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Preliminary evaluation of antinociceptive and antipyretic activities of aqueous extract of the leaves of *Xylopia parviflora*

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Abstract

The purpose of this study was to evaluate the antinociceptive and antipyretic activities of orally administered aqueous extract of the leaves of *Xylopia parviflora* (Benth) using the acetic acid, formalin and the hot plate-induced pain and the antipyretic tests. These were carried out in albino mice or rats of both sexes using standard procedures. The results obtained indicated that the aqueous extract of the leaves of *Xylopia parviflora* significantly ($p<0.05$) reduced pain induced by administration of 0.1ml/kg acetic acid (0.6%) in mice at oral doses of 140 and 210mg/kg. Similarly, the extract at doses of 140 and 210mg/kg significantly ($P<0.05$) reduced Formalin – induced nociception in both early and late phases in the rat model compared to control. However the reduction was not significant ($P>0.05$) at a lower dose of 70mg/kg of the extract in both phases. Results of the hot-plate test in the mice model showed that the extract significantly increased the reaction time in all groups administered the extract. It was also observed that the extract possessed an antipyretic effect in the rat model ($P<0.05$). These effects were observed to be dose-dependent. Based on these results it can be reported that the aqueous extract of the leaves of *Xylopia parviflora* possesses a dose -dependent analgesic and antipyretic activities. These findings may justify its traditional use in febrile disease conditions such as malaria and typhoid fever.

Keywords: *Xylopia parviflora*, Antinociception, Antipyretic, Traditional medicine.

Introduction

The use of medicinal plants by traditional healers based on mere observation of non-specific symptoms or poor description of disease conditions by patients or their relations is a serious medical concern. In spite of this, the practice seems to have popularised some medicinal plants for indication in some disease conditions.

Xylopia parviflora (Benth) (Family Annonaceae), is one of such medicinal plants. It grows freely in the forest and mountainous regions of Nigeria (Burkill 1985). It is claimed to be effective in its crude form in the treatment of malaria and other febrile conditions such as typhoid fever (Azija, 1998). Documented pharmacological profiles of *Xylopia parviflora* seem to be lacking.

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However, the crude extract of *Xylopia parviflora* has been found to posses inhibitory effect on contractile responses of Guinea pig ileum to ACh, KCl and histamine (Bukar 2001) and antimalarial effect in mice effected with *Plasmodium yoelli* (Bukar *et al.*, 2002-unpublished report).

Though, fever is a prominent symptom of malaria, it is also a non-specific symptom of several diseases (Harinasuta and Bunag 1988, Peters 1992). The use of analgesic/antipyretic agents in febrile conditions such as malaria is rational and common, but it is still an inadequate treatment. However, in treatment of malaria, some individuals still use analgesic/ antipyretic agents to treat symptoms such as pyrexia and pain without actually treating the cause of the disease (Peters 1992, Bukar 2001, unpublished report). While the anti malarial property of *Xylopia parviflora* is currently being scientifically evaluated the present study aims at concurrently evaluating possible analgesic /antipyretic activity of *Xylopia parviflora.*

Experimental

Animals. Albino mice (18-24g) and rats (150-180g) of both sexes (three weeks old) were used in this study. They were obtained from the Animal House, University of Jos. They were isolated and fed freely with standard pellets and water before the beginning of the experiment.

Drugs/ chemicals. Normal saline 0.9% w/v, Morphine (Antigen Ltd), Acetic acid, 0.6% (M &B), Formalin 1% (B&B), Brewer's yeast (ICH), Acetylsalicylic acid (Sigma), indomethacin. (Sigma).

Collection of plant materials. The leaves of *Xylopia parviflora* were collected in Jos by a herbalist, (Mrs.) Azamya Sule of Babale village, near Jos in the month of October. The leaves were identified and authenticated by Dr. Hussein of the Department of Botany, University of Jos, and a voucher specimen was deposited at their herbarium.

Preparation of extract. The leaves were washed, dried under the shade and powdered. 1000g of the powder was weighed and soaked in 250ml of distilled water and extraction was carried out continuously in a soxhlet extractor for 72 hours. The extract was evaporated to dryness and the yield was found to be 7.46%w/w of dry weight of sample. The extract was preserved at – 5^0C until the beginning of the experiment. The doses of the extract to be used were determined in an earlier pilot study using the acute toxicity test (LD_{50}) results in mice.

Screening for antinociceptive activity

(a) Acetic acid test in mice. The method of Koster *et al.* (1959) was adopted. Mice weighing 18-24g were divided into five groups of six animals each. The extract was administered in different doses of 70, 140 and 210mg / kg by oral gavages while morphine at a dose of 1mg/kg was administered by intraperitoneal route (IP). The animals were then allowed to stay for thirty minutes after which acetic acid was intraperitoneally administered at a dose of 0.1ml/10g. Abdominal contortions (writhes) were observed and counted for a period of 20 minutes. Animals of control group were similarly pre-treated with normal saline 0.9% w/v (1ml/100g) intraperitoneally before the administration of acetic acid.

(b) Formalin-induced nociception in rats. The method of Dubuisson *et al.* (1977) as described by Elisabetsky *et al.* (1995) was used. The rats (150- 180g) were divided into five groups of five animals each. They were placed into glass chambers for 30 minutes. Reference drug (morphine1mg /kg, IP) and extract at oral doses of 70, 140 and 210mg / kg were administered accordingly and each animal was then allowed to stay for 20 minutes. Thereafter, formalin at a dose of 0.05 ml/100g was administered by injection into the plantar surface of one hind paw of

each animal. Control groups were similarly administered normal saline(1ml/100g ip) 20 min before the administration of formalin. Animals were subsequently placed in glass chambers and carefully observed for licking of the injected hind paw. The time (in seconds) each animal spent licking the injected paw was observed and recorded for two periods of 0-5min and 20 – 25min in post formalin administration.

(c) Hot plate test in mice. Albino mice (18-24g) were divided into five groups of six animals each. They were then individually placed on a hot plate adapted to a water bath at a temperature of 50 ± 0.5^0 C as described by Jacob & Ramabadran (1978). Reaction times to licking of the hind paw or jumping off the plate of 5 minutes before, and 30, 60 and 90 minutes after oral administration of the extract at oral doses of 70, 140 and 210 mg / kg were recorded for each group. Morphine (ip) was used as a reference drug while normal saline (ip) was used in control groups. A cut off time of 60 sec was used to avoid tissue damage.

(d) Antipyretic test in rats. The method described by Williamson *et al.* (1996) was used. Forty (40) rats weighing between 158 – 172g were screened by first recording their rectal temperatures (before administration of brewers' yeast) by means of a clinical thermometer carefully inserted into the rectal cavity for two minutes. Induction of pyrexia was then carried out by subcutaneous (sc) administration of 1mg/kg of 20% Brewers yeast suspension to each animal. The animals were then starved for 18hrs after which their rectal temperatures were recorded (post brewers' yeast administration). Twenty five of them that had increased temperature of 1.0^0C and above were selected and divided into five groups for treatment with the extract at 70, 140 and 210 mg / kg oral doses, and acetylsalicylic acid (Aspirin) at 16mg / kg, po. Normal saline (1ml/kg) was administered (ip) to animals in the control group. Their temperatures were determined and recorded 5 minutes before and 1h, 2h, 3h and 4h after administration of the extract, aspirin and normal saline.

Statistical analysis. Results obtained were subjected to statistical analysis using the ANOVA test at P = 0.05 significance level.

Results and Discussion

The results indicate that the mean contortion following acetic acid reduced to 31.2 ± 4.8, 28.8 ± 4.5 and 25.2 ± 3.0 when the doses of the extract were increased from 70, 140 and 210 mg / kg respectively (Table 1). The mean contortion for all the doses used were significantly lower compared to that of the control, 54.2 ± 4.4 ($P< 0.05$). The effect was dose-dependent.

The extract at oral doses of 140 and 210 mg / kg reduced the time each animal spent licking the injected hind paw to 102.2 ± 8.90 sec and 93.6 ± 17.78 sec respectively in the first phase compared to that of the control, 119.6 ± 13.4 sec and this further reduced to 71.2 ± 12.19 and 60.12 ± 6.83 respectively in the second phase. These results were significantly lower than those of the control in both phases ($P< 0.05$). However, at a dose of 70 mg /kg the extract reduced the licking time to 108.2 ± 11.37 sec in first phase and 96.6 ± 7.99 in the second phase. These reductions are not significantly different compared to those of the control ($P> 0.05$).

Table 3 present the results of the hot plate test in mice. The results indicate that the mean reaction time was highest at 60 min after administration of the extract with all the doses used compared to those of 30 and 60 min. The reaction time increased with corresponding increase in the doses of the extract. These increases were significantly higher compared to that of the control ($P< 0.05$). The effect of the extract on the hot-plate test was therefore dose and time-dependent.

The effect of the extract on the antipyretic test are presented in Table 4. The results show that the extract significantly reduced Brewers' yeast induced hyperthermia with all the doses used at 4 hrs after oral administration. The reduction in the induced hyperthermia was also observed to be dose – dependent in all the time periods of 1 – 4 hrs. However, the reduction in hyperthermia was not significant (P> 0.05) at dose of 70 and 140 mg / kg within 1 – 3 hours after administration but significant with 210 mg / kg as compared to the control (P< 0.05).

The antinociceptive and antipyretic activities of the aqueous extract of the leaves of *Xylopia parviflora* were evaluated in mice or rats using standard methods.

Table 1. Effect of *Xylopia parviflora* on acetic acid-induced pain in mice.

Group	Treatment	Mean (± SD, n=6) no. of contortions in 20 min	% Inhibition
I	Normal saline (1ml/100g)	54.2 ± 4.4	-
II	Morphine (1.0mg/kg)	24.3 ± 2.9*	55.2
III	Extract (70mg/kg)	31.2± 4.8*	42.4
IV	Extract (140mg/kg)	28.8 ± 4.5*	46.9
V	Extract (210mg /kg)	25.2± 3.0*	53.5

F (4,20) = 11.82, *= P< 0.05

Table 2. Effect of *Xylopia parviflora* on formalin induced nociception.

Group	Treatment	Time spent licking (sec) (0-5min)	% Inhibition	Time spent licking (20-25min)	% Inhibition
I	Normal saline (1ml/100g)	119.6 ± 13.14	-	106.8 ±10.40	-
II	Morphine (1mg/kg)	82.8 ± 15.51*	22.41	63.4 ± 11.30*	40.64
III	Extract (70mg/kg)	108.2 ± 11.37^{+}	5.35	96.6 ± 7.99^{+}	9.55
IV	Extract (140mg/kg)	102.2 ± 8.90*	14.55	71.2 ± 12.19*	33.33
V	Extract (210mg/kg)	93.6 ±17.78*	17.56	60.2 ± 6.83*	43.63

*= P<0.05 anova vs control. $^{+}$P> 0.05

Table 3. Effect of *X. parviflora* on the hot plate reaction time in mice.

Group	Treatment	- 5 min.	30 min.	60 min.	90 min.
		Reaction time (mean ± SD; n=6)			
I	Normal saline (1ml/100g)	11.6.± 2.5	11.5± 3.4	11.4±2.0	11.5±1.8
II	Morphine (1mg/kg)	11.5 ± 2.2	20.6±2.9	29.3±5.4.	34.6±5.0
III	Extract (70mg/kg)	11.5±1.6	13.1±1.8	13.8±1.9	14.4±1.0
IV	Extract (140mg/kg)	11.3±1.5	13.7±2.3	19.8±1.6	15.3±2.7
V	Extract (210mg/kg)	11.6±1.3	16.4±1.2	32.3±1.3	22.1±1.5

F (3,12) = 5.15, P<0.05, Anova vs control

Table 4. Antipyretic effect of *X. parviflora* on brewers yeast induced hyperthermia in rats.

Group	Treatment	Before	18h after	-5 min	1 h	2 h	3 h	4 h
		Rectal temperatures (°C; mean ± SD; n=5)						
I	Normal saline (1ml/100g)	38.7±0.21	40.6±0.41	40.8±0.36	40.8±0.27	40.6±0.24	40.5±0.26	40.2±0.15
II	Morphine (1mg/kg)	38.9±0.18	40.3±0.34	40.5±0.25	39. ±0.34	39.2±0.12*	38.8±0.14*	38.7±0.11*
III	Extract (70mg/kg)	38.5±0.22	40.8±0.32	41.3±0.38	40.4±0.48	40.3±0.41^{+}	40.2±0.41^{+}	39.4±0.23*
IV	Extract (140mg/kg)	38.9±0.15	40.1±0.29	41.1±0.31	40.5±0.37	39.8±0.27^{+}	39.6±0.30^{+}	38.8±0.21*
V	Extract (210mg/kg)	38.6±0.24	40.5±0.44	40.7±0.42	40.0±0.26	39.5±0.33*	39.2±0.17*	38.8±0.16*

F (4,24) = 7.82, Anova * = P<0.05 + = P>0.05

The results of the acetic acid-induced pain showed that the extract at orally administered doses of 70, 140 and 210mg / kg reduced the mean number of contortion or writhes in mice to 31.2 ± 4.8, 28.8 ± 4.5 and 25.2 ± 3.0 respectively compared to that of the control (54. 2 ± 4.4) which received normal saline 1ml / 100g. These reductions correspond to 42.4, 46.9 and 53.5% inhibition of the acetic acid-induced nociception in the model at the respective doses of 70, 140 and 210 mg / kg compared to the control. These results show that the extract significantly reduced the acetic acid – induced nocicention in a dose dependent manner ($P<0.05$). Mice that received morphine intraperitoneally at a dose of 1 mg / kg had the highest reduction in the mean number of contortions which was 24.32 ± 2.87 corresponding to 55.2% inhibition of nociception compared to the control. The acetic acid induced nociception, though not very selective (Loux *et al.,* 1978), is more sensitive to opioid analgesics (Bentley 1983). Notwithstanding, the results revealed that the extract of *Xylopia parviflora* may possess an antinociceptive property since all analgesics inhibit abdominal cramp (Loux *et al.,* 1978).

The extract administered at oral doses of 140 and 210 mg / kg reduced the mean time the rats spent licking their injected hind paw to 102.2 ± 8.90 seconds and 93.6 ± 17.78 seconds respectively in the first phase of the formalin test compared to that of the control of 119.6 ± 13. 14 seconds. A similar finding was observed during the second phase which showed that the licking time for the doses of 140 and 210 mg / kg was further reduced to 71.2 ± 12.19 seconds and 60.2 ± 6.83 seconds respectively compared to that of the control of 106.8 ± 10.40 seconds. The decrease in the licking time corresponding to the dose of 140 and 210 mg in both phases were significantly different compared to those of the control group ($P<0.05$). However, at a lower dose of 70 mg / kg, the reduction in the licking time was not significant in both phases compared to control ($P> 0.05$). Consequently the extract was found to possess significant antinociceptive effect at oral doses of 140 and 210 mg / kg but not so at a dose of 70 mg / kg. The formalin test essentially involves the induction of minor tissue injuries. The pain due to such injuries has been classified as either inflammatory which occur during the second phase and is mainly due to sensitization or non-inflammatory which occur in the early phase and is mainly due to direct stimulation of nociceptors (Le Bars *et al.,* 2001). Opioid, analgesics are reported as being effective in abolishing nociception in the second phase while the non-steroidal anti inflammatory agents suppress only the second phase (Abbot *et al.,* 1986, Jourdan *et al.,* 1997). Against this background the exact mechanism for the inhibition of nociception by the extract remains to be established. This is moreso that most opioid analgesics act both centrally and peripherally (Tyers 1980) and also many other agents are capable of reducing the formalin-induced nociception in rats (Coderre *et al.,* 1984, Tjolsen *et al.,* 1992).

Results obtained with the hotplate test revealed that the extract of the leaves of *Xylopia parviflora* at oral doses of 70, 140 and 120 mg/ kg significantly increased the reaction time of the animals compared to control especially at 60 and 90 min after the administration of the extract ($P<0.05$). This effect was observed to be dose – dependent. The hot plate test is essentially employed in screening for centrally mediated analgesic activity (Le Bars *et al.*, 2001). Consequently, the hot plate test is mainly affected by opioid analgesics, but can also be affected by other analgesics such as acetylsalicylic acid especially when the jumping reaction is said to be the only monitor (Ankier 1974, Carter 1991). In spite of this observation, and from

the results, it can be said that the extract may possess a central analgesic activity. The antipyretic test revealed that the aqueous extract of the leaves of *Xylopia parviflora* significantly reduced Brewer's yeast-induced hyperthermia at all the oral doses of 70, 140 and 210 mg / kg after 4 hrs of administration ($P<0.05$). This effect was also observed to be dose and time-dependent.

The observations reported in this study suggest that the aqueous extract of the leaves of *Xylopia parviflora* possesses anti nociceptive and antipyretic activities. This probably justifies the traditional use of *Xylopia parviflora* for the treatment of febrile disease conditions such as malaria and typhoid fever (Azija, 1988).

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