Full Length Research paper

Preliminary phytochemical, analgesic and anti-inflammatory studies of the methanol extract of *Anisopus mannii* (N.E.Br) (Asclepiadaceae) in rodents

A. M. Musa[1]*, A. B. Aliyu[2], A. H. Yaro[3], M. G. Magaji[4], H. S. Hassan[1] and M. I. Abdullahi[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Pharmacology, Bayero University, Kano, Nigeria.
[4]Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria.

The methanol extract of the aerial parts of *Anisopus mannii* was evaluated for its analgesic and anti-inflammatory activities. The analgesic effect was studied using acetic acid-induced abdominal constriction test in mice, while the anti-inflammatory effect was investigated using carrageenan induced paw oedema in rats. The results of the study showed that the extract (40 mg/Kg) exhibited significant (P < 0.01) analgesic effect. It also exhibited significant (P < 0.01) anti-inflammatory effect at a dose of 20 mg/kg. Phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins. The extract was found to have an intraperitoneal LD_{50} of 282.8 mg/kg in mice. The results showed that the extract contains some pharmacologically active principles and lend pharmacological credence to the ethnomedical use of the plant in the management of pain and inflammatory conditions.

Key words: *Anisopus mannii,* phytochemical screening, analgesic activity, anti-inflammatory activity, toxicity.

INTRODUCTION

Pain is an unpleasant sensation that is a consequence of complex neurochemical processes in the central and peripheral nervous systems (Howland and Mycek, 20-06). Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively. These drugs have serious limitations due to their side effects. Opioids cause respiratory depression, euphoria, tolerance and dependence while non-steroidal anti-inflammatory drugs produce gastrointestinal irritation and renal damage (Howland and Mycek, 2006). There is therefore, a need to intensify research with the aim of developing efficacious agents with low toxicity profile. Herbal medicine is still the mainstay of therapy for about 75 - 80% of the whole population in developing countries for primary health care. This is because of better cultural acceptability, affordability, better compatibility with the human body and fewer side effects;

in addition, the last few years have seen a major increase in the use of herbal remedies in developed countries (Parekh et al., 2005). The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficacy of traditional medicine (WHO, 2000). World Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases (Amos et al., 2001).

Many medicinal plants are used in developing countries for the management of pain and inflammatory conditions. The validation of the folkloric claims of these medicinal plants will provide scientific basis for the conservation of tropical medicinal resources, the deployment of the beneficial ones as phytomedicine in the primary healthcare and the development of potential bioactive constituents. These could provide novel compounds or precursors in drug development, and utilization of isolated compounds as investigative, evaluative and other research tools in drug development and testing processes. One of such medicinal

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*Corresponding author. Email: alimsa69@yahoo.com. Tel.: +2348023637971.

plants with ethnomedical claims in pain and inflammatory conditions is *Anisopus mannii* popularly called Kashe zaki by Hausas of Northern-Nigeria. It is a glabrous twining shrub with leaves petiolate, elliptic, ovate and shortly cuspidate at apex up to 15 cm or more long and 12 cm broad, and the stem twining to a height of 3.7 - 4.6 cm (Hutchinson and Dalziel, 1963). In ethnomedicine, the decoction of the whole plant is use to treat diabetes, hypertension, diarrhea and painful conditions like haemorrhoids (Personal communication). Recently, phytochemical and antimicrobial screening of the stem aqueous extract of *A. mannii* has been reported (Sani et al., 2009). However, survey of literature on *A. mannii* revealed that there is no any report on the analgesic and anti-inflammatory activities of the plant. This study was, therefore, carried out to evaluate the effect of the methanol extract of the aerial parts of the plant on pain and inflammation so as to establish a scientific basis for its use in treatment of painful conditions.

MATERIALS AND METHODS

Animals

Adult Swiss albino mice weighing between 19 - 23 g and rats weighing 150 - 220 g obtained from the animal house facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for these studies. The animals were housed in a standard cage at room temperature in a 12/12 h light and dark cycle, and were supplied with food and water *ad libitum*. All experiments were conducted in accordance with animal use ethics as accepted internationally.

Plant material

The plant sample of *A. mannii* was collected in Samaru, Zaria, Nigeria in the month of February, 2006. The plant was authenticated by Mallam Musa Muhammed of the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where a voucher specimen (No. 217) was preserved for future reference.

Preparation of the extract

Aerial parts of *A. mannii* were air dried, powdered and sieved. The powder (600 g) was defatted exhaustively with petroleum ether (60 - 80°C) in a soxhlet apparatus. The defatted marc was subsequently extracted with 95% methanol. The extract was concentrated *invacuo* with a Büchi rotary evaporator at 45°C to obtain a dark green mass (45 g) subsequently referred to as "methanol extract". The extract was kept in a desiccator before use.

Drug and chemicals

Ketoprofen injection manufactured by Lek pharmaceuticals Company Slovenia, Acetic acid manufactured BDH Poole, England and Carrageenan.

Phytochemical screening

The methanol extract was subjected to phytochemical screening for the presence of alkaloids, flavonoids, saponins, tannins and steroids/ triterpenes, according to standard procedures (Trease and Evans, 1996).

Test for sterols/terpenes

Liebermann-Buchard test: 1 ml of anhydrous acetic acid was added to 1 ml chloroform and cooled to 0°C then one drop of concentrated sulphuric acid was added to the cooled mixture followed by the extract. The solution was observed for blue, green, red or orange colour that changes with time.

Salkowski test: A little quantity of the extract was dissolved in 1 ml chloroform and to it 1 ml of concentrated sulfuric acid was added down the test tube to form two phases. Formation of red or yellow coloration was taken as an indication for the presence of sterols.

Test for flavonoids

Shinoda test: To an alcoholic solution of the extract three pieces of Magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

Sulphuric acid test: The sample was dissolved in concentrated sul-furic acid and the colour change was observed.

Ferric chloride test: Extract was boiled with water and filtered to 2 ml of the filtrate, two drops of freshly prepared ferric chloride solution was added; green, blue or violet colourations indicate the presence of phenolic hydroxyl group.

Sodium hydroxide test: 2 ml of the extract was dissolve in 10% aqueous sodium hydroxide solution and filtered to give yellow colour, a change in colour from yellow to colourless on addition of dilute HCl indicate the presence of flavonoids.

Test for Alkaloids

0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. 3 ml of the filtrate was divided into three. To the first 1 ml few drops of freshly prepared Dragendoff reagent was added and observed for formation of orange to brownish precipitate. To the second, 1 drop of Mayer reagent was added and observed for formation of white to yellowish or cream colour precipitate. To the third 1 ml 1 drop of Wagner reagent was added to give a brown or reddish or reddish-brown precipitate.

Test for tannins

A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of a blue-black, or green precipitate was taken as evidence for the presence of tannins.

Test for anthraquinones

Free anthraquinones: The extract was shaken with 10 ml of benzene, the content was filtered, and 5 ml of 10% ammonia solution was added to the filtrate, the mixture was shaken. Presence of a pink, red or violet colour in the ammoniacal layer (lower phase) indicates the presence of free anthraquinone.

Combined anthraquinones: The extract was boiled with 10 ml of aqueous sulphuric acid and filtered hot. The filtrate was shaken with 5 ml benzene, the benzene layer was separated and half its own volume, 10% NH4OH was added. A pink, red or violet colouration in

Table 1. Phytochemcial analysis of the methanol extract of *Anisopus mannii*.

Constituents/test	Results
Alkaloids	
Dragendoff's	+
Mayers	+
Wagner's	+
Flavonoids	
Shinoda	+
Sodium hydroxide	+
Saponins	
Frothing	+
Tannins	
Ferric chloride	+
Lead acetate	+
Steroidal nucleus	
Salkwoski	+
Liberman-Burchard	+

+ = Positive indicating presence

the ammonia phase (lower phase) indicates the presence of combined anthraquinone or anthraquinone derivative.

Test for saponins: About 0.5 g of the extract was shaken with water in a test tube. Frothing which persisted for 15 min indicates the presence of saponins

Route of Administration

The extract and the standard drug were administered to the animals intraperitonealy in all the experiments.

Acute toxicity study

The method of Lorke (1983) was adopted. The study was divided into two phases. In the first phase, nine mice of either sex were divided into three groups of three mice each. Group 1 received 10 mg/kg extract while group 2 and 3 received 100 and 1000 mg/kg extract respectively. The mice were observed for signs and symptoms of toxicity and mortality for twenty four hours after treatment. In the second phase, 4 mice were divided into 4 groups of one mouse each, the first received extract at a dose of 50 mg/kg while the second, third and fourth groups received the extract at doses of 100, 200 and 400 mg/Kg respectively. The mice were also observed for 24 h. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose that is. The geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Acetic acid-induced abdominal constrictions in mice

The method described by Koster et al. (1959) was used. 20 albino male and female mice were divided into 4 groups of 5 mice each. Group 1 was injected with 10 ml/kg of normal saline (negative control). Group 2 was injected with ketoprofen 10 mg/kg (positive control). Groups 3 and 4 were injected with 20 mg/kg and 40 mg/kg of the extract respectively. Thirty minutes later, each mouse was injected with 10 ml/kg of aqueous solution of acetic acid (0.6%). The number of abdominal constrictions for each mouse was counted 5 min after injection of acetic acid for a period of 10 min. The percenttage inhibition of abdominal constrictions was calculated using the following formula:

Inhibition (%) = $\dfrac{\text{Mean No. of writhes (Control)} - \text{Mean No. of writhes (Test)}}{\text{Mean No. of writhes (Control)}} \times 100$

Carrageenan-induced paw oedema in rats

The method described by Winter et al. *(*1963) was adopted. 20 rats were divided into 4 groups of 5 mice each. Group 1 received 10 ml/Kg normal saline group 2 received ketoprofen 10 mg/kg *(*positive control), while groups 3 and 4 received extract at doses of 20 and 40 mg/kg respectively. 30 min later, 0.1 ml of sterile saline solution of 1% carrageenan was injected into the sub plantar surface of the left hind paw. Paw size was measured using vernier caliper at time 0, 1, 2, 3 and 4 h after the carrageenan administration.

Statistical analysis

All data were expressed as mean ± S.E.M. The mean values of control groups were compared with the mean value of treated groups using student′s t-test. Results were considered significant at $P < 0.05$.

RESULTS

Phytochemical screening

Preliminary phytochemcial screening of the extract revealed the presence of alkaloids, flavonoids, saponins, tannins and steroids (Table 1).

Acute toxicity study

The intraperitonial LD_{50} of the extract was found to be 282.8 mg/kg. The animals presented with weakness and respiratory distress prior to death.

Analgesic activity study

The extract at doses of 20 and 40 mg/kg significantly ($P < 0.05$) reduced the number of acetic-acid-induced abdominal constrictions by 32.40 and 56.3% respectively. Ketoprofen (10 mg/kg) produced 48.9% reduction in abdominal constriction (Table 2).

Anti-inflammatory study

In the normal saline treated animals, sub plantar injection of 1% carrageenan suspension produced a local oedema reaching its maximum at 2 h. the percentage anti-inflammatory effect (at the peak of carrageenan-induced oedema) of the extract at the lower dose tested (20 mg/-

Table 2. Effect of methanol extract of *Anisophus manni* on acetic acid-induced abdominal constrictions in mice.

Treatment	Dose (mg/kg)	Mean number of writhes ± SEM	Inhibition (%)
Normal saline	10	14.2 ± 0.86	-
Ketoprofen	10	7.25 ± 1.97[a]	48.9
Extract	20	9.6 ± 1.57[a]	32.4
Extract	40	6.2 ± 1.77[b]	56.3

[a]$P < 0.05$; $P < 0.01$ (compared with control, students' t-test).

Table 3. Effect of the methanol extract on carrageenan induced paw oedema in rats.

Treatment	Dose (mg/kg)	Mean paw diameter (cm) Time (h)			
		1	2	3	4
Normal saline	10	0.15 ± 0.01	0.23 ± 0.02	0.23 ± 0.02	0.18 ± 0.03
Ketoprofen	10	0.07 ± 0.01[b] (53.3)	0.08 ± 0.01[c] (65.2)	0.10 ± 0.01[c] (56.5)	0.06 ± 0.01[b] (66.7)
Extract	20	0.05 ± 0.02[b] (66.7)	0.11 ± 0.02[b] (52.2)	0.13 ± 0.02[b] (43.5)	0.11 ± 0.01[a] (38.9)
Extract	40	0.09 ± 0.01[a] (66.7)	0.19 ± 002[NS] (17.4)	0.18 ± 0.03[NS] (21.7)	0.14 ± 0.03[NS] (22.2)

[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$; NS = Not significant (compared with control, students' t-test). Figures in parentheses represent percentage inhibition of inflammation.

kg) was 52.2% compared to 65.2% for ketoprofen (10 mg/kg), the standard anti-inflammatory agent (Table 3).

DISCUSSION

The results of preliminary phytochemical screening of the methanol extract revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins. Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported (Das et al., 1989), hence the analgesic and anti-inflammatory effects produced by the extract may be attributed individually or collectively to the flavonoids and tannins the intraperitoneal LD_{50} obtained with this extract suggests that the extract is moderately toxic (Lorke, 1983).

Acetic acid-induced abdominal constriction test is used for the evaluation of pheripheral analgesic activity (Gene et al., 1998). The extract showed analgesic activity in acetic acid induced writhing test in mice. This indicates that the extract possessed peripheral mediated analgesic activity. The abdominal constriction response is thought to involve in part local peritoneal receptors (Bentley et al., 1983), so the extract may have interfered with these peritoneal receptors to bring about analgesia. Acetic acid-induced writhing test has been associated with increase in the levels of prostaglandins E_2 and $F_2\alpha$ in peritoneal fluid (Deradt et al., 1980) as well as lipooxygenases (Levini et al., 1984), so the mechanism of activity of the extract may be linked to cyclooxygenases and/or lipooxygenases. The extract caused marked inhibition of carrageenan induced oedema in rats. Carrageenan induced inflammation is believed to be biphasic, the early phase (1 - 2 h) is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged

tissue surroundings, the late phase is sustained by prostaglandins released and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Brito and Antonio, 1988). The inhibitory effect of the extract 20 mg/kg on carageenan induced inflammation over period of 4 h is similar to the effect of most non-steroidal anti-inflammatory drugs. This suggests that it acts in later phase probably involving arachidonic acid metabolites which produce oedema dependent on neutrophils mobilization (Just et al., 1998).

Conclusion

In conclusion, the results of the study showed that the extract of *A. mannii* has both analgesic and anti-inflammatory activities which explain the basis of its use in traditional medicine to manage pains. It also contains some biologically active constituents worthy of further investigations.

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