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Phytochemical Screening and Evaluation of Analgesic and Anti-inflammatory Activities of the Methanol Leaf Extract of *Cissus polyantha*

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Cissus polyantha is used in African traditional medicine is the management of pain and inflammatory conditions. This study was therefore designed to evaluate the analgesic and anti-inflammatory activities of the methanol extract of the leaf of *Cissus polyantha,* as well as to establish the class of phytochemical constituents present in the extract. The analgesic effect was studied using acetic acid-induced writhing and hot plate tests in mice, while anti-inflammatory effect was investigated using carrageenan-induced hind paw oedema in rats. The results of the study showed that the extract significantly (50, 100, 200 mg kg^{-1}) ($p<0.001$) and dose-dependently inhibited acetic acid-induced writhing. The extract at dose of 100 mg kg^{-1} increased the mean pain responses by 69.25% compared to control. At the end of third hour after carrageenan administration, the various doses of the extract offered 65.67, 70.15 and 67.16% inhibition of hind paw oedema, respectively. These effects were more remarkable than those produced by ketoprofen (63.8%). Preliminary phytochemical screening revealed the presence of steroids, flavonoids, saponins, tannins and anthraquinones. The intraperitoneal mean lethal dose (LD_{50}) of the extract in mice was estimated to be 774.6 mg kg^{-1}. The findings of this study showed that the methanol leaf extract of *Cissus polyantha* contains some pharmacologically active principle(s) with analgesic and anti-inflammatory activities and lend credence of the ethnomedical use of the plant in the management of pain and inflammatory conditions.

Key words: *Cissus polyantha*, phytochemical analysis, analgesia, inflammation

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INTRODUCTION

Traditional practitioner and birth attendants have been reported to provide health care for about 80% of the world population living in developing countries (Bannerman, 1983). Medicinal plants constitute the cornerstone of the practices employed by these groups of practitioners due to their affordability, availability and their use is linked with ancestral experiences (Marin-Bettulo, 1980). Medicinal plants occupy a central position in traditional medical practice and have been a viable source of some beneficial drugs in use today.

Pain is an unpleasant sensation associated with a number of disease conditions and could be the only symptom of diagnosis of some of these diseases. The opioids have ruled the world in the management of severe pain, while non-steroidal anti-inflammatory drugs (NSAID) have been quite beneficial in the management of mild to moderate pain. However, there use is associated with some serious limitation due to side effects such as gastrointestinal irritation (NSAIDs), tolerance and dependency (opioids) (Howland and Mycek, 2006).

From generation to generation, herbal therapies have been employed by man as remedies for pain (Ahmadiani *et al*., 1998) and medicinal plants occupies a central role in the formulation of these remedies due to their availability, affordability and perceived better safety profile. A number of medicinal plants have enjoyed patronage among the traditional practitioners in the management of pain and inflammatory conditions. There is therefore a need for scientific validation of their folkloric claims with the aim of deploying the beneficial ones as phytomedicines and subject them to bioassay guided isolation of bioactive principles which could serve as "lead" compounds in drug development process. One of such medicinal plants with ethnomedical claims in painful and inflammatory conditions is *Cissus polyantha*. *Cissus polyantha* is a semi-woody climber; of the close forest from Sierra Leone to Southern Nigeria. And it is also from Eastern Cameroun to Ubangi (Burkill, 2000). Many species in the genus *Cissus* have been reported to possess wide range of uses to mankind ranging from medicinal and feeds for livestock (Burkill, 2000). Sap from macerated leaves of *C. polyantha* has been used in Liberia for treatment of conjunctivitis (Burkill, 2000), while the decoction of the underground root and the leaves is used as analgesic and anti-inflammatory medication (Muhammad Maiwada, Personal communication). To our knowledge, there is no report of the analgesic and anti-inflammatory activities of the leaf of *Cissus polyantha*. This study therefore, aimed at conducting phytochemical analysis and evaluating the analgesic and anti-inflammatory activities of the methanol leaf extract of *Cissus polyantha* in laboratory animals.

MATERIALS AND METHODS

Plant material: The plant material was collected from Turunku town, Igabi Local Government Area of Kaduna State. Nigeria, in the month of June, 2010. The plant was identified by Mallam Musa Muhammad of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria-Nigeria. A voucher specimen (No. 616) was deposited for future reference.

Extraction of plant material: The leaves were air dried at room temperature for 14 days and then crushed into coarse powder with a pestle and mortal. A total of 311.2 g of the powdered leaves of the *Cissus polyantha* was extracted with 5 L of methanol by continuous maceration for ten days. The methanol extract was then concentrated at reduced pressure to give 58.23 g of dark green solid subsequently referred to as *Cissus polyantha* extract (CPE).

Preliminary phytochemical screening: The presence of carbohydrates, glycosides, anthraquinones, saponins, flavonoids, tannins, steroids/triterpenes and alkaloids was determined by the simple and standard qualitative methods described by Trease and Evans (1989) and Sofowora (1993).

Animals: Swiss Albino mice (20±2 g) and Wistar rats (200±20 g) of either sex were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria. They were housed in polypropylene cages at room temperature and maintained on standard rodent feed and water *ad libitum*. All experimental protocols were in accordance with the Ahmadu Bello University Research policy; and ethic and regulations governing the care and use of experimental animals as contained in "Principles of laboratory animal care" (NIH Publication No. 85-23, revised 1985).

Drugs/chemicals and treatment: CPE, normal saline and ketoprofen were administered via the intraperitoneal routes. All administrations were at volumes equivalent to 10 mL kg^{-1} (for mice) and 1 mL kg^{-1} (for rats).

Groupings: For each pharmacological studies, the animals were divided into 5 each consisting of six animals. First group served as control and was treated with normal saline. The second, third and fourth groups were treated with CPE (50, 100 and 200 mg kg^{-1}). The fifth group received the standard agent.

Acute toxicity (LD_{50}) test: The method previously described by Lorke (1983) was used in estimating the LD_{50} of the extract. Briefly, the procedure comprises of two phases. In the first phase, mice were divided into three groups each consisting of three mice and were treated with CPE at doses of 10, 100 and 1000 mg kg^{-1} of their body weight, intraperitoneally. They were observed for signs and symptoms of toxicity and death within 48 h period. In the second phase, four groups (one mouse each) were treated with CPE at 600, 1000, 1600 and 2900 mg kg^{-1}, based on the result of the first phase. The LD_{50} value was estimated by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

Acetic acid-induced writhing test in mice: Thirty minutes post treatment with normal saline (10 mL kg^{-1}), CPE (50, 100 and 200 mg kg^{-1}) or ketoprofen (10 mg kg^{-1}), each mouse in each group was treated with (0.6% acetic acid of 1 mL per 100 g i.p.). The mice were placed individually into plastic cages. The number of writhes produced in these animals was counted for ten minutes after a 5 min latency period. The percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhes (control)} - \text{Mean No. of writhes (test)}}{\text{Mean No. of writhes (control)}} \times 100$$

Hot plate test in mice: The hot plate was maintained at 50±1°C. Each mouse was gently placed on the plate and the time taken by mouse to lick its paw or jump off was taken as the latency to pain response. To avoid tissue damage the cut-off time or latency response in the control was taken as 15 sec. The determination of latency of pain response was performed before the administration of the extract (50, 100 and 200 mg kg^{-1}), pentazocine (10 mg kg^{-1}) or normal saline (10 mL kg^{-1}) and repeated at intervals of 30, 60, 90 and 120 min (Turner, 1965).

Carrageenan-induced hind paw oedema in rats: The method previously described by Winter *et al.* (1962) was employed in the study. Thirty minutes post treatment with normal saline, CPE (50, 100 and 200 mg kg^{-1}) or ketoprofen (10 mg kg^{-1}), 0.1 mL of freshly prepared carrageenan suspension (1% w/v in 0.9% normal saline) was injected into the sub plantar region of the left hind paw of each rat. The paw diameter was measured with the aid of a vernier caliper at 0, 1, 2, 3 and 4 h after the injection of carrageenan. The difference between the readings at time 0 h and the different time intervals was taken as the thickness of edema. The percentage inhibition of inflammation was calculated for each dose at different hours.

Statistical analysis: The data were expressed as Mean±SEM. The results were analysed using one way ANOVA followed by Dunnet *post hoc* test for multiple comparison. p values less than 0.05 ($p<0.05$) were considered indicative of significance.

RESULTS

Preliminary phytochemical screening: The preliminary phytochemical screening of CPE revealed the presence of flavonoids, tannins, saponins, cardiac glycosides and steroids (Table 1).

Acute toxicity study: The intraperitoneal median lethal dose of CPE in mice was estimated to be 774.6 mg kg^{-1}.

Acetic acid-induced writhing test in mice: CPE significantly ($p<0.001$) attenuated the acetic acid-induced writhing in mice with the highest protection (83.75%) produced at the highest dose (200 mg kg^{-1}) tested. The standard drugs (ketoprofen) afforded 55% protection at the dose of 10 mg kg^{-1} (Table 2).

Hot plate test in mice: CPE produced a significant ($p<0.001$) and dose dependent increase in mean pain latency on the hot plate at the end of the 120 min. These effects were greater than that of the standard drug used (Table 3).

Carrageenan-induced hind paw oedema in rats: CPE significantly inhibited carrageenan-induced hind paw oedema at all the doses tested. However, its effect was not dose-dependent. At the end of third hour, the extract

Table 1: Phytochemical constituents of the methanol extract of the leaves of *Cissus polyantha*

Tests	Results
Carbohydrates	+
Glycosides	+
Saponins	+
Steroids and Triterpenes	+
Flavonoids	+
Tannins	+
Anthraquinones	-
Alkaloids	-

+: Present, -: Absent

Table 2: Effect of methanol extract of *Cissus polyantha* on acetic-acid induced writing in mice

Treatment	Mean No. of writhes	Protection (%)
Normal saline (10 mL kg^{-1})	16.0±1.30	
Extract (200 mg kg^{-1})	2.6±0.75*	83.75
Extract (100 mg kg^{-1})	6.8±0.97*	57.5
Extract (50 mg kg^{-1})	9.2±0.73*	42.5
Ketoprofen (10 mg kg^{-1})	7.2±0.97*	55

Data represented as Mean±SEM, n = 5 (One way ANOVA followed by *post-hoc* t-test for multiple comparison); *$p<0.001$

Table 3: Effect of methanol extract of *Cissus polyantha* on thermally induced pain in mice

	Mean pain latency (sec) (min)			
Treatment	30	60	90	120
N/Saline (10 mL kg^{-1})	0.93±0.1	0.88±0.6	0.95±0.03	0.89±0.05
CPE (50 mg kg^{-1})	2.25±0.2^{a}	2.17±0.3^{a}	2.00±0.3^{c}	1.69±0.8^{a}
CPE (100 mg kg^{-1})	1.72±0.2^{b}	2.15±0.6$^{N/S}$	2.92±0. 8^{c}	2.63±0.6^{a}
CPE (200 mg kg^{-1})	2.19±0.5^{c}	1.56±0.2^{c}	2.97±0.7^{c}	2.16±0.3^{b}
Pentazocine (10 mg kg^{-1})	1.74±0.2^{b}	1.89±0.2^{b}	1.98±0.25^{b}	1.82±0.1^{a}

Data represented as Mean±SEM; $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$; n = 6 student t-test; CPE: Methanol leaf extract of *Cissus polyantha*, N/S: Not significant

Table 4: Effect of methanol extract of *Cissus polyantha* on carrageenan induced inflammation in rats

	Mean paw diameter (cm) (h)			
Treatment (mg kg^{-1})	1 h	2 h	3 h	4 h
N/saline (mL kg^{-1})	0.12±0.01	0.22±0.01	0.27±0.02	0.17±0.01
CPE 50	0.07±0.01* (32.17%)	0.08±0.01** (62.03%)	0.09±0.02** (65.67%)	0.08±0.01** (56.14)
CPE 100	0.06±0.02* (46.96%)	0.07±0.01** (99.66%)	0.08±0.01** (70.15%)	0.07±0.01** (60.23%)
CPE 200	0.06±0.01* (46.95%)	0.07±0.03** (66.20%)	0.09±0.03** (67.16%)	0.08±0.02** (51.46%)
Ketoprofen (10)	0.08±0.01^{NS} (32.17%)	0.08±0.02** (61.57%)	0.1±0.01** (63.80%)	0.08±0.01** (54.97%)

Data presented as Mean±SEM; standard Error of mean; values in parentheses represent percentage inhibition of inflammation; *p<0.05, **p<0.001 (compared with control)

at the dose of 100 mg kg^{-1} produced 70.15% inhibition which was greater than that produced by ketoprofen (63.8%) (Table 4).

DISCUSSION

The preliminary phytochemical screening of the methanol extract revealed the presence of flavonoids, steroids and triterpenes, carbohydrates, cardiac glycosides saponins and tannins. The intraperitoneal LD_{50} (774.6 mg kg^{-1}) obtained with this extract suggests that the extract is relatively toxic (Matsumura, 1985). However, it is safe at the doses used in the study.

Acetic acid-induced abdominal constriction test is used for the evaluation of peripheral 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 (Gene *et al*., 1998). The analgesic effect of the extract at 200 mg kg^{-1} tested in acetic acid-induced pain was significant compared to that of Ketoprofen (10 mg kg^{-1}) ($p<0.001$). The abdominal constriction response is thought to involve in part local peritoneal receptors (Bentley *et al*., 1981), so the extract might 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 $F_{2\acute{a}}$ in peritoneal fluid (Deraedt *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.

Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Parkhouse and Pleuvry, 1979). The ability of the extract to prolong the reaction latency to pain thermally-induced in mice suggests that the extract has some central analgesic activity.

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 prostaglandin in the damage tissue areas, the late phase is sustained by prostaglandins release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Brito and Antonio, 1988). The inhibitory effect of the extract on carrageenan induced inflammation over a 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 produces oedema dependent on neutrophils mobilization (Just *et al*., 1998).

Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported (Das *et al*., 1989). Saponins have also been reported to possess analgesic activity (Choi *et al*., 2005), hence the analgesic and anti-inflammatory effects produced by the extract may be attributed individually or collectively to the flavonoids, steroids and saponins.

CONCLUSION

In conclusion, the results of the study showed that the methanol extract of *Cissus polyantha* has analgesic and anti-inflammatory activities which explain the basis of its use in traditional medicine to manage pain and inflammatory conditions.

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