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Original Research Article

Evaluation of the antidiarrheal effects of the methanol extract of *Waltheria indica* L. (Sterculiaceae) leaves in albino mice and rats

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Abstract

Purpose: *Waltheria indica* is a medicinal plant used in ethnomedicine to control dysentery and diarrhea. This work was therefore carried out to evaluate the effects of the leaves on the gastrointestinal tract using animal models.
Methods: After phytochemical screening, the powdered leaves were extracted with methanol using a Soxhlet apparatus. The acute toxicity of the methanol extract (1-5 g/kg) was evaluated in mice. Using charcoal meal intestinal transit and castor oil induced diarrhea models, the effect of the extract (100-400 mg/kg) on intestinal motility was evaluated in mice and rats respectively. The extract (20-80 mg/mL) was further evaluated on Ach-induced ileum contraction.
Results: The leaves of *W. indica* tested positive to the presence of alkaloids, saponins, tannins, cardiac glycosides, flavonoids and anthraquinones. Mice tolerated the extract up to 5.0 g/kg. The extract was observed to significantly ($p < 0.05$) reduce the motility of the charcoal meal in mice gastrointestinal tract. In the control animals, the charcoal moved up to 71.83 % of the intestine, whereas in the animals treated with 400 mg/kg, the movement was reduced to 42.87 % compared to 39.53 % in animals treated with atropine (0.1 mg/kg). In castor oil-induced diarrhea, the onset of stooling was 10 ± 1.0 min in the control animals after administration of the castor oil and 5.2 ± 0.9 wet stools were produced. The extract at 100 mg/kg delayed the onset of stooling to 62.33 ± 2.3 min; reduced the number of stools to 1.2 ± 0.6 while the animals treated with Loperamide (5.0 mg/kg) did not produce any stool. The extract significantly ($p < 0.05$) attenuated the ileum contraction produced by Ach in a concentration-dependent manner.
Conclusion: The results confirm the ethnomedicinal application of *W. indica* leaf in the management of diarrhea.

Keywords: *Waltheria indica*, phytochemical screening, antidiarrheal effect

Indexing: Index Copernicus, African Index Medicus

Introduction

Medicinal plants have always served as alternative means of combating health challenges and conditions that cause discomfort or pose a threat to the wellbeing of humans and animals. They have provided health solutions to many discomforting situations like diarrhea which has long been described as the major cause death in children [1] and the need to urgently reduce the scourge by two-thirds before 2015 was emphasized as part of Millenium Development Goals [2]. Diarrhea is more prevalent among adults who are exposed to children and non-toilet-trained infants, particularly in a daycare setting. It is also more prevalent in travelers to tropical regions, persons with underlying immunosuppression, and those living in non-hygienic environments who are exposed to contaminated water or foods [3]. In addition to improved hygiene, many synthetic drugs are available for effective control of

diarrhea particularly before it gets to advanced stages. However, non-availability of such drugs to rural dwellers as well as lack of sufficient health personnel to manage rural health facilities may contribute to the use of medicinal plants to manage the ailment. Among the plants used by the herbal medicine practitioners in the South Western part of Nigeria is *Waltheria indica* L. (Sterculiaceae). It is commonly called Sleepy morning. The local names in Nigeria include "Korikdi, Omodan" in Yoruba; "Hankufa" in Hausa; "Kakafi" in Fulani; "Ngamzina" in Kanuri and "Ewe-epo" in Ibo [4].

W. indica is commonly used in traditional medicine in Africa, South America and Hawaii against pain inflammation, diarrhea, dysentery, conjunctivitis, wounds, abscess, epilepsy, convulsions, anemia, erectile dysfunctions, bladder ailments and asthma [5]. In Nigeria, it is used for the treatment of sexually transmitted infections, urinary tract infections and a variety of infant illnesses. Its root's extract are reported to treat ailments such as diarrhea, wounds and stomach ache [6]. Whole plants may be used to treat cough, haemorrhage, fever and malaria amongst others [7]. In this part of the world, there is no scientific information on the probable effect of this plant on the intestinal motility. This work was therefore carried out to ascertain the claimed ethnomedicinal use of the plant in diarrhea treatment.

Methods

Collection and preparation of the plant materials

The leaves of *Waltheria indica* were collected in June 2012 by a plant collector, Alabi Najimdeen, at Iwo in Osun State. The identity of the plant was authenticated by Dr. Olufemi Soshanya of Forest Research Institute of Nigeria (F.R.I.N) Ibadan where herbarium specimen was deposited and FHI109766 issued as the herbarium number. Another herbarium specimen of the plant was also deposited at the herbarium section, Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.
The leaves were spread on the laboratory table to dry for three days and further dried in the oven maintained at 50^{O}C for 4h after which it was reduced to powder form using a laboratory milling machine (Chris Norris, England, UK). The powdered material was kept in an airtight container for subsequent use.

Preliminary phytochemical screening

Phytochemical screenings were performed using standard procedures [8,9].

Extraction of the plant materials

The powdered plant material (1.5 kg) was extracted using Soxhlet apparatus with 7.5 L of methanol for 4-5 h in the batch of 180-200 g at room temperature. The extract obtained was concentrated to dryness using water bath maintained at 50 °C to obtain a soft extract which was kept in a refrigerator maintained at 4 °C until required.

Source and maintenance of experimental animals

Male and female albino mice (20-25 g) and rats (180-230 g) were purchased from Pharmacology Department in Ambrose Ali University Ekpoma, Nigeria. The animals were maintained in animal house of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin with adequate access to food (Top Feeds, Benin City) and water before use.

Proximate content analysis

Batches of 20 mice (20-25 g) of either sex and were divided randomly into four groups of five mice each. Group one served as control and was administered distilled water (1.0 mL) and the other groups received 1, 2 and 5 g/kg of the methanol extract respectively. The general signs of symptoms of toxicity and mortality were observed for 24 h [10].

Determination of antidiarrheal effects

Effects of the methanol extract on mice small intestinal transit using charcoal model

Mice subjected to 24 h fasting were divided into five groups of five animals each. Group 1 animals were administered 1mL distilled water orally whereas groups 2, 3 and 4 received plant extract at doses of 100, 200 and 400 mg/kg respectively. Group 5 was given atropine (0.1 mg/kg i.p.). 1.0 ml of marker (10 % activated charcoal suspended in distilled water) was administered orally to each animal after treatment with plant extract. The mice were sacrificed after 1 h according to International Protocols via chloroform anesthesia. After opening the animals, the distance travelled by

charcoal meal from the pylorus was measured as percentage of total length of the intestine from the pylorus to caecum. The results were expressed as percentage distance travelled compared with the negative control [10].

Effects of the methanol extract on castor oil-induced diarrhea

Albino Wistar rats (180-230 g) were divided into five groups of five animals each. Group 1 which served as control was given 2 mL/kg distilled water orally, while groups 2, 3 and 4 received 100, 200 and 400 mg/kg orally of the extract respectively. Group 5 was administered atropine (5 mg/kg), 1 h before oral administration of 0.5 mL castor oil. The numbers of both wet and dry diarrheal droppings were counted every hour for a period of 4 h. Mean number of stools passed by each of the groups treated were compared with that of the control group i.e. animals given distilled water. Also noted was the average onset of stooling for each group while weights of the stool produced were recorded [10].

Effects of the methanol extract on isolated ileum

Albino wistar rats weighing (180-230 g) were sacrificed using chloroform as anesthesia. The abdominal cavity of the animals was immediately opened and the ileum 1-1.5 cm in length was removed and trimmed from surrounding tissue. The contents of the intestine were washed with Physiological Salt Solution (PSS) Tyrode solution. Segments of the ileum were tied with silk threads at both ends (ileum tied in opposite directions) and suspended in a thermo-regulated 25 mL organ bath containing Tyrode solution maintained at 37 °C. The ileum was attached to a tissue holder at the base of the organ bath and the other end to the isometric channel recorder through an isometric transducer. The tissues were constantly bubbled acetylcholine or the particular plant extract or the standard drugs. After the initial equilibrium period, acetylcholine (Ach) at concentrations of 20-80 µg was added to the organ bath and the control concentration-response curve for Ach was determined. Each time the Ach was left in contact with the tissues for 30 s before adding the next concentration. Then the tissue was washed two times with Tyrode solution at the interval of 90 s. The effect of methanol extract on the isolated ileum was evaluated by simultaneous administration of the various concentrations of the Ach and 0.2, 0.4 and 0.8 mL of 100 mg/mL of the extract. These implied 20, 40 and 80 mg of the extract respectively. Atropine (25 µg/mL) was used as standard while the speed of the channel recorder was maintained at 5 mm/min [10].

Statistical Analysis

All the data obtained were expressed as mean ± SEM (standard error of mean) and n represents the number of animals used. Where applicable, the data were compared using one way analysis of variance (ANOVA), Graph pad InstatR version 2.05a software (UK). The level of significance was from $P < 0.05$.

Results

W. indica leaves was observed to test positive to the presence of alkaloids, anthraquinones, tannins, flavonoids, saponins and cardiac glycosides (Table 1).

Table 1: Summary of results of the phytochemical screening of the leaf of *W. indica.*

Constituents	Leaf
Alkaloids	+
Anthraquinones	+++
Flavonoids	+++
Tannins	+++
Saponins	+++
Cardiac glycoside	+++

+ Trace +++ Abundant

Results of antidiarrheal effects of methanol extract of W. indica

Toxicity test

After oral administration of extract (1-5 g/kg) to the mice followed by observation for 24 h, no death was recorded. This implied the safety of the extract within the period.

Small intestinal transit (Charcoal model)

The extract and atropine (0.1 mg/kg) were observed to significantly ($P < 0.05$) reduce the intestinal movement of the charcoal meal in mice. In the control animals, the activated charcoal was observed to move a distance of 33.9 ± 0.1 cm out of 42.28 ± 0.9 cm implying 71.83 % of intestinal transit. However, in animals treated with 100mg/kg of the extract, the intestinal transit was reduced to 44.71 %, and in

the animals treated with 400 mg/kg, the charcoal movement was further reduced to 42.87 % of intestinal length. Atropine (0.1 mg/kg) reduced the charcoal movement to 39.53 % (Table 2).

Castor oil induced diarrheal

While the animals in the control group produced 5.2 ± 0.9 stools which weighed 1.16 ± 0.1g at the end of the experiment, the methanol extract (100-400 mg/kg) significantly ($p < 0.05$) delayed the onset of stooling, reduced the number of stools produced and as well as the weight of stools below the controls. For instance, the animals treated with 400 mg/kg of the methanol extract produced 0.8 ± 0.6 stools which weighed 0.23 ± 0.2 g. While the control animals started stooling at 10 ± 1 min after the castor oil administration, the treated animals did not produce stools until after 1 h, and those treated with Loperamide did not stool throughout the period of experiment (Table 3).

Table 2: Effects of the methanol extract on intestinal transit

Doses (mg/kg)	Mean length of intestine (cm)	Distance moved by charcoal (cm)	% Intestinal transit
Control	42.28 ± 0.9	33.96 ± 0.1[a]	71.83[a]
100	45.62 ± 0.9	20.40 ± 0.4[b]	44.71[b]
200	44.46 ± 1.5	19.80 ± 0.4[b]	44.53[b]
400	45.72 ± 1.3	19.60 ± 0.5[b]	42.87[b]
Atropine (0.1 mg/kg)	47.46 ± 0.6	18.76 ± 0.4[b]	39.53[b]

Values with different letters are significantly different from one another ($p < 0.05$); n = 5

Table 3: Effects of the methanol extract on castor oil - induced diarrhea

Doses (mg/kg)	Onset of stooling (min)	Number of stools	Weight of stools (mg)
Control	10 ± 1[a]	5.2 ± 0.9[a]	1.16 ± 0.1[a]
100	62.33 ± 2.3[b]	1.2 ± 0.6[b]	0.29 ± 0.1[b]
200	61.5 ± 1.5[b]	1.2 ± 0.1[b]	0.29 ± 0.2[b]
400	60 ± 0[b]	0.8 ± 0.6[b]	0.23 ± 0.2[b]
Loperamide (5 mg/kg)	0[c]	0[c]	0[c]

Values with different letters are significantly different from one another ($p < 0.05$); n = 5

Results of the effect of methanol extract on isolated ileum

Ach was observed to evoke concentration – dependent contractile effects on the isolated rat ileum with the highest effect, C_{max} produced by 80 µg/mL of the drug. The contractile effect of each concentration of the Ach was attenuated by 20, 40 and 80 mg of the crude extract. These inhibitions of contractions consequently and significantly reduced the C_{max} of the Ach. For example with the presence of 20mg of the extract, the C_{max} was reduced to 69.70 ± 8.32 %. 80 mg of the extract further inhibited the contraction produced by the Ach by reducing the C_{max} to 48.32 ± 7.14. (Fig 1).

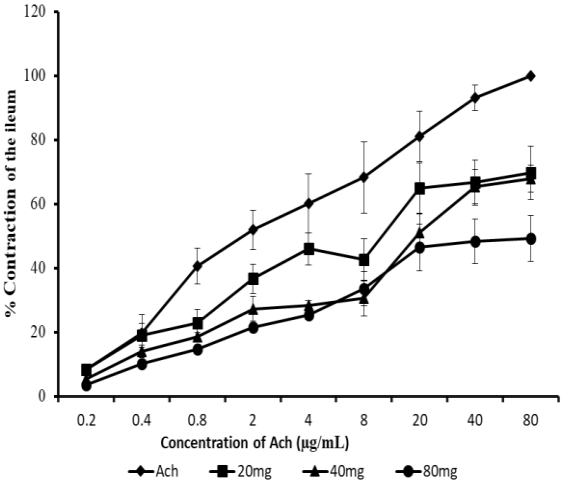

Fig. 1: Inhibitory effects of methanol extract of *W. indica* leaf on Ach-induced rat ileum contraction. (n=5).

Discussion

As none of the animals died within the period of experiment, the extract of *W. indica* can be regarded safe between 1-5 g/kg. The distance moved by the charcoal marker is a function of the rate at which the intestine contracts which in turn is a product of functionality of muscarinic agents and receptors. It is possible the constituents found in the extract affected the contractile agents in the intestine in order to bring about remarkable reduction in the extent the charcoal meal traveled. The inhibitory effect of the extract can be likened to that of atropine which is a known antimuscarinic agent [11].

The probable inhibitory effect of the extract on the intestinal motility was also suggested by the delay in the onset of stooling in treated animals as compared to the control animals. Although, there was no remarkable difference in the activity of the different doses, the fact that significant difference was observed between extract-treated animals and the control implied the relaxant effect of the extract. The absence of remarkable difference in the activity of the extract at the different doses may be due to the saturation of the active sites or receptors in the intestine by 100 mg/kg. All the stools produced by the control animals were more in number and wet in nature which could explain the high weight recorded.

It has been noted that diarrhea is a product of various pathological mechanisms. These include increased active secretion and decreased absorption of water and electrolytes which result in reduced transit time and abnormal motor activity usually observed in patients with diarrhea [12]. Diarrhea caused by small intestine disease is typically high volume, watery and often associated with mal-absorption. Dehydration is frequent. Diarrhea caused by colonic involvement is more often associated with frequent small-volume stools and a sensation of urgency [13].

The mechanisms of action of castor oil in inducing diarrhea have been variously reported to include stimulation of intestinal secretions and reduction of absorption through inhibition of Na^+, K^+-ATPase enzyme [14,15]. It has also been observed to involve the stimulation of prostaglandins [16]. All these have been attributed to the presence of ricinoleic acid - the major constituent [17] which exerts laxative effects by activation of intestinal muscles via EP_3 prostanoid receptors [18]. The entire activities of the extract of *W. indica* can be explained within these contexts. The early onset of stooling observed in the control animals may be due to stimulation of the intestinal secretions by the castor oil coupled with induction of peristalsis. It can be inferred that the extract possibly prolonged the onset of stooling by reducing or inhibiting the rate of intestinal secretion and enhancing the absorption of water and electrolytes. These invariably resulted in production of dry stools with reduced weight and number. The loperamide - treated animals did not produce any stool throughout the period of experiment. The pharmacological effect of loperamide is due to its antimotility and antisecretory properties [19]. It can be inferred that the constituents of the crude extract possibly mediate their effects through similar mechanism.

Extracts of plants that contain flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) thereby inhibiting prostaglandin production [20]. The activation of LOX is induced by fatty meals while COX-1 and COX-2 is by diarrhea-genic agents [21]. The extract may have interfered in the activities of these enzymes which culminated in the results obtained. Furthermore, the extract inhibited spontaneous contraction of rat ileum induced by the Ach. The inhibitory effects were more pronounced on the lower concentrations of the Ach. Ach is known to be stimulator of muscarinic receptors in the intestine and this explains its use in the investigation of contractile or relaxant effect of drugs and tissues like the intestine.

The fact the crude extract at all concentrations (20, 40, 80 mg/ml) attenuated the contractile effects of the Ach on the ileum further suggested that the constituents of the extract were acting like anti muscarinic agents as earlier observed. The IC_{50} (the concentration of Ach to produce 50 % contraction) for the Ach alone was 1.80 µg/ml. This increased to13.25, 20, 80 µg/ml in the presence of 20, 40 and 80 mg/ml of the extract respectively. All these point to the fact the methanol extract of *W. indica* possesses constituents capable of exerting relaxant effects on a diarrheal intestine which forms the basis for its use in ethnomedicine as an antidiarrheal crude drug. As the plant was observed to contain different groups of secondary metabolites,

further work would be required to ascertain the particular constituent(s) that could be responsible for the antidiarrheal activity.

Conclusion

There is a continuous need to search and investigate the efficacy and safety of medicinal plants used in ethnomedicine. Aqueous extract of *W. indica* leaves claimed to be useful in mitigating or completely stopping diarrhea was investigated and observed in this work to prolong onset of diarrhea, reduce number and weight of stools in the laboratory animals administered with castor oil. The intestinal relaxant effect of the extract was further established on isolated tissue experiments thus justifying its use in ethnomedicine.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was carried out by the authors named in this article all liabilities pertaining to claims relating to the content of this article will be borne by the Authors. BAA conceived and designed the work, OJO supervised the pharmacological evaluation and ISU carried out the work while MIC is a research collaborator.

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