CRUDE AQUEOUS EXTRACT OF THE ROOT BARK OF *ZANTHOXYLUM XANTHOXYLOIDES* INHIBITS WHITE BLOOD CELLS MIGRATION IN ACUTE INFLAMMATION

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SUMMARY

Background: Crude aqueous extract of *Zanthoxylum xanthozyloides* is used locally to treat inflammatory conditions. Previous study confirmed that the extract has anti-inflammatory activity and also reduced vascular response in inflammation.

Objective: To identify the effect of the extract on migration of white blood cells to the site of inflammation.

Method: The extract was obtained by Soxhlet extraction and rotatory evaporation, followed by freeze-drying. Cohorts of Wistar rats (150g – 200g) were randomly assigned to 6 treatment cells, and were given, per os, three different treatments: indomethacin (20mg/kg and 40mg/kg), the extract (2000mg/kg and 4000mg/kg), and 0.9% saline (two groups of control). Inflammation was induced with carrageenin in the hind paw of the treated groups of rats and one group of the control (positive control), one hour after treatment. Inflammatory exudates from the inflamed paws were collected and the white blood cells (WBCs) counted.

Results: Carrageenin increased the total WBC count (in the paw fluid) which was reduced by the extract and indomethacin ($p<0.05$). Neither the extract nor indomethacin had any effect on total WBC count in the non-carrageenin treated control rats.

Conclusion: The extract did not affect the pre-existing WBC population at the site of inflammation but rather inhibited migration of the cells to the site.

Keywords: Root bark extract, *Zanthoxylum xanthoxyloides*, inflammation, migration, white blood cells

INTRODUCTION

Crude aqueous extract of the root bark of *Z. xanthoxyloides* is used widely in Ghana and Nigeria for treating various inflammatory conditions.[1] Previous study on carrageenin-induced inflammation of the rat paw established the anti-inflammatory activity of the extract.[2]

As an anti-inflammatory agent, the extract has been found to reduce vascular response in inflammation.[3] Since it is common for non-steroidal anti-inflammatory drugs (NSAIDs) to interrupt the inflammatory process at more than one step[4], it became necessary to explore further the mechanism(s) underlying the anti-inflammatory activity of the extract.

A plausible mechanism, aside modification of the vascular response, is modulation of recruitment of inflammatory cells at the site of inflammation. A drug that interrupts the inflammatory process at this level (recruitment) would be expected to reduce WBC count in inflammatory exudates. The study was undertaken to identify the effect of the extract on WBC count in inflammatory exudates and, by inference, the migration of WBCs to the site of inflammation.

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 one 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.[6]

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 (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 $-18^{o}C$ until when needed. Reconstituted freeze-dried powder in 0.9% saline is referred to as "the extract" in this text.

Collection of paw fluid from treated rats

Thirty Wistar rats (150g- 200g) of both sexes were randomly assigned to 6 groups of 5 rats each (cohort).

The rats received, per os, three different treatments: two groups were given normal saline (control); another two groups received two dose levels of indomethacin, 20 mg/kg and 40 mg/kg respectively; and the remaining two groups, two dose levels of the extract 2000 mg/kg and 4000 mg/kg respectively. One hour after treatment, inflammation was induced by injecting 1% (w/v) carrageenin in normal saline, 0.1 ml, into the subplantar surface of the hind paw of one group of the control and the treated groups of rats. The carrageenin treated control rats served as positive control. Three hours after the administration of the inflammatory agent, the plantar aponeurosis of the inflamed paw was inuncted with 2% xylocaine, incised, and the paw fluid of each rat aspirated (using 26G hypodermic needle) and slowly squirted into a test tube. The residual fluid was gently squeezed out, ensuring that blood did not mix with the fluid. Any fluid that had blood in it was discarded. The fluid was examined under microscope (x40) for any sign of breakages of the blood cells.

Total WBC count in paw fluid

The paw fluid, 0.02 ml, was mixed with WBC fluid (3% acetic acid with crystal violet dye), 0.38 ml, in a test tube. The mixture was transferred into a counting chamber, and the total number of WBC counted under a microscope (x40). The total number of WBCs counted was calculated, using the formula:

WBCs = number of cells counted x depth factor (10) x dilution factor (20) x area factor (0.25).

Statistical analysis

Analysis of variance was used to compare the means of WBC count of the different treatment groups. Post hoc analysis (after-test ANOVA) was employed to compare the means of paired observations (inter-groups and intra- groups). Differences at $p<0.05$ were considered significant

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RESULTS

Carrageenin treatment significantly increased WBC population in the inflammatory exudates as indicated by higher WBC count in control group 2 than control group 1 (Table 1). The total WBC count of control group 2 was significantly reduced ($p<0.05$) by indomethacin at both dose levels, 20 mg/kg and 40 mg/kg, just as did the extract at both dose levels, 2000mg/kg and 4000 mg/kg. Low dose extract (2000mg/kg) caused 16% reduction in the WBC count while high dose extract (4000mg/kg) reduced the count by 26%. In the case of indomethacin, the low dose (20mg/kg) caused 36% reduction in the WBC count and the high dose (40mg/kg), 47% reduction.

Table 1: Effect of the extract and indomethacin on the WBC count ($x10^3$/ml) in the paw fluid of non-carrgeenin treated (Control 1) and carrageenin-treated (Control 2) rats.

TREATMENT	CONTROL 1 (non-carrageenin treated rats)		CONTROL 2 (carrageenin-treated rats)	
	WBC count ($x10^3$/ml) (Mean ± SEM)	% Reduction	WBC count ($x10^3$/ml) (Mean ± SEM)	% Reduction
0.9% Saline	1.78 ± 0.14		2.66 ± 0.12	
Extract, 2000mg/kg	1.71 ± 0.29	4	2.23 ± 0.42	16
Extract, 4000mg/kg	1.61 ± 0.18	10	1.96 ± 0.20	26
Indo. 20mg/kg	1.60 ± 0.26	10	1.71 ± 0.13	36
Indo. 40mg/kg	1.76 ± 0.21	1	1.41 ± 0.18	47
Indo. = Indomethacin				

Table 2: Paired observations (inter-groups and intra-groups) in groups of carrageenin-treated rats given different dose levels of the extract and indomethacin.

Paired Observation (mean ± SEM)		p-value
0.9% Saline (2.66 ± 0.12)	: Extract 2000mg/kg (2.23 ± 0.42)	0.0825
0.9% Saline (2.66 ± 0.12)	: Extract 4000mg/kg (1.96 ± 0.20)	0.0001
0.9% Saline (2.66 ± 0.12)	: Indomethacin 20mg/kg (1.71 ± 0.13)	< 0.0001
0.9% Saline (2.66 ± 0.12)	: Indomethacin 40mg/kg (1.41 ± 0.18)	< 0.0001
Extract 2000mg/kg (2.23 ± 0.42)	: Extract 4000mg/kg (1.96 ± 0.20)	0.0357
Extract 2000mg/kg (2.23 ± 0.42)	: Indomethacin 20mg/kg (1.71 ± 0.13)	0.0080
Extract 2000mg/kg (2.23 ± 0.42)	: Indomethacin 40mg/kg (1.41 ± 0.18)	0.0012
Extract 4000mg/kg (1.96 ± 0.20)	: Indomethacin 20mg/kg (1.71 ± 0.13)	0.1739
Extract 4000mg/kg (1.96 ± 0.20)	: Indomethacin 40mg/kg (1.41 ± 0.18)	0.0059
Indomethacin 20mg/kg (1.71 ± 0.13)	: Indomethacin 40mg/kg (1.41 ± 0.18)	0.0312

Post-hoc analysis showed that the extract or indomethacin caused significant ($p<0.05$) dose-dependent percentage reduction in paw fluid WBC count of carrageenin-treated rats (Table 2). The percentage reduction also differed significantly ($p<0.05$) among the groups of rats receiving different dose levels of the extract and

indomethacin. However, there was no significant difference ($p = 0.1739$) between the percentage reduction in the WBC count caused by 4000mg/kg extract and 20mg/kg indomethacin. Even though low dose extract (2000mg/kg) caused 16% reduction in WBC count, the effect was not significant ($p = 0.0825$).

Compared to the extract, indomethacin caused a significantly greater percentage reduction ($p<0.05$) in total WBC count at the two dose levels. However, neither indomethacin nor the extract treatment had any significant effect ($p>0.05$) on the total WBC count in control group 1 (Table 1).

DISCUSSION

The carrageenin-induced increase in total WBC count in the inflammatory exudates of the rat paw is consistent with the fact that increase in WBC population occurs at the site of inflammation. The increase in the total WBC count could mean either there was migration of WBCs to the site or increase in the population of the white cells pre-existing at the site of inflammation. Significantly, neither the extract nor indomethacin had any effect on the total WBC count in control group 1 (Table 1), suggesting neither the extract nor indomethacin had any effect on the population of the WBCs present at the site prior to the inflammation. The decrease in the WBC count caused by the extract and indomethacin suggests that the two agents inhibited migration of WBCs to the site of inflammation.

It is established that migration of inflammatory cells is effected by cell adhesion molecules[6] and chemical mediators.[7,8,9] NSAIDs inhibit migration of inflammatory cells by: inhibiting the release of chemical mediators[10,11,12]; inhibiting the expression of cell adhesion molecules [13,14]; and inhibiting cell motility.[15] Indomethacin exhibits all these three mechanisms. Considering the similarities between the effects of the extract and indomethacin, a prototype of NSAIDs, it is possible that the extract inhibited migration of the WBCs by one or more of the aforementioned mechanisms.

CONCLUSION

The results showed that inflammation induced in rats by carrageenin is associated with increased WBC count at the site of inflammation. Although the extract did not affect the pre-existing WBC population at the site of inflammation, it did inhibit migration of the inflammatory cells to the site. This can contribute to the anti-inflammatory activity of the extract.

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