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Antidiarrhoeal Properties of *Acacia Nilotica* Leaves on Ileum of Guinea Pig and Castor Oil-induced Diarrhoea in Rats

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Abstract
Acacia nilotica is a dicotyledonous plant used in traditional medicine in Nigeria and Senegal for treating stomach and intestinal disorders. The antispasmodic activity of the leaf extract was assessed on contraction of isolated ileum of Guinea pig induced by acetylcholine and amechol. The effect of the leaf extract of *Acacia nilotica* on castor oil induced diarrhoea in rats and was undertaken. The study showed that *Acacia nilotica* is a good relaxant of Guinea pig isolated ileum. In addition to antispasmodic activity in vitro, the leaves extract inhibited castor oil-induced diarrhoea in rats. The LD_{50} was also calculated to be 1890mg/kg which is higher than the minimum recommended according to the Hodgel and Sternel scale. The inhibition effect of contractile activity of the ileum is basis for the treatment of some gastrointestinal disorder. The result showed that the plant leaves have clinical benefits in the treatment of diarrhoea and dysentery related diseases.

Introduction

Diarrhoea and dysentery epidemics are mostly common where the living conditions are crowded and hygiene is poor (IFGA 2008). Dysentery and diarrhoea are caused by microbial infections of gastrointestinal tract. Symptoms include fever, vomiting and abdominal pain and vomiting which often contains blood and pus (GBD 2008). Diarrhoea could also be caused by some diet and reactions to some drugs. The onset of the disease usually occurs within 2-3 days after infections and can last for several weeks.

Dehydration occurs rapidly especially in children and can cause death if treatment is not given. Infectious diseases such as dysentery and diarrhoea are the main causes of high mortality rate in developing countries. About five million children under the age of five die annually from severe diarrhoeal diseases (Akuodor et al. 2011). Limited access to formal and adequate health care services makes it imperative for the rural dwellers to seek help from traditional healers who provide alternative health care services. Indigenous plant remedies are widely used in treating these diseases conditions.

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Acacia nilotica has been used in folk medicine due to its wide range of biological activities (Hemamalini et al. 2013). It is a dicotyledonous plant of the family *Leguminoseae* sub-family *Mimosacea* (Haytham et al. 2013). The genus Acacia is a large genus consisting about 700-800 species. It is widely distributed in Africa, Arabia, Mexico and Indo–Pakistan subcontinent. *Acacia nilotica* are widely distributed in Africa due to its use in re-forestation programmes to check desert encroachment. It is also used in dehairing as well as tanning of leather (Ezeamagu et al. 2011). In this study, antispasmodic activity of this plant extract was evaluated using an animal model.

Materials and Methods

Plant Material

The leaves of *Acacia nilotica* were collected at the road side leading to the main campus of Usmanu Danfodiyo University, Sokoto-Nigeria in September, 2001 and the leaves were air dried under shade. Taxonomic identification of the plant was carried out and authenticated at the Botany Unit, Department of Biological Sciences of Usmanu Danfodiyo University, Sokoto. The dried leaves were powdered and stored in polythene bags and kept safely at room temperature until needed for analysis.

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Preparation of Extract
Two hundred grams (200g) of powdered leaves were subjected to extraction with methanol in a soxhlet extractor at 80^{o}C. The solvent was evaporated using a rotary evaporator; the viscous liquid obtained was then dried on a water bath (Odebiyi and Sofowora 1979). The residue obtained was weighed and the percentage extraction by dry mass was computed and recorded. This extract was labelled methanolic extract of *Acacia nilotica*.

Castor Oil-induced Diarrhoea in Rats
The method followed here was the method used by Awouters et al. (1978) with modification in terms of fasting period. In the present study, rats of both sexes (100-140g), were fasted for 18 hours. The animals were housed in six perforated steel cages, each containing five rats. The methanolic extract was administered orally at doses of 200, 400, 600 and 800mg/kg body weight as suspension to first four groups of animals. The fifth group received diphenoxylate (5mg/kg) orally as suspension; the standard drug for comparison. The sixth group received 2% (w/v) aqueous Acacia suspension only (pre-treatment). After one hour administration of the extract, 2ml of castor oil (p.o) was orally administered. The animals were placed in their individual cage lined with adsorbent white paper. The animals were then observed for 4 hours for the characteristics diarrhoea droppings.

Studies on Isolate Guinea-pig Ileum
Two male Guinea-pigs bought from Sokoto Central market (Nigeria) were made to fast for a day and were sacrificed. Portion of their ileum was removed immediately and placed in oxygenated Tyrode's solution at room temperature. The connective tissues were carefully trimmed from the intestinal tissue (2cm long) and then suspended in a 20cm^3 organ bath containing an aerated Tyrode solution at 37^{0}C from a resting tension of 1g. Responses were recorded on a smoked kymograph paper using an isotonic frontal writing lever (Magnitude 7 time). After 60 minutes of equilibration period, responses of acetylcholine drug, histamine drug and amechol were recorded in the absence of the extract. Each contractive agent (drugs) has a concentration of 0.002mg/ml and was used on a separate preparation. Extracts were added directly to the organ bath in volumes of 0.2, 0.8, 1.6, 2.4, 3.2 and 6.4cm^3 at any given minute and then washed out before another drug was added. Volume of the extract (2.4cm^3) was added together with acetylcholine sulphate to determine the level of relaxation relative to effect by acetylcholine sulphate alone.

Acute Toxicity Studies
This was carried out in vivo using white albino rats of both sexes weighing between 100-140g. All solutions were prepared using distilled water and all administrations were by intraperitoneal route. Initial pilot studies were carried out to determine the maximum dose of leaf extract of *A. nilotica* that did not produce death and the minimum dose that produced 100% death. In between this dose range, three doses were selected at geometric intervals for the studies with equal number of rats in each group dose range. Each group of rats were placed in clean rat cage and injected with the leaf extract at varying doses. A control group also comprising of five rats were injected with 2cm^3 of distilled water. The symptoms of toxicity in the rats were observed in order of severity and recorded. The number of rats that died within 24hrs was also noted. The LD_{50} of aqueous leaf extract was calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982) with the formula;

$LD_{50} = LD100-(Dd \times MD)/n$

Dd = Dose difference, MD = Mean death,
n =No of death

Results

Castor Oil-induced Diarrhoea Test (Dropping Test)
The oral treatment of rats with 2% aqueous suspension of the plant extracts at 0hrs

(control) after castor oil induction yielded appreciable feacal droppings of 29.0±1.97 while the Standard diphenoxylate (5mg/kg) had mean dropping of 13.1±2.97. The mean droppings were highest in Castor oil-induced rats treated with acacia extract (200mg/kg) while, mean dropping was least when the dose was increased to 800mg/kg (Table 1).

Table 1: Effect of Methanol leaf Extract of *Acacia nilotica* on Castor Oil induced Diarrhoea in Rats

Treatment	Doses	Mean No of feacal droppings 4hrs±S.D
pre-treatment at 0hr	Control: Acacia suspension (2%)	29.0±1.97
	Standard diphenoxylate (5mg/kg)	13.1±2.97
Acacia extract	200 mg/kg	22.8±1.8
	400 mg/kg	19.4±2.04
	600 mg/kg	17.3±2.00
	800 mg/kg	14.9±1.80

Effect of acacia extract on smooth muscle

The relative relaxation of the smooth muscle of Guinea pig ileum increased with increasing concentrations of the methanolic leaf extract of *Acacia nilotica* (Table 2). The relative contraction on smooth muscle of Guinea pig ileum when standard drugs (Histamine and Amechol) were used for relaxation study was similar, with Acetylcholine producing highest relaxation (Table 3). However, there was a drastic reduction in relaxation effect when a consortium of Acetylcholine and leaf extract was used for the experiment.

Table 2: The Result of the Relative Relaxation of the Smooth muscle of Guinea Pig Ileum with changing Concentrations of the Methanolic Leaf Extract of *Acacia nilotica*

Vol. cm^3	mg/cm^3	Length of relaxation (cm)	% Relaxation
0.1	0.002	0.30	12.0
0.8	0.016	0.60	24.0
1.6	0.032	0.90	36.0
2.4	0.048	1.10	44.0
3.2	0.064	1.15	46.0
6.4	0.0128	1.15	46.0

Table 3: Concentration of Standard Drugs and their Relative Contraction on Smooth Muscle of Guinea Pig Ileum

Drugs (2mg/cm^3)	Length of relaxation (cm)
Histamine	1.1
Amechol	1.1
Acetylcholine	2.5
Acetylcholine + 48mg/cm^3 of extract	0.4

Acute Toxicity test

The Acute Toxicity studies indicated that when 900, 1800 and 3600mg/kg doses of the leaf extract were administered into the rats, zero, three and five rats died respectively. The toxicity level (LD_{50}) of the extract was calculated as 1890mg/kg according to Table 4.

Table 4: Arithmetic Method of Calculating the Lethal Dose (LD_{50})

Plant extract dose (mg/kg) body weight	Dose Difference (D.d) (mg)	Death	Mean Death (MD)	D.d ×MD
900	450	0	0	0
1800	900	3	1.5	1350
3600	1800	5	4	7200
				8550

LD_{50} = 360-(8550/5) =1890mg/kg

Discussion

The methanol extract of *Acacia nilotica* has shown dose-dependent anti-diarrhoeal activity in castor oil induced diarrhoea in rats. This was in conformity with Awouters et al. (1978). Methanolic extract of the Acacia leaves also indicated a decrease in the intestinal propulsive movement in vitro. The gradual increase in concentration of the extract gave a relative relaxative effect on the smooth muscle of a Guinea-pig. The methanolic leaf extract of the *Acacia nilotica* significantly inhibited the frequency of defecation when compared to untreated control rats.

Phytochemical studies earlier carried on the plant methanolic extract of the leaves showed that saponins, tannins, phenolics and resins were present (Ezeamagu et al. 2011). Tannins and related phenolics have been reported ealier to react with protein to form protein tannate, which makes the intestinal mucosa more resistant and reduce secretion by virtue of which many different species have been reported to possess antidiarrhoeal effect. (Tripath 1994; Mukherjee et al. 1995). Also, prostaglandin contributes to the patho-physiological function of the gastrointestinal tract and also acts on the local electrical and mechanical activity of ileac circular muscles

(Odo et al. 2013). Castor oil increase persistent activity and produces permeability changes in the intestinal mucosal membranes to electrolyte and water (Ezeonwumelu et al. 2012). Induction of diarrhoea by castor oil is through elevated prostaglandin biosynthesis. Strong chelation properties were shown by thyroxin, histamine, prostaglandins and chelation action of these prostaglandins with tannins, saponins and phenolics could be responsible for the reduction in propulsive movement of the small intestinal tract or may inhibit the local or mechanical activity of circular muscle of ileum which consequently results in the reduction of fluid accumulation and diarrhoea (Hagerman et al. 1998).

Toxicity/safety assessment of the leaf extract indicated that the LD_{50} was relatively high compared to the minimum level of 1000mg/kg on the Hodge and Sternel Scale. However, another research indicated that leaf extract of *A nilotica* showed low toxicity (Ahmed et al. 1998). According to this author, there were no death, significant changes in serum parameters of hepatic renal function and histological changes in liver section. Since, the extract contains both tannins and saponins, the toxicity level can be tolerated because tannins are chelating agent as well as antioxidant (Hagerman et al. 1998). It chelates not only toxic metals, but also anti-metabolites where the products become insoluble for easy excretion (Oakenfull 1990). Saponins on the other hand cause depletion of body cholesterol by binding with it and preventing re-absorption into the body system and thus enhancing excretion (Oakenfull 1990).

Conclusion

Since, the methanolic extract from previous antimicrobial studies (Ezeamagu et al. 2011) showed that the extract has effect on diarrhoea and dysentery causative agents such *E. coli*, in addition to relaxation effect of castor oil induced diarrhoea in rat, it is an indication that the extract can be an alternative to the conventional chemo-therapeutic options for effective treatment of both diarrhoea and dysentery related diseases.

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