nash P.R., Willis D., Chivers J., Paul-Clarke M.J., and Willoughby D.A. Inducible cyclooxygenase may have anti-inflammatory properties, *Nat. Med.,* 1999; 5(6): 621-622
12. Vane J.R. Inhibition of PG synthesis as a mechanism of action for the aspirin-like drugs. *Nature New Biol (London)* 1971, 231:232-235.
13. Vogel, A.L. A textbook of practical organic chemistry including qualitative organic analysis, Fifth edition, 1989; pp 164-5, Longman Group.
14. Chard T. An introduction to radioimmunoassay and related Techniques, Fourth edition, 1990; Amsterdam: Elsevier.
15. Raz, A., Wyche, A., and Siegel, N. Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. *J. Biol. Chem.,* 1988; 263:3022-3028