

Preliminary Anti-diarrhoeal Activity of Hydromethanolic Extract of Aerial Part of *Indigofera Pulchra* in Rodents

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Abstract: *Indigofera pulchra* is used as remedies for many ailments such as in the treatment of diabetes, prophylactic against snake-bites and as anti-inflammatory, but has not been investigated for its anti-diarrhoeal activity. This study was therefore aimed at investigating the hydromethanolic portion of *Indigofera pulchra* aerial part extract for antidiarrhoeal activity, using castor oil-induced diarrhoeal model in mice. The effect of these extract on perfused isolated rabbit ileum were also evaluated. The hydromethanolic extract produced a dosed dependent relaxation of the rabbit ileum. The hydromethanolic leaf extract produced a dose dependent protection against the castor oil-induced diarrhoeal with the highest protection (80%), obtained at 500mg/kg comparable to that of loperamide (5mg/kg), the standard agent. The preliminary phytochemical analysis revealed that the extract contained tannins steroids, alkaloids, saponins and flavonoids. The acute toxicity test revealed the median lethal dose (LD50) value for the extract is greater than 5000mg/kg. The result obtained revealed that the leaves possess pharmacological activity against diarrhoeal.

Key words: *Indigofera Pulchra*, antidiarrhoeal, castor oil, isolated tissue, hydromethanolic extracts

INTRODUCTION

Diarrhoeal diseases are one of the leading causes of childhood morbidity and mortality in developing countries. An estimated 1000 million episodes occur each year in children under 5 years of age. Diarrhoeal causes an estimated 5 million death in children fewer than 4 years of age per year (Carlos and Saniel, 1990). Incidence of diarrhoeal diseases still remains high despite intervention of government agencies and international organisation to halt the trend. The use of herb drugs in the treatment of diarrhoeal diseases is a common practice in many African countries (Agunu *et al.*, 2005). Despite immense technology advancement in medicine, many people in developing countries still rely on traditional healing practices and medicinal plant for their daily health care need (Ojewole, 2004). The world health organisation (WHO) encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices (Atta and Mouneir, 2004). There is therefore an urgent need for the intensification of research into medicinal plant claim to be effective in the management of diarrhoeal diseases.

A number medicinal plant have been used traditionally in the management of diarrhoeal diseases, and one of such medicinal plant is *Indigofera pulchra*. It belongs to the family Papilionaceae of the order Fabales. It is an annual non climbing herb or shrub that can grow up to 1m tall. It is widely distributed throughout west-Africa (Herper, 1976). In ethno medicine, the leaves are used to treat infected wound (Herper, 1976; Burk hill, 1995) while the decoction of the aerial part is used as prophylactic against snake-bites (Sule *et al.*, 2003) and as anti-inflammatory (Abubakar *et al.*, 2007). Recent studies

have shown that it also possesses anti diabetic activity (Tanko *et al.*, 2008). The common vernacular name of *indigofera pulchra* is Bákín búnúú in Hausa.

The present study was carried out to test the potential effects of hydro methanolic portion of *Indigofera pulchra* extract on gastrointestinal (GIT) motility in mice and intestinal smooth muscle using rabbit ileum with particular emphasis on the anti-diarrhea effects.

MATERIALS AND METHODS

Collection of Plant materials: *Indigofera pulchra* sample was collected from Samaru-Zaria in the month of October 2007 and was authenticated by A.U. Gallah of the herbarium section in the department of Biological Sciences, Ahmadu Bello University Zaria-Nigeria where a voucher specimen (No.6558) was deposited at the herbarium for future references.

Preparation of plant materials: The *Indigofera pulchra* was air dried under shade and then ground to powder. 3kg of *Indigofera pulchra* was put in a bucket and 2 liters of methanol (75%) and 75cl (25%) of water was poured into it and it was allowed to soak for 5 days. Then it was filtered using filter paper, beaker and funnel. The filtrate was then transferred to an evaporating dish and was evaporated using a water bath. The extract was obtained after evaporation from the water bath and refrigerated till the day of the experiment. Solutions of the extracts were prepared freshly for each study.

Animals: New Zealand rabbit (800g) and Swiss albino mice (16-30g) maintained in the Animal House Facility of the Department of Pharmacology and Clinical Pharmacy,

Ahmadu Bello University Zaria, Nigeria were used in these experiments. The animals were maintained on standard animal feed and water *ad libitum*. This research was carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

Drugs: Acetylcholine and Adrenaline (Sigma chemical, USA) Castor oil (Bell Sons and Co., England) and Loperamide (Janssen)

Phytochemical procedure: The preliminary phytochemical screening of the crude extract of *Indigofera pulchra* was carried out in order to ascertain the presence of its constituents by utilizing standard conventional protocols (Trease and Evans, 1989).

Acute Toxicity Study: The method previously described by Lorke (1983) was adopted using 13 mice. In the first phase, three doses of the hydromethanolic extract (10, 100 and 1000mg/kg were administered to three groups each containing three mice). In the second phase, more specific doses were administered to four groups each containing one mouse. The median lethal dose (LD₅₀) was determined as the geometric mean of the highest non lethal dose and lowest lethal dose of which there is 0/3 and 0/1 survival.

Effects on isolated rabbit ileum: The rabbit was made unconscious by a powerful strike at the back of the neck (stunning). The abdomen was immediately opened using forceps and part of the ileum was quickly removed.

The ileum was introduced immediately into a Petri dish of saline containing Tyrode solution and each end of the ileum was tied with a thread. The ileum was then suspended into the organ bath, which contains supply of oxygen and air to the ileum in organ bath. One end of the ileum was attached to the transducer which measured the mechanical impulse of the tissue and converted it to electrical impulses which was then recorded on microdynamometer. The temperature of the inner bath was kept at temperature 37^oc by the thermo regulator. Subsequently, solutions of acetylcholine and adrenaline

Indigofera pulchra extracts were added at intervals to the isolated perfuse chamber. After application of each drug, the tissue was washed three times with the Tyrode solution to remove every trace of the drug.

Effects of castor oil induced diarrhea in mice: The mice were fasted for 12 hours prior to the commencement of the experiment and were randomly divided into five groups of five mice each. The mice in the first group received 10mlkg⁻¹ normal saline intraperitoneally while the mice in the second received 5mlkg⁻¹ Loperamide as a standard positive control, the third, fourth and fifth 100, 250 and 500mg/kg, respectively. After 30 minutes of administration of the extract, castor oil 0.2ml/ mouse were administered orally. The animals were placed on individual special cages over white clean whatman filter,

three hours after castor oil challenge. The cages were inspected for the presence of the characteristic diarrhoea droppings. The absence was recorded as a protection from diarrhoea (Diurno, *et al.*, 1996) and the percentage protection was calculated (Akah and Offiah, 1996).

Statistical analysis: The results were analysed by chi square (X²). Values were considered significant with P < 0.05 for both isolated tissue and castor oil induced diarrhoea.

RESULTS

Phytochemical analysis: The preliminary phytochemical screening of the extract revealed the presence of alkaloids, saponins, flavonoids, tannins and steroids.

Acute Toxicity study: The median lethal dose of the extract was found to be greater than 5000mg/kg bodyweight.

Effect of hydro-methanolic extract of *Indigofera pulchra* On isolated rabbit ileum: Fig. 2 showed the effects of the plant hydromethanolic extracts on the isolated rabbit ileum were dose related the extract relaxed the spontaneous contraction of the rabbit ileum.

Effect of *Indigofera pulchra* on castor oil induced diarrhoeal: The extract produced a dose dependent protection against the castor oil- induced diarrhoeal with the highest protection (80%) obtained at the highest dose tested (500mg/kg) comparable to that of loperamide, the standard anti diarrhoeal agent.

DISCUSSION

The median lethal dose of the extract was greater than 5000mg/ kg .bodyweight Castor oil causes diarrhea due to its active metabolite, ricinoleic acid (Ammo,*et al.*, 1974; Watson and Gordon,1962), which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin (Galvez *et al.*, 1993).

Acetylcholine (Ach) cause increased contraction of the isolated jejunum as shown in Fig. 1. However, *Indigofera pulchra* significantly reduced intestinal transit time as observed by the decrease in intestinal motility of isolated rabbit jejunum as shown in Fig. 2. The relaxation effects of the extract is also similar to adrenalin as shown in Fig. 3. The effects of both the extract and adrenalin were blocked by propranolol, which is a beta blocker as shown in Fig. 4. This suggests that the extract may be acting via beta receptors. Phytochemical screening revealed the presence of alkaloids, tannins and sterols. Earlier studies showed that anti-dysenteric and anti-diarrhea properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids and sterols

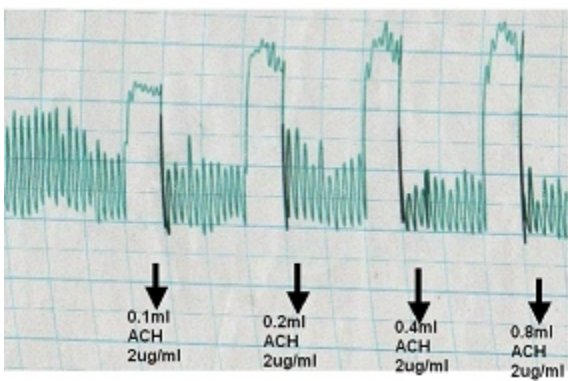


Fig. 1: Effects of Acetylcholine (ug/ml) on isolated rabbit ileum

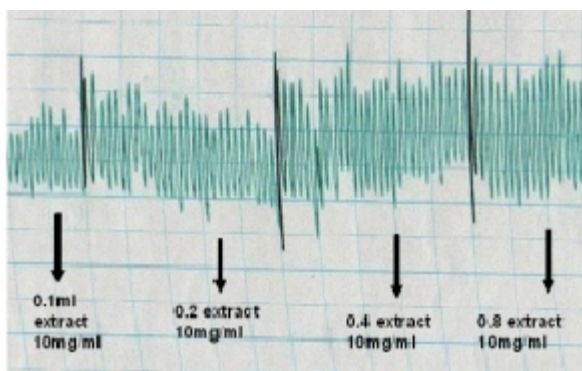


Fig. 2: Effect of Hydromethanolic extract of aerial part of *Indigofera pulchra* on isolated rabbit ileum

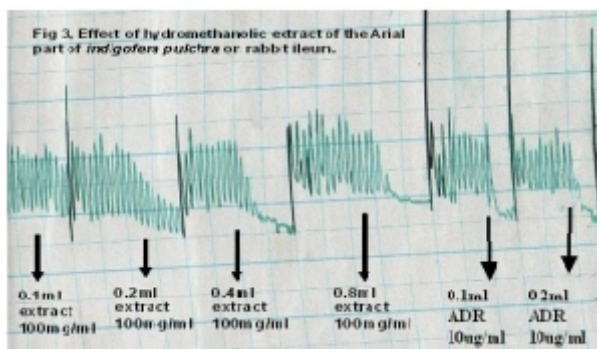


Fig. 3: The effect of hydromethanolic extract of aerial part of *Indigofera pulchra* and Adrenaline on rabbit ileum

(Galvez *et al.*, 1991; Loganga *et al.*, 2000). Hence, tannins, sterols, alkaloids may be responsible for the mechanism of action of *Indigofera Pulchra* anti-diarrhea activity. The anti-diarrhea activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistance and hence, reduce secretion. This can be due to the fact that the extract increased the reabsorption of by decreasing intestinal motility in isolated rabbit ileum.

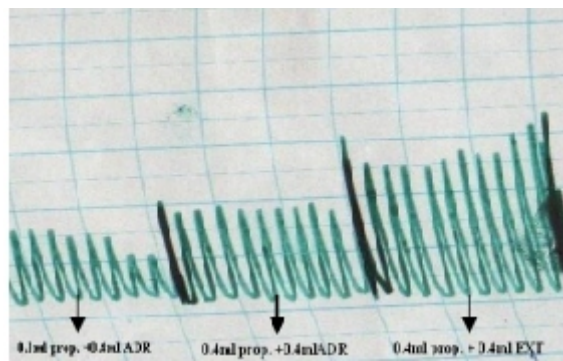


Fig. 4: Effects of Propanolol, Adrenaline and *Indigofera Pulchra* Extract on Isolated Rabbit Ileum

Table 1: Effects of hydro-methanolic extract of *Indigofera pulchra* on castor oil Induced diarrhoeal in mice

Treatment	Dose (mg/kg)	No of mice with Diarrhoeal	Protection (%)
Normal saline	10ml/kg	5/5	0.0
Extract	100	3/5	40
Extract	250	2/5	60
Extract	500	1/5	80
Loperamide	5	0/5	100

Results were analyzed by Chi-square (χ^2). Values were considered significant when $P < 0.05$ compared with Normal saline group, $n=5$

Mice administered with 100, 250 and 500mg/kg hydro-methanolic extract of *Indigofera pulchra* had diarrhea in 3/5, 2/5 and 1/5 respectively (40, 60, and 80% protection respectively) as shown in Table 1

In this study, hydro-methanolic extract of *Indigofera pulchra* exhibited a significant anti-diarrheal activity. Its effect depended on the dose. The results were similar to that of the standard drug Loperamide 5mg/kg with regard to the severity of diarrhea.

The observed relaxation exhibited by the leaves extract further explains its ability to protect the mice against diarrhea induced by castor oil.

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