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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(4): 351-356

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Evaluation of analgesic and anti-inflammatory activities and phytochemical screening of the leaves extract of *Paullinia pinnata* (Sapindaceae)

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ABSTRACT

The ethanolic extract of the leaves of Paullinia pinnata was evaluated for its analgesic and anti-inflammatory activity. The analgesic effects were studied using two models: acetic acid-induced abdominal constriction test and hot plate method, in mice and rats respectively. The anti-inflammatory effect was investigated using egg white induced paw oedema in rat. The result of the study showed that P. pinnata extract (200 mg/kg and 400 mg/kg) exhibited significant ($p<0.05$) analgesic effect in the two models. It also exhibited significant ($p<0.05$) anti-inflammatory effect at a dose of (400 mg/kg). This indicates that P. pinnata extract is effective in the management of peripherally and centrally induced pain. Phytochemical analysis of the extract revealed the presence of saponins, tannins, glycosides and flavonoids. The intraperitoneal LD50 of P. pinnata was found to be 1131 mg/kg. These results support some of the use of the plant in folk medicine.

Keywords: *Paullinia pinnata* , analgesic, anti-inflammatoryactivity, phytochemical screening.

INTRODUCTION

Paullinia pinnata (linn), Family *Sapindaceae* is a native to Tropical Africa, South and Central America [1]. The plant is easily recognized from the leaves that have five serrated leaflets with prominent veins and a winged rachis and petiole [2]. The plant is regarded in tropical Africa as a terrible poison with a slow but surely fatal effect; present day observers have felt to corroborate this. The chief medicinal use in West Africa seems to be an agent to stop bleeding. In general an infusion of the leaves is used for fever accompanied by aches, the effect being to cause sweating

and diarhoea. In Sierra Leone, the Timnes use it for toothaches; [3] Previous investigation of the extract showed some anti-malaria activity [4] and some Diuretic action [5]. The plant is also used locally for conditions such as arthrithes and rheumatism. Majority of human population worldwide is getting affected by the inflammation related disorders. It is believed that current analgesia inducing drugs such as opiates and NSAIDS are not useful in all cases, because of their side effects like gastrointestinal irritation, liver dysfunction and many others [6]. In this work the ethanolic extract of *P. pinnata* was screened for analgesic and anti inflammatory effects as used traditionally.

EXPERIMENTAL SECTION

Animals

Adult albino rats of either sex (weighing 200 - 250g) and Wister albino mice of either sex (weighing 25 - 30g), were bred and kept in cages under standard environmental conditions in the animal house of the University of Jos Nigeria. They were fed with standard animal pellets (Pfizer Feeds, Nigeria) and water ad libitum. Experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Plant Materials

The leaves of *P. pinnata* were collected from Toro LGC of Bauchi state of Nigeria by an herbalist in Terminus herb market Jos Plateau State, Nigeria. It was identified and authenticated by a taxonomist Dr. A.I. Kareem of College of Forestry, Jos Plateau State.

Preparation of extract

The leaves were cleaned and dried under shade and reduced to powder using mortar and pestle, and stored in air tight containers until use. The powdered plant parts were soxhlet extracted using a mixture of water and ethanol (30:70). The resultant extracts were filtered and evaporated to dryness on steam bath at a temperature of 80 ^{o}C. the dried extract was weighed; the percentage yield was determined and it was preserved in the refrigerator until use.

Acute Toxicity Testing

The LD_{50} values of the extract were determined in mice following intraperitoneal administration as described by Lorke [7].

Tests for analgesic activity

Writhing reflex test in mice

Mice of either sex were divided in to 4 groups of 5 each. Group 1 received normal saline (control), another group received standard drug (Aspirin 5 mg/kg) and the other 3 groups received 3 doses of *P. pinnata* extract (100 mg/kg, 200 mg/kg and 400 mg/kg) intraperitoneally. 0.1 ml of 1% acetic acid was injected intraperitoneally, 30 minutes later and the number of writhing movements was observed for 15 minutes beginning 5 minutes after injection of acetic acid. The percentage inhibition of writhing movement was then calculated.

Hot Plate Test

Rats were divided into 5 groups of 5 rats each, group 1 served as control and received normal saline, another group received standard drug (pentazocine 5 mg/kg), and the other groups

received 3 doses of *P.pinnata extract* (100 mg/kg, 200 mg/kg and 400 mg/kg). The pretreated rats were kept individually in a glass beaker on a hot plate having a constant temperature of 55±1°C the time taken for either paw licking or jumping were recorded.

Anti-Inflammatory Test

Animals used for this test were fasted for 12 hours and deprived of water only during the experiment. The rats were divided into 5 groups of 5 animals each. One group received normal saline, another received standard drug (Piroxicam 5 mg/kg), while the other groups received 2 doses of the extract (100 mg/kg, 200 mg/kg and 400 mg/kg) administered intraperitonealy, 30 minutes before the induction of inflammation. Acute inflammation was induced by injecting egg albumin into the sub- planter surface of the rat hind paw linear circumference, edema was assessed in terms of difference in zero time linear circumference at the injected paw and it circumference at 30 minutes interval after egg albumin injection.

Phytochemical Screening of The Plants

The method described by Trease and Evans [8] were used to chemically analyze the extract for the presence of alkaloids, saponins, tannins, flavonoids resins and carbohydrate.

Statistical Analysis

The group means ± SEM was calculated for each analyte and reported. Significant differences between means were evaluated by one way analysis of variance (ANOVA). Post hoc test analysis was done using Dunnet test with SPSS version 16.0 package. Values of $P < 0.05$ were considered as statistically significant.

RESULTS

Acute Toxicity Testing

The intraperitoneal LD_{50} of *P. pinnata extract* was found to be 1.131 g/kg.

Analgesic Activity

The extract at dose a dose of 100 mg/kg insignificantly ($P>0.05$) reduced the number of acetic acid induced writhings by 57.8%. while doses of of 200 mg/kg and 400 mg/kg reduced the number of acetic acid induced writhings by 74.6% and 83.8 % respectively. Aspirin 5 mg/kg caused 67.6% reduction in the number of writhings. These values were significant at $P<0.05$ when compared with positive control. In hot plate test, the extract at 200 mg/kg and 400 mg/kg significantly ($P<0.05$) increased the after treatment reaction time.

TABLE 2: Effect of ethanolic extract of *S. guineense on* acetic acid induced writhing test on mice

Treatment	Dose (mg/kg)	Mean	% Inhibition
Normal Saline		35.8±1.393	
EXTRACT	200	5.6±1.24	56.4
EXTRACT	500	15.4 ±0.678	57.0
EXTRACT	1000	4.60±0.927*	87.2
Aspirin (Standard)	5	10.8±0.927	69.8

***** = *Significant at P < 0.05. Significantly different compared to negative control*

Hot Plate Test

The extract significantly P<0.05 increased the pain reaction time in hot plate.

TABLE 2: Effect of ethanolic extract of *P. pinnata* on pain reaction time in hot plate test in rats

Treatment	Dose (mg/kg)	30	60	90	120
Normal Saline		2.80±0.29	2.5±0.60	2.1±0.57	2.5±0.48
EXTRACT	100	4.64±0.19	6.4±0.29	4.7±0.30	3.1±0.33
EXTRACT	200	4.89±0.19	6.8±0.32*	5.3±0.30*	3.2±0.40*
EXTRACT	400	5.35±0.29*	7.8±0.39*	5.2±0.30*	3.7±0.22*
PENTAZOCINE	5	5.49±0.54	9.1±0.57	6.1±0.74	3.9±0.45

***** = Significant at P < 0.05. Significantly different compared to negative control***

Anti-Inflammatory Activity

The extract at doses of 200 mg/kg and 400mg/kg significantly (P<0.05) reduced the induced paw oedema in rats.

TABLE 3: Effect of ethanolic extract of *P. pinnata* on egg albumin induced paw oedema in rats

Treatment	Dose (mg/kg)	1hr	% inhibition	2hr	% inhibition	3hr	% inhibition
Normal Saline		3.60±0.11		3.55±0.22		3.47±0.14	
EXTRACT	100	3.40±0.06	06	3.27±0.20	08	3.10±0.20	11
EXTRACT	200	3.24±0.16*	10	2.93±0.08*	17	2.60±0.04*	25
EXTRACT	400	2.17±0.04*	40	1.89±0.07*	47	1.21±0.03*	65
PIROXICAM	5	2.21±0.01	39	1.96±0.06	45	1.28±0.16	63

***** = Significant at P < 0.05. Significantly different compared to negative control***

Phytochemical tests of ethanolic extracts

The preliminary phytochemical screening reveal the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides as shown below (Table 4)

Table 4

TEST	*P. pinnata*
ALKALOIDS	+
CARDIAC GLYCOSIDES	++
SAPONINS	++
FLAVONOIDS	+++
TANNINS	+++
CARBOHYDRATES	++

KEY:* + *indicates presence
- indicates absence

DISCUSSION

The intraperitoneal LD_{50} of *P. pinnata* was found to be 1,131 mg/kg. Scientifically plants are assayed for LD_{50} values to establish their safety. Acute toxicity test gives an idea of the effect of long-term use of the drug on body organs and system. The result for this study showed that the intraperitoneal LD_{50} are within safety margins, this suggests that the extract is safe for

consumption. The extracts inhibit the acetic acid induced writhing reflex on mice. Plants that are effective in this test have peripheral analgesic activity [9]. Acetic acid induced writhing test have been associated with increase in the level of prostaglandins in peritoneal fluid [10], so the mechanism of activity of the extract may be linked to inhibition of cyclooxygenases. The result of the hot plate test showed that *P. pinnata* extract significantly increased the pain reaction time of the rats on hot plate. Hot plate test is a model for assaying effects of drugs on central pain. Drugs that are effective in this model have central analgesic effect [11]. *P. pinnata* showed significant anti inflammatory activity on the fresh egg-albumin induced inflammation as against the progressive increase in the rat paw circumference in the control, it suppressed the increase in rat paw oedema in a dose dependent manner two or three hours after the injection of inflammatory agent. This implies that the extracts will be useful in the management of inflammatory pain [12]. Pain and inflammation are complementary and always occur together. Phytochemical screening of the plants revealed that *P. pinnata* extract contain flavonoids, tannins, saponins and carbohydrate. Alkaloids and cardiac glycosides are also present. These phytochemical constituents are physiologically active compounds possessing great potential for therapeutic and prophylactic uses. Analgesic and anti-inflammatory effects of flavonoids and tannins have been reported [13].The flavonoids exhibits potent anti-inflammatory activity by inhibiting prostaglandin synthesis [14]. These might be responsible for the analgesic and anti-inflammatory activities of the plant extract seen in this study.

The above result therefore supports their use by traditional healers for various forms of pains and inflammatory conditions such as arthrithis and rheumatism.

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