1-2003

Anti-nociceptive and Anti-inflammatory Effects of the Methanol Extract of *Annona senegalensis* Root Bark

B. Adzu

Samson Amos
Cedarville University, samos@cedarville.edu

M. Adamu

K. S. Gamaniel

Vol. 3/1 (2003) 63 - 67

JOURNAL OF NATURAL REMEDIES

Anti-nociceptive and Anti-inflammatory effects of the methanol extract of *Annona senegalensis* root bark

B. Adzu, S. Amos*, M. Adamu, K. Gamaniel

Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, P. M. B. 21, Abuja, Nigeria.

Received 01 March 2002 ; Accepted 05 August 2002

Abstract

Objective: To investigate the antinociceptive and anti-inflammatory effect of *Annona senegalensis* in mice and rats. Materials and Methods: The analgesic effects of the methanolic extract were studied using acetic acid-induced writhing test, hot plate test and formalin test, while the anti-inflammatory effect was evaluated using the egg-albumin induced hind paw oedema in rats. Results: The methanolic extract exhibited antinociceptive activity against the acetic acid writhing test, hot plate test and the late phase of formaline induced nociception and significant anti-inflammatory activity. Conclusion: The analgesic and anti-inflammatory effect of the methanolic extract might be through peripheral mechanisms and thus justifying its folkloric use in the treatement of rheumatic pain.

Key words: *Annona senegalensis*, analgesia, anti-inflammatory, rheumatism

1. Introduction

Annona senegalensis PERS (Annonaceae) is a small fire resistant plant found throughout the savannah region of northern Nigeria. Its flowers are strongly aromatic and are used to flavour food. The fruit, yellow in colour with a sweet clear jelly, when ripe is edible and has a pleasant flavour. Medicinally, the root and leaves are sold in Nigeria as native medicines [1] for chest pain, intestinal problems, vermifuges [2], cancer treatment [3-5] diarrhoea, dysentery [6], arthritis and rheumatism [7], and to fight Leishmaniasis [8]. The plant has been reported to contain mucilage, wax, tannin, glycosides, proteins, amino acids and anthraquinones [2,6]. In this study, we investigated the pharmacological basis for the use of *Annona senegalensis* in the treatment of rheumatism using egg-albumin induced oedema in rats, and formalin test as well as acetic acid induced abdominal constriction and hot-plate tests in mice.

* Corresponding author
E-Mail: samsonamos@yahoo.co.uk

2. Materials and Method

2.1 Preparation of plant material

A consultant herbalist, Mal Achaba Lugudu at Midlu, Adamawa state in March 2001, collected the roots of the plant. The plant was authenticated at the Taxonomy unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, NIPRD, Abuja, Nigeria. A voucher specimen was deposited at the herbarium unit of the Institute. The root bark was removed, cleaned and shade dried for a week.

The dried material was powdered using pestle and mortar. 500 g of the powdered material was extracted with 2 L of methanol (BDH, England) using a soxhlet extractor (Gallenkamp, England) and the solvent was evaporated under reduced pressure using a rotary evaporator. This gave a yield of 9.7% w/w. The extract was freshly prepared to desired concentration with distilled water before use. Parallel control experiments were done to correct possible effects caused by vehicle alone. Preliminary phytochemical screening were carried out using standard procedures. [9]

2.2 Animals

Adult Wistar rats (200 - 230 g) and Swiss albino mice (28 - 34 g) obtained from the Animal Facility Centre, NIPRD, were used for the study. They were kept under standard conditions of 12:12 h light and dark cycle kept in plastic cages and fed with standard diet (Ladokun Feeds Ltd, Ibadan, Nigeria). Experiments were performed based on the "principles of laboratory animals care" NIH Publication No. 85 - 23, revised (1985).

2.3 Analgesic activity

2.3.1 Acetic acid induced writhing test

The method described by Koster *et al* [10] and Freire [11] were used. Thirty mice were divided into 5 groups (n = 6). Group 1 received normal saline (10 ml/kg i.p.), groups 2, 3 and 4 received the extract (50, 100 and 200 mg/kg i.p.) respectively, while group 5 received acetylsalicylic acid, (ASA 100 mg/kg p.o). [12]

Thirty minutes after treatment each mouse was given 10 ml/kg of 0.7% aqueous solution of acetic acid injected intraperitoneally. Each mouse was then placed in a plastic transparent observation cage and number of abdominal constrictions were cumulatively counted from 5-15 min. Results were expressed as % inhibition of analgesia. [13]

2.3.2 Hot - plate test

This study was carried out according to the method of Eddy and Leimback [14], as modified by Hunkaar *et al* [15]. Mice that showed nociceptive responses within 20s when placed on Ugo Basile DS – 37 (Italy) hot plate maintained at 55 ± 0.50°C, were selected. The mice so selected were then grouped into 5 (n = 6), and treated either with saline (10 ml/kg, p.o.), methanolic extract (50, 100 and 200 mg/kg, p.o.) or ASA (100 mg/kg, p.o.). Each mouse was placed gently on the plate and its reaction time noted. Reaction time was taken as the interval between the time the animal was placed on the plate till the moment it began to lick its fore paws or jumps. [16]

2.3.3 Formalin test

The method of Tjolsen *et al* [17] was adopted. Pain was induced in rats with 2.5% formaldehyde, injected into the sub-plantar surface of the rats left hind paw. Pain was induced 30 min following treatment with either saline or methanolic extract (100 mg and 200 mg/kg p.o). Severity of pain was measured as scores [18] for the two distinct phases i.e. 0 – 10 and 15 – 60 min post formalin injection in the following manner; 0, normal weight bearing on the injected paw; 1, light resting of the paw on the floor;

2, elevation of the injected paw; 3, licking, biting and grooming of paw.

2.4 Anti-inflammatory activity

This was evaluated in 24 rats divided into 4 groups (n = 6) using fresh egg-albumin (100 %) as a phlogisic agent [19]. Briefly, 0.1 ml of raw egg albumin was injected into rat left hind paw 30 min after treatment with either saline control, methanolic extract (100 and 200 mg/kg i.p.) or ASA. Oedema was measured in duplicates at an interval of 20 min for a total of 120 min using Letica plethysomometer (LE 7500, Spain) calibrated with 0.1% Triton X - 100 solution. Results were expressed as percentage inhibition of inflammation in the treated groups compared to the saline control.

2.5 Data analysis

Results were all expressed as mean ± SEM. Differences between more than two means were determined by ANOVA followed by Dunnet's test for multiple comparison among groups. Data were considered significant at $p < 0.05$.

3 Results and discussion

The methanolic extract of *Annona senegalensis* root bark gave positive test for saponins, tannins and resins. The extracts caused significant inhibition of acetic acid induced writhing in mice, by 23.29%, 38.93 %, 62.04 % at doses of 50, 100 and 200 mg/kg respectively when compared to the saline control. ASA caused a 89.41% inhibition in the same study (Table 1).

Table 1.
The methanol extract of *A. senegalensis* on acetic acid induced writhing in mice.

Treatment	Dose	Writhing count	Inhibition (%)
Control (saline)	10 ml/kg i.p.	34. 67± 3.52	-
A. senegalensis	50 mg/kg i.p	28.12 ± 3.13	23.29
A. senegalensis	100 mg/kg, i.p	21.17 ± 2.56	38.93
A. senegalensis	200 mg/kg i.p.	13.16 ± 1.25	62.04
ASA	100 mg/kg, p.o.	3.67 ± 0.47	89.41

Value are mean ± SEM (n=6)

Table 2.
Effect of the methanol extract of *A. senegalensis* on hot plate nociception test in mice.

Time	Reaction Time (s)				
(min)	Control (saline) 10 ml/kg	Extract 50 mg/kg	Extract 100 mg/kg	Extract 200 mg/kg	ASA 100 mg/kg
0	9.6 ± 0.68	8.9 ± 0.83	9.1 ± 0.45	10.2 ± 0.04	9.8 ± 0.38
15	9.8 ± 0.35	10.12 ± 0.42	10.27 ± 1.62	10.4 ± 0.35*	12.13 ± 0.64*
30	10.6 ± 0.72	11.02 ± 0.47	11.9 ± 0.91	12.73 ± 0.83 *	23.76 ± 2.11*
45	10.9 ± 1.33	11.34 ± 0.91	11.53 ± 1.30	13.55 ± 1.36*	27.73 ± 1.89*
60	10.8 ± 0.76	11.88 ± 0.32	11.17 ± 0.73	13.97 ± 0.59*	22.26 ± 1.35*
90	11.8 ± 0.69	12.14 ± 0.33	12.47 ± 0.45*	16.47 ± 0.49*	19.17 ± 1.07*
120	12.26 ± 1.70	12.41 ± 0.63	13.77 ± 1.09*	19.17 ± 0.49*	18.13 ± 1.07*

Values expressed as mean ± SEM (n = 6). * ($p<0.05$) control *vs.* treated groups

Table 3
Effect of the methanol extract of *A. senegalensis* on formalin-induced pain in rats

Treatment	Dose	Score of Pain			
		Early phase	% Inhibition	Late phase	% Inhibition
Saline	10 ml/kg	2.4 ± 0.25	-	2.2 ± 0.32	-
A. senegalensis	100 mg/kg	2.2 ± 0.38	8.33	1.2 ± 0.37	45.45
A. senegalensis	200 mg/kg	2.0 ± 0.32	16.67	0.8 ± 0.39	63.64

Table 4
Effect of the methanol extract of *A. senegalensis* on egg albumin-induced oedema in rats

Treatment	Dose	% Inhibition of Oedema (min.)			
		30	60	90	120
Saline	10 ml/kg	-	-	-	-
A. senegalensis	100 mg/kg	18.18	30.90	27.78	19.04
A. senegalensis	200 mg/kg	27.27	43.63	33.33	34.92
ASA	100 mg/kg	40.00	45.45	29.62	44.44

The extract may therefore possess analgesic properties. Acetic acid induced writhing is a sensitive method of screening anti-nociceptive effect of compounds.

It causes an increase in peritoneal fluid concentration of PGE_2 and $PGF_2\alpha$ [20, 21]. The activity of the extract may therefore be exerted via inhibition of prostaglandin synthesis. The extract also increased the latency of pain reaction in mice following hot plate stimulation thereby supporting the peripheral mechanisms of pain inhibitions (Table 2).

Formalin produces biphasic pain reaction; agents acting primarily *via* central mechanisms inhibit both phases while peripherally acting agents inhibit the late phase [22]. The extract significantly ($p < 0.05$) inhibited only the late phase of the formalin induced nociception (45.45% and 63.64% for 100 and 200 mg/kg respectively; Table 3). This suggests that the action of the extract in relieving pain is primarily peripheral.

It is therefore clear from the foregoing that the root extract of *Annona senegalensis* may have strong analgesic properties, which are likely to be mediated peripherally. Paw oedema induced by phlogistic agents is a widely accepted model for the evaluation of anti-inflammatory effect of drugs [22, 23]. The methanolic extract of *Annona senegalensis* showed a significant suppression of rat paw oedema in a dose depended manner (Table 4).

Such ability to significantly ($p < 0.05$) reduce the increase in oedema induced by egg albumin is an indication of an anti-inflammatory activity [19]. We conclude thus that the root extract of *Annona senegalensis* has anti-inflammatory and anti-nociceptive properties that may justify the traditional use of the plant in Nigeria for the treatment of rheumatism and other pain related diseases [7].

Bio-activity guided isolation of the active principle is on going in our laboratory with the

aim of isolating the active principle mediating the observed biological activity.

4. Acknowledgements

The authors are grateful to the authorities of the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, for the use of their equipments; Hauwa Abdullahi and Sunday Dzarma for their technical assistance and Charles Balogun for preparing the manuscript.

References

1. Dalziel JM. (1937) *The useful plants of West Tropical Africa.* The crown agent for the Colonies, London; Oxford: 2-3.

2. Burkill, HM. (1985) *The useful plants of West Africa*. Royal Botanical Gardens, Kew, 103-105.

3. Durodola JI. (1975) *Planta Med.* 28: 359.

4. Durodola JI. (1975 *Planta Med.* 28: 32.

5. Adesogan EF, Durodola JI. (1976) *Phytochemistry* 15: 1311-1312.

6. Ekpendu TOE, Obande OD, Anyogo PO, Attah AD. (1998) *J. Pharm. Res. Dev.* 3 : 37-46.

7. Audu JA. (1989) *The Nigerian Field* 54: 157-168.

8. Akendegue B, Ngou-Milana, E, Laurens A, Hocquemiller R. (1999) *Parasite* 6: 3-8.

9. Trease GE, Evans WC. (1996) *Textbook of Pharmacognosy.* Brailliere Tindall Press: London; 565-566.

10. Koster R, Anderson M, Beer EJ. (1959) *Fed. proceedings* 18 : 412.

11. Freire SMF, Emim JAS, Lapa AJ, Souccar C and Torres LMB (1993) *Phytother. Res.* 7: 408-414.

12. Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AKN. (2000) *J. Ethnopharmacol.* 72 : 265-268.

13. Adzu B, Amos S, Wambebe C, Gamaniel K. (2001) *Fitoterapia* 72: 344-350.

14. Eddy NB, Leimback D. (1953) *J. Pharmacol. Exp. Ther.* 3: 385-393.

15. Hunkaar S, Berge OG, Hole K. (1986) *Behav Brain Res.* 21: 101-108.

16. AL-Ghamdi MS. (2001) *J. Ethnopharmacol.* 76 : 45-48.

17. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. (1992) *Pain* 4, 161-174.

18. Dubuisson D, Dennis SG. (1977) *Pain* 51: 5-17.

19. Akah PA, Nwambie AE. (1994) *J. Ethnopharmacol.* 42: 179-184.

20. Deraedt R, Jougney S, Delevalcee F, Falhout M. (1980) *Eur. J. Pharmacol.* 79: 125-134.

21. Bentley GA, Newton SH, Starr J (1983) *Br. J. Pharmacol.* 79: 125-134.

22. Shibata M. Ohkubo T, Takahashi H, Inoki R. (1989) *Pain* 38: 347-352.

23. Castro J, Sasame H, Sussman H, Bullette P. (1968) *Life Science* 7: 129- 136.