Antidiarrhoeal Activity Of The Methanolic Extract Of The Leaves Of Paullina Pinnata Linn (Sapindaceae)
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Citation

Abstract
Various parts of the plant Paullinia pinnata has been used for the management of various illnesses by various tribes in Africa. This work attempts to provide the pharmacological basis for the ethno medical use of the plant in the treatment of diarrhoea. The methanolic leaf extract was screened for anti-diarrhoeal activity on whole mouse gastrointestinal tract. The Phytochemical screening of the plant and acute toxicity were also carried out. Two models were adopted for anti diarrhoea evaluation: Measure of intestinal transit time and Measure of Inhibition of castor oil induced diarrhoea. The results obtained in the intestinal transit model showed that the percentage distances travelled by the charcoal were: 83.82%±4.6, 52.75%±5.8, 33.68%±10.1, 17.31%±4.9, and 36.12%±9.5 for groups A, B, C, D and E respectively. While the results obtained in the castor oil induced diarrhoea model showed the average number and weight of stool per group over 4 hours were: 14.25±0.48 : 0.3391g, 6.50±0.86 : 0.59g, 6.75±2.175 : 0.2793g, 2.25±0.75 : 0.1834g and 7.75±0.75 : 0.2353g for groups A, B, C, D and E respectively. This result shows that the extract inhibits gastrointestinal motility and its antidiarrhoeal property is dose dependent. Phytochemical analysis revealed the presence of carbohydrate, reducing sugars, Saponins, anthracene derivatives, tannins and cardiac glycosides. The extract at 4g/kg produced no acute toxicity even after being monitored for 5 days.

The Research work was done in the Faculty of Pharmacy, University of Benin, Benin City Nigeria.

INTRODUCTION
Diarrhoea is increased fluidity, frequency or volume of bowel movements. It may be acute or chronic. Diarrhoea can be very serious in infants and elderly people because of the risk of severe, potentially fatal dehydration. Diarrhoea occurs worldwide and causes 4% of all deaths and 5% of health loss to disability. Diarrhoea, a very common symptom of HIV/AIDS affects 90% of PLWHA and results in significant morbidity and mortality. In Africa, diarrhoea is four times more common among children with HIV and seven times more common among adults with HIV than their HIV-negative household members.

In the past 2 decades, there has been a search for drugs that might inhibit the process of diarrhoea development especially the secretory process. Although a number of drugs have emerged, none has found a place in the routine management of diarrhoea.

Local herbalists have depended on medicinal plants as a reliable means of treating diarrhoea. Hence the use of medicinal plants that possess anti-diarrhoeal activities has been explored as a measure that could be of benefit in combating widespread diarrhoea infections especially in third world countries.

Numerous studies have validated the traditional use of antidiarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which have antisecretory effects, delay intestinal transit, suppress gut motility, stimulate water absorption or reduce electrolyte secretion.

Of the numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes) present in active extracts, tannins and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption. Others act by inhibiting intestinal motility.

As some of the active ingredients are potentially toxic, there is a need to evaluate the safety of plant preparations. A few clinical trials have evaluated the safety and tolerability of traditional and herbal medicine preparations used to treat diarrhoea and generally indicate that minimal side effects are observed. However, with increased popularity of plant derived medicines in Western Society, the benefits and
potential dangers of these medicines must be considered.

Paullinia pinnata Linn (family sapindaceae) is an African woody vine whose fruits are widely eaten and its leaves are used in traditional medicine for the treatment of malaria. A woody or sub-woody climber of damp sites and stream banks of the forest and jungle re-growth in the savannah zone; originally of tropical America and now naturalised and common throughout the West African region and in all parts, except the driest of tropical Africa.

MATERIALS AND METHOD

PLANT MATERIAL

Fresh leaves of Paullinia pinnata were collected from Ugbowo, Benin City, Edo state, Nigeria in July 2008. Botanical Authentication was done by Mr Sunny Nweke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. Voucher sample was prepared and deposited in the herbarium for reference. Immediately after collection, the leaves were dried under shade for two weeks. The dried leaves were pulverised into fine particles, weighed and kept for further analysis.

ANIMALS

Swiss Albino mice weighing between 20-30g of either sex were obtained from the Physiology Department of the University of Ibadan, Ibadan, Oyo State, Nigeria. The animals were maintained in a 12 hour light and dark cycle and had access to feed (grower’s marsh) and water ad libitum.

The animals were allowed to acclimatize for 14 days before being subjected to experimental protocol.

METHODS

PLANT EXTRACTION

The powdered plant material (300g) was macerated in 1.5 litres methanol for 72 hours. The mixture was stirred at 6 hourly intervals using a glass stirrer. The extract was filtered and evaporated to near dryness using a rotary evaporator. The concentrated extract was weighed, stored in an air-tight container, labelled and refrigerated at 4°C prior to use.

The stock solution was extemporaneously prepared using distilled water to a concentration of 100mg/ml for administration to the experimental animals. All reagents used were of analytical grades.

PHYTOCHEMICAL SCREENING

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, reducing sugars, Saponins, tannins and alkaloids were carried out using standard procedures.

PHARMACOLOGICAL SCREENING

Two models were employed in evaluating the antidiarrheal activity.

A. SMALL INTESTINAL TRANSIT TIME IN MICE

Adult mice of both sexes weighing between 20-30g were divided into five groups of four mice each. Group A which was the control group received normal saline intraperitoneally at a dose of 10ml/kg. Group B, C and D received 50mg/kg, 100mg/kg and 200mg/kg of the extract respectively intraperitoneally; while Group E received Atropine 0.2mg/kg also intraperitoneally. 30 minutes after administration, charcoal meal was administered using an Orogastric tube at a dose of 0.2mls/mouse. 30 minutes after the administration of the charcoal meal, each animal was placed under chloroform anaesthesia and then sacrificed by cervical dislocation. The mice were dissected and the stomach and small intestines removed and stretched out on a clean surface. The distance travelled by the charcoal meal from the stomach in relation to the total length of the intestine was measured. Thereafter values were expressed as a percentage and the percentage inhibition calculated.

B. INHIBITION OF CASTOR OIL-INDUCED DIARRHOEA

Mice were divided into 5 groups of four animals each. Group A (control) received 10ml/kg normal saline intraperitoneally, Groups B, C and D received 50mg/kg, 100mg/kg and 200mg/kg of the extract respectively intraperitoneally also. While Group E received 0.2mg/kg Atropine intraperitoneally.

After 30 minutes, all the mice in each group received 0.2mls castor oil orally. The mice were then placed in separate cages lined with filter paper. The following parameters were observed over the next 4 hours:

i. The time elapsed between the administration of castor oil (cathartic agent) and the excretion of the first diarrhoeic stool

ii. The total number of both wet and dry diarrhoea droppings (this was counted every 30 minutes for 4 hours)

iii. The total weight of both the wet and dry diarrhoeal stool in this period of time (by weighing the filter paper before
and after the experiment).

**ACUTE TOXICITY**

Mice (16) were randomly selected into 4 groups of 4 mice each. The animals were starved for 12 hours prior to testing. Groups A, B, C, and D were orally administered with 0.5g, 1g, 2g and 4g of the methanolic leaf extract of Paullinia pinnata respectively.

General symptoms of toxicity like jerks and writhes were observed over 24 hours and up to 5 days.

**STATISTICAL ANALYSIS**

The data were compared using one way analysis of variance (ANOVA) and Tukey Kramer multiple comparison test. Graph pad instat version 2.05 software (UK). All data were expressed as mean ± SEM (standard error of mean).

**RESULTS AND DISCUSSIONS**

**PHYTOCHEMICAL ANALYSIS**

Table 3.1: The qualitative analysis of the leaves of Paullinia pinnata revealed the presence of the following secondary metabolites:

The result of the phytochemical screening of the leaves of Paullinia pinnata showed the presence of the following constituents; carbohydrates, reducing sugars, saponins, tannins cardiac glycosides and anthracene derivatives. Of these phytochemicals tannins are thought to be responsible for antidiarrhoeal action by increasing colonic water and electrolyte reabsorption while others may act by inhibiting intestinal motility.

**PHARMACOLOGICAL SCREENING**

Table 3.2: Inhibitory effects of the methanolic extract of the leaves of Paullinia pinnata on small intestinal transit time in mice as compared with atropine.

Values are expressed as mean of percentages travelled by the charcoal meal in relation to the full small intestinal length±SEM (n=4/group).

Level of significance: P value is 0.0002 which is very significant.

Table 3.3: Antidiarrhoeal activity of the extract and atropine on castor oil-induced diarrhoea in mice.

Figure 1: Effect of methanolic extract of Paullinia pinnata on the number of stools passed with time

Paullinia pinnata at a dose of 50mg/kg significantly (p < 0.01) decreased the total number of stools passed (6.50 ± 0.866) as compared to the castor oil treated control groups (14.25 ± 0.479), while the 200mg/kg caused a near blockade of diarrhoea induced by castor oil with only 2.25±0.75 stools passed. Atropine at a dose of 0.2mg/kg (i.p) also produced a marked antidiarrhoeal effect (7.75 ± 0.75). Thus the effect of the extract at 200mg/kg was significantly greater than that produced by Atropine. Besides decreasing the number of stools passed.It also inhibited the castor oil induced diarrhoea by delaying the onset of diarrhoea, with the 200mg/kg dose giving the highest effect (153.75 ± 27.207 minutes). While the 50mg/kg and 100mg/kg doses delayed the onset of diarrhoea by 82.0 ± 20.01 and 92.25 ± 19.76 minutes respectively. The onset of diarrhoea time is in minutes and this is the time interval between the administration of cathartic agent and the first diarrhoeic stool. The total weights of stools were also significantly lower compared with the control group.

The induction of diarrhoea by castor oil is attributed to its active ingredient ricinoleic acid, which stimulates the production of several mediator substances that include prostaglandins, nitric oxide, and platelet activating factor, cAMP and tachykinins. The induction of diarrhoea by castor oil can also be attributed to the liberation of prostaglandins by colonic cells. Inhibitors of prostaglandin synthesis such as ibuprofen and aspirin can reduce significantly, the release of prostaglandins and the volume of fluid loss induces by castor oil. Since the extract was capable of inhibiting the castor oil induced diarrhoea, Paullinia pinnata may also have the ability to inhibit prostaglandin synthesis and posses anti-inflammatory activity.

The effect of the methanolic extract of Paullinia pinnata on intestinal transit time.

The extract, besides producing an antisecretory effect, was found to inhibit the intestinal transit in mice providing 79.34% inhibition at 200mg/kg dose.

In this model, it was observed that the extract at 50mg/kg, 100mg/kg and 200mg/kg, significantly (p > 0.05, p < 0.01 and p < 0.001) inhibited the transit of charcoal meal along the intestine by 37.06%, 59.81% and 79.34% respectively. This was compared to the control group, and standard (Atropine 0.2mg/kg) group which caused 56.91% inhibition. The effect of the extract at 100mg/kg is as or more potent
than Atropine and at 200mg/kg the inhibition produced is
22% more than that produced by Atropine.

Although there was no investigation into the mechanism of
action of the extract, this reduction in percentage distance
travelled can be used to establish the intestinal smooth
muscle relaxation and antisercretory effect of the extract, this
smooth muscle relaxation may be responsible for the use of
the plant ethnomedically in treatment of menstrual cramps
and in preventing miscarriages in pregnant women.

ACUTE TOXICITY

The extract was well tolerated by the animals as no sign of
acute toxicity like restlessness, dizziness or seizures were
observed over a 24 hour period and even up to 5 days. There
were no deaths after the administration of 0.5g/kg, 1g/kg,
2g/kg and 4g/kg of the methanolic extract of Paulinnia
pinnata.

CONCLUSION

This work has demonstrated that the Methanolic extract of
the leaves of Paulina pinnata has anti diarrhoea activity
comparable to those of Atropine and hence may be
potentially useful in the management of diarrhoea and could
serve as a lead to new antidiarrhoea agents in humans, a
validation of its traditional use in the treatment of diarrhoea
in traditional medicine.

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