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Full Length Research Paper

Pharmacological justification for the ethnomedicinal use of *Amblygonocarpus andongensis* stem bark in pain relief

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Amblygonocarpus andongensis **(family: Mimosaceae) is ethnomedicinally used in Northern Nigeria for the relief of pain. The methanolic extract of the plant stem bark was evaluated for anti-nociceptive activity using acetic acid-induced writhing model and formalin test in mice. Anti-inflammatory property was tested on egg albumin-induced oedema in rats while agar dilution method was used for antimicrobial effect. The acute toxicity effect (LD_{50}) was also determined via intraperitoneal route. The results showed the LD_{50} value to be 547.7 mg/kg i.p. There was a significant ($P < 0.05$) dose-dependent reduction of acetic acid-induced pain at 50, 100, 200 mg/kg i.p. The extract at the same doses significantly ($P < 0.05$) inhibited pains in both early and late phases of the formalin test. However, the extract showed neither anti-inflammatory nor anti-microbial effects. The results corroborate the folkloric use of the plant.**

Key words: *Amblygonocarpus andongensis*, anti-nociception; anti-inflammation, acute toxicity, antimicrobial effect.

INTRODUCTION

Amblygonocarpus andongensis (Mimosaceae) is widely spread in tropical Africa, mostly in the Savannah areas. It is a tree usually 30–40 feet high, but reaching 60 feet and 5 feet girth in moist areas with a wide flat open crown. The bole is clean and straight. The bark is grey to brown, rough, flaking off in irregular patches leaving reddish scars; slash dark brown, crumbly lighter beneath (Keay et al., 1964). The leaves are mostly at the ends of the erect twig and entirely glabrous with leaflets pale-blue-green. The flowers are white or yellowish and sweetly scented. The fruits are dark brown, 4–5 inches long by about 1 inch across, hanging on thick stalks 2–3 inches long (Keay et al., 1964). From the ethnobotanical knowledge of traditional medicine in Adamawa State, Northern Nigeria, an infusion of the bark is taken to relieve pains in the breast. The objective of this study was to establish the scientific basis on which this claim is made.

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MATERIALS AND METHODS

Plant collection and extraction

The stem bark was collected from Adamawa State, Northern Nigeria in July 2001. The identification of the plant was carried out at the Forestry Research Institute, Ibadan, Nigeria. The stem bark

of the plant was air-dried and grounded to powder in a mortar with the pestle. 375 g of the powdered material was cold macerated in 1 L of methanol at 25°C for 24 h. The sample was then suction-filtered through Whatman #1 filter paper. The filtrate was then evaporated to near dryness with a rotary evaporator at 90–100°C to give a dark brownish crude extract. The crude extract was brought to complete dryness over water bath and the yield was 1.8% (w/w).

Animals

Swiss Albino mice (22.1–28.2 g) and Wistar rats (208.3–220.0 g) of both sexes were used for the studies. The animals were bred in the Animal Facility Centre (AFC), Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The animals were maintained under normal environmental conditions. They were fed *ad libitum* with standard feed (Ladokun Feeds PLC, Ibadan, Nigeria) and water from the Abuja Municipal Area Council except when starvation was otherwise needed during the investigations.

Chemicals

Glacial acetic acid (Searle, Essex, England), formaldehyde 40% w/v (M & B, England), triton X-100 (GE Healthcare, UK), nutrient agar (BBL, USA), nutrient broth (BBL, USA) were used for the studies.

Test organisms

The microorganisms used in this study include *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 13709), *Escherichia coli* (ATCC 9637), *Candida albicans* (ATCC 10231), *Klebsiella pneumoniae, Proteus mirabilis*, *Bacillus subtilis* and *Salmonella typhi* all of which were clinically isolated, standardized and stored by the Department of Microbiology, Human Virology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

Acute toxicity study

This involves the estimation of the median lethal dose (LD_{50}), which is the dose that will kill 50% of the animal population within 24 h post treatment with the extract. The method of Lorke (1983) was modified and used. Swiss Albino mice were starved of feed but allowed access to water 24 h prior to the study and were then grouped (four mice per group). They were treated intraperitoneally with different doses of the extract (500, 600, 700, 800, 900, 1000, 1500 mg/kg). The animals were then observed for 24 h for any behavioural effects such as nervousness, excitement, dullness, in-coordination or even death. The LD_{50} was estimated from the geometric mean of the dose that caused 100% mortality and the dose, which caused no lethality at all.

Acetic acid-induced writhing in mice

The study was carried out according to the method of Siegmund et al. (1957) as modified by Koster et al. (1959). The mice were grouped into four (of five mice each). Three different groups of mice were pre-treated with the extract at doses of 50, 100 and 200 mg/kg i.p., respectively. The fourth group of mice received normal saline (10 ml/kg i.p.). 10 ml/kg of 0.75 % glacial acetic acid was then administered intraperitoneally to every mouse 30, 60, 90 and 120 min post extract/normal saline treatment. Each mouse was placed in a transparent observation box. 5 min post acetic acid administration, the number of abdominal constrictions (writhes) made within the next 5 min by every mouse was counted using a manual table counter. The percentage of the writhes for every time interval (30, 60, 90, 120 min) was calculated as follows:

Writhes (%) = (Test mean for every interval/Control mean for the same interval) x 100

Also, the percentage dose-effect of the extract on the abdominal constriction was calculated thus;

Dose-effect (%) = (Total abdominal constrictions per dose/Total abdominal constrictions for control group) x 100

The difference between 100 and the calculated percent abdominal constriction is considered to be the dose-pain inhibition effect (%) of the extract. The values were all compared statistically with the normal saline control group.

Formalin test

The modified method of Dubuisson and Dennis (1977) was adopted for this study. Four groups of mice (of five mice each) was given the extract (50, 100, 200 mg/kg i.p.) and normal saline (10 ml/kg i.p.), respectively. 30 min post treatment, 50 µl of 2.5% formalin was injected into the sub-plantar surface of the left hind paw of every mouse. The severity of pain exhibited by every mouse was observed and rated as scores:

0 = mice walked or stood firmly on the injected paw
1 = partially elevated the paw from the floor
2 = elevated the paw without contact with the floor
3 = licked, bit or shook the paw

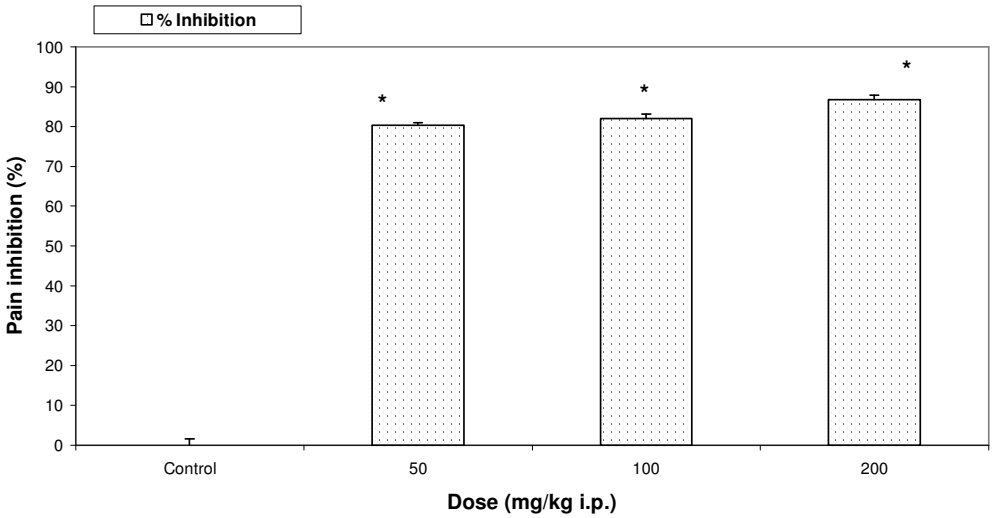
These observations were recorded every 2 min for the first 10 min (early phase) and at every 5 min between the 10 and 60 min interval (late phase).

Anti-inflammatory study

The extract was tested for its ability to inhibit or suppress inflammation using fresh egg albumin-induced oedema model in rats. This was in accordance with the technique of Winter et al. (1962) as modified by Akah and Nwambie (1994). Both male and female rats used for the investigation were fasted overnight. They were deprived of water during the experiment to ensure uniform hydration and to minimize variability in edematous response (Winter et al., 1963). The rats were separated into groups (of five rats each) and were treated with the extract (25, 50, 100 mg/kg i.p.) and normal saline (10 ml/kg i.p.), respectively. 30 min post treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin (phlogistic agent) into the sub-plantar surface of the right hind paw of the rats. The measurement of the paw volume (cm^3) was done on the principle of volume displacement using LETICA Digital Plethysmometer (LE 7500) which was earlier calibrated with 0.1 % Triton X-100. The readings were taken before and at 20 min intervals after the injection of egg albumin for a period of 2 h. The oedema at every interval was calculated in relation to the paw volume before the injection of the phlogogen.

Antimicrobial activity

The agar dilution method of Jonas et al. (1989) was employed to determine the antimicrobial activity. Nutrient agar was prepared according to the manufacturer's instruction while the extract (4 mg)



* P < 0.05

Figure 1. The dose-pain inhibition effects of *A. andongensis* stem bark on glacial acetic acid-induced abdominal constriction in mice.

was dissolved in 1 ml of sterile distilled water to give an extract concentration of 4 mg/ml. The later solution was then diluted with 19 ml of the prepared nutrient agar to provide a required concentration of 2000 µg/ml. Each of the test organisms was cultured overnight in nutrient broth to approximately 5 X 10^7 to 9 X 10^7 cfu/ml. A 1:20 dilution of the later was then made in normal saline for inoculation. Surface streaking of each innoculum was done on the extract-containing nutrient agar plate using a wire loop with a capacity of 0.002 ml. The organisms were also streaked on plates containing only nutrient agar (organism viability control) and on plates containing nutrient agar and sterile distilled water, which also served as the control. The plates were kept overnight in an incubator at 37°C. They were then observed for microbial growth inhibition.

Statistical analysis

All the results were expressed as mean ± SEM. The significance of difference between the control and treated groups were determined using two-way analysis of variance (ANOVA), followed by Student t-test. P-values < 0.05 were considered to be statistically significant.

RESULTS

Acute toxicity study

All the mice became dull within 10–15 min post extract administration. However, only mice treated with doses ≥ 600 mg/kg i.p died within 24 h of treatment. The median lethal dose (LD_{50}) was estimated to be 547.7 mg/kg i.p.

Acetic acid-induced writhing in mice

The stem bark extract of *A. andongensis* (50, 100, 200 mg/kg i.p.) significantly (P < 0.05) reduced the degree of acetic acid-induced abdominal constrictions in mice at all doses and at every interval. The dose-pain reduction effect of the extract was calculated to be 80.3, 82.0 and 86.7% for the 50, 100 and 200 mg/kg i.p groups, respectively. This shows a dose-dependent reduction of acetic acid-induced pain (Figure 1). The study revealed anti-nociceptive effect of the extract from the period of 30 to 120 min. However, the maximal anti-nociceptive effects for 100 and 200 mg/kg i.p doses were seen at the 60 min having writhing of 5.22 and 0.00 % (i.e. percent inhibition of writhes of 94.8 and 100.0 %), respectively (Figure 2). The values were all significantly (P < 0.05) different from the control.

Formalin test

The study revealed that the stem bark extract of *A. andongensis* had a significant (P < 0.05) activity in both the early (0–10 min) and late (15–60 min) phases of the formalin test. In the early phase, the activities were seen to be higher with decreasing doses. In this phase, the doses of 200, 100 and 50 mg/kg i.p showed a mean severity of pain of 4.8 ± 2.9, 4.3 ± 0.9 and 2.5 ± 1.0, respectively. In the late phase however, the activity was least with the lowest dose of 50 mg/kg showing mean severity of pain of 12.8 ± 5.4 as against 7.8 ± 2.9 and 8.3 ± 2.3 for 100 and 200 mg/kg i.p doses, respectively. All the values were significant (P < 0.05) compared with the control (Table 1).

Anti-inflammatory study

The stem barks extract of *A. andongensis* (25, 50, 100 mg/kg i.p.) did not suppress or inhibit fresh egg albumin-

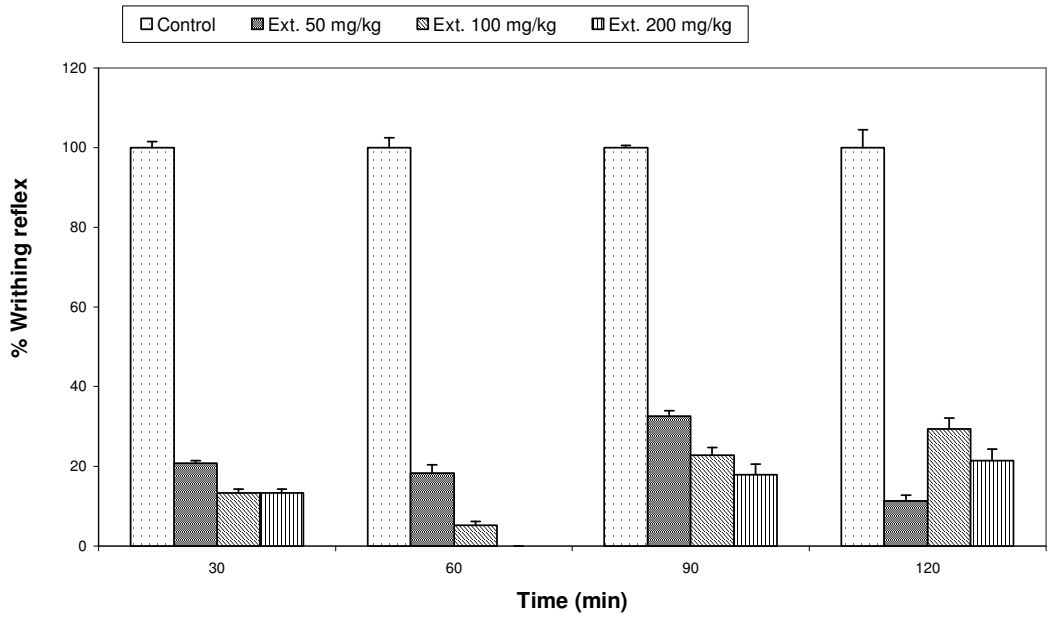


Figure 2. The time effect of methanolic extract of A. andongensis stem bark on glacial acetic acid-induced abdominal constriction in mice.

Table 1. Effect of methanolic extract of *A. andongensis* stem bark on early and late phases of formalin-induced pain in mice.

Treatment Group (n = 5)	Early Phase		Late Phase	
	Score of pain ± SEM	% Inhibition	Score of pain ± SEM	% Inhibition
Normal saline (10 ml/kg i.p.)	11.00 ± 1.0	-	18.75 ± 3.2	-
A. andongensis **(Mg/kg i.p.)**				
50	2.50 ± 1.0***	77.27	12.75 ± 5.4	32.00**
100	4.25 ± 0.9***	61.36	7.75 ± 2.9	58.67*
200	4.75 ± 2.9*	56.82	8.25 ± 2.3	56.00***

Student t-test, *P < 0.05, **P < 0.01, ***P < 0.005 vs normal saline group.

induced inflammation in rats (P > 0.05). The statistical comparison was done with the normal saline treated rats (Figure 3).

Antimicrobial activity

The methanolic extract (2000 µg/ml) did not show antimicrobial activity against in all the test organisms used in the study.

DISCUSSION

The present study revealed some of the pharmacological basis for the ethnomedicinal use of stem bark of *A. andongensis* in pain relief. Acetic acid-induced abdominal constriction model adopted for anti-nociceptive study is a sensitive method in detecting analgesic effect of medicinal agents. It is able to detect anti-nociceptive effect of compounds/dose levels that may be inactive in other methods like the tail-flick test (Collier et al., 1968; Bentley et al., 1981). The mechanism for the abdominal constriction is postulated to partly involve local peritoneal receptors (Bentley et al., 1983) caused by peritoneal fluid concentration of PGE_2 and $PGF_2\alpha$ (Deraedt et al., 1980). The extract showed a significant dose-dependent reduction in the number of acetic acid-induced writhes in mice. This probably means that the extract is able to reduce the receptor sensitivity to the chemically (acetic acid)-induced pain in a dose-dependent manner. This therefore shows anti-nociceptive activity.

This result was further corroborated by the formalin test results. According to Dubuisson and Dennis (1977) and Tjølsen et al. (1992), the nociception induced by formalin occurs in two distinct phases. The early phase represents the phasic pain while the late phase represents the tonic pain. The first phase ensues immediately after formalin injection and continues for 5 min, after which nociception

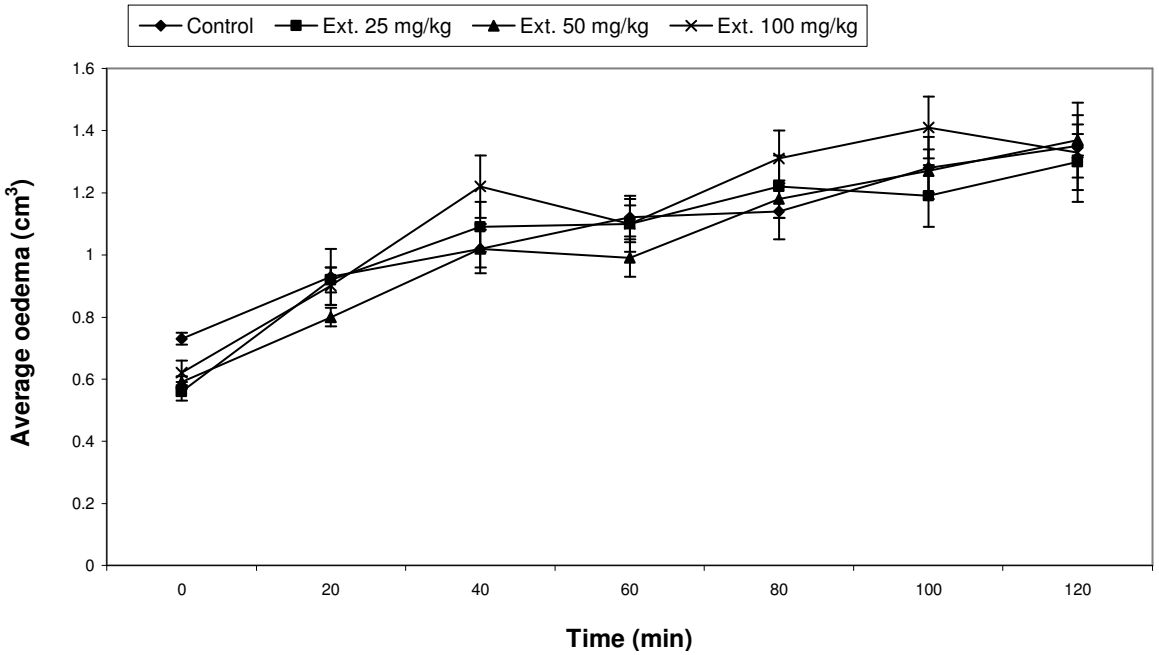


Figure 3. Effect of methanolic extract of A. andongensis stem bark on egg albumin-induced paw oedema in rats.

appears to diminish. In the second phase, nociception returns to high levels beginning 15–20 min after formalin injection and continuing for ~ 60 min. The first phase is believed to be a direct result of stimulation of nociceptors in the paw while the second phase may reflect the inflammation process and at least to some degree, the sensitization of central nociceptive neurons (Coderre et al., 1990; Coderre and Melzack, 1992).

The present study revealed that the extract showed activity on both the early and late phase of nociception. This did not only support the anti-nociceptive activity of the extract but also depicted its possible mechanisms of action. Tjølsen et al. (1992) reported that formalin test method is useful for elucidating the mechanism of pain and analgesia. Drugs which act mainly centrally such as narcotics, inhibit both phases of formalin-induced pain, while drugs such as aspirin, hydrocortisone and dexamethasone which are primarily peripherally acting, only inhibit the late phase (Chen et al., 1995; Elisabetsky et al., 1995; Santos et al., 1995). The activity of the extract on both early and late phases of pain therefore suggests the possible involvement of the central mechanism in the pain inhibition. Also, the late phase of formalin test involves peripheral inflammatory process and since the extract was able to inhibit this phase involving inflammation, it might also mean an involvement of the peripheral mechanism in anti-nociceptive effect. However, the later assumption of involvement of the peripheral mechanism may not be true since anti-inflammatory study carried out showed that the extract at the tested doses of 25, 50, 100 mg/kg i.p did not reduce egg albumin-induced inflammation in rats. This method is an *in vivo* model of inflammation used to screen agents for acute inflammatory effect (Akah et al., 1993; Akah and Nwambie, 1994; Amos et al., 2002).

Some drugs are known to have clinically effective analgesic and anti-inflammatory properties. This is well documented for various non-steroidal anti-inflammatory drugs (NSAIDs) especially with salicylates and their congeners (Reuse, 1978; Beuoist and Misse, 1979; Famaey, 1983). Others are potent anti-inflammatory agents but lack or have only weak analgesic properties; such includes phenylbutazone (Insel, 1996). Some are however effective analgesics, but lack significant anti-inflammatory properties, e.g. phenacetin, acetaminophen (Insel, 1996). The extract therefore belongs to the later classification since it showed anti-nociceptive activity and no anti-inflammatory effect.

The intraperitoneal median lethal dose of the extract estimated to be 547.7 mg/kg probably suggests that the extract may not have a wide safety margin. Lorke (1983) considered LD_{50} values > 1000 mg/kg body weight as being safe. This therefore means that care needs to be taken on the frequency and dose of intake of the stem bark infusion. It is possible that cumulative toxic effects may occur if the extract is taken over time. Some reports have shown the need for sub-chronic data in the prediction of hazards of long term, low dose exposure to a particular compound (McNamara, 1976). Also worth noting is the dullness observed in the treated mice in the acute toxicity study. This probably confirms the suggested central activity mechanism of anti-nociception. The lack of microbial growth inhibition observed in the anti-microbial study probably shows that the organisms were

not susceptible to its activity or that the extract does not have antimicrobial activity at all. However, it is possible that other microorganisms may be susceptible to the effects of the plant extract.

In conclusion, these studies have shown that the stem bark extract of *A. andongensis* contains some active principles with the potentials of being good analgesics. This was demonstrated in its ability to inhibit pain in both acetic acid-induced writhing and formalin tests. These results conform to the folkloric use of the plant and also revealed its potential for the development of putative herbal analgesic remedies. Efforts are ongoing to ascertain the long-term toxicity profile of the plant.

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