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Full Length Article

A controlled study to investigate anti-diarrhoeal effect of the stem-bark fractions of *Terminalia avicennioides* in laboratory animal models

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ABSTRACT

Due to the shortcomings associated with modern synthetic antidiarrhoeal drugs, it is important to find newer, safer and cheaper antidiarrhoeal agents from natural sources. The study was conducted to evaluate the anti-diarrhoeal activity of the fractions of the stem-bark of *Terminalia avicennioides* in laboratory animal models. The effect of different concentrations (1.0×10^{-3}, 2.0×10^{-3}, 4.0×10^{-3} and 8.0×10^{-3} mg/mL) of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HXF) fractions of *T. avicennioides* were tested against spontaneous and acetylcholine-induced contractions of rabbit jejunum as well as on histamine-induced contraction of guinea pig ileum. Similarly, the effects of the AMF on gastro-intestinal transit time, castor oil-induced diarrhoea and castor oil-induced enteropooling were evaluated. The AMF, EAF and HXF at concentrations of 1.0×10^{-3}, 2.0×10^{-3}, 4.0×10^{-3} and 8.0×10^{-3} mg/mL attenuated the contractile effects of both the spontaneous and acetylcholine-induced contractions of rabbit jejunum and that of histamine-induced contraction of guinea pig ileum in a concentration-dependent manner. The AMF at doses of 200, 300 and 500 mg/kg produced significant ($p < 0.05$) reductions in gastrointestinal transit time of charcoal and incidence of castor oil-induced diarrhoea in mice relative to the untreated control. Similarly, at doses of 300 and 500 mg/kg, AMF significantly ($p < 0.05$) reduced the weight and volume of intestinal fluid in the treated mice when compared to the untreated animals. The results of this study showed that the stem-bark of *T. avicennioides* possesses spasmolytic effect and could be a potential antidiarrhoeal agent. However, detailed pharmacological trials are required to justify the clinical use of the plant for treating diarrhoea.

1. Introduction

Diarrhoea is characterised by increased frequency of bowel, wet stool and abdominal pains [1]. It is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral, parasitic organisms and other non-infectious causes. [2]. The World Health Organisation (WHO) estimated that 3–5 billion cases of diarrhoea occur each year (1 billion in children less than 5 years of age) [3]. Similarly, Ahmed et al. [4] reported that diarrhoea is the foremost fatal outcome among children in Nigeria under the age of five. Despite the availability of different approaches for diarrhoeal management, vast majority of the people in developing countries rely on herbal drugs for the management of diarrhoea [5]. The use of modern drugs for treating diarrhoea are usually associated with unwanted side effects (e.g. dry mouth and urinary retention often observed with the use of antimuscarinic drugs as atropine and headache, and nausea with calcium channel blockers). The synthetic opioid drugs (diphenoxylate and loperamide) cause severe constipation and may significantly slow gastrointestinal transit and increase the absorption of bacterial toxins in infectious diarrhoea [6]. Due to these shortcomings associated with modern antidiarrhoeal drugs, it is important to find newer, safer and cheaper antidiarrhoeal agents from natural sources [7].

Terminalia avicennioides (Combretaceae) is found in the savannah region of West Africa [8]. In Nigeria, the plant is locally called "*baushe*", "*Idi*", "*kpace*", "*kpayi*" and "*Edo*" in Hausa, Yoruba, Nupe, Gwari and Igbo languages, respectively [9,10]. Different parts of the plant have been used traditionally to manage conditions such

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as gastric ulcer, gastro-intestinal disorders (diarrhoea), bloody sputum, cough, and gastro-intestinal helminth parasites [11–13]. Evaluation of the antidiarrhoeal effects of this plant is an attempt not only to validate the traditional claim but that is also hoped to provide an alternative source for an effective treatment against diarrhoea.

2. Materials and methods

2.1. Plant material

The stem-bark of *T. avicennioides* was obtained from the wild, around Zaria, Nigeria. Samples of the flowers, leaves and seeds of the plant were sent to the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, for identification. A voucher specimen with number 900239 was deposited at the Herbarium for reference purposes.

2.2. Experimental animals

Five adult New Zealand white rabbits weighing between 2.0 and 2.5 kg and 5 adult guinea pigs of 300 and 400 g weight were purchased from a local market in Zaria. One hundred adult swiss albino mice of both sexes weighing between 22 and 23 g were obtained from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were acclimatized to laboratory conditions for two weeks and were fed on commercial rodent diet. In addition, water was provided *ad libitum*. All animal experiments were done according to the ethical guidelines on laboratory animal use and care policy, which is in line with Ahmadu Bello University Research Policy (revised in 2010).

2.3. Equipment and laboratory materials used

Locally made cages; Wooden mortar and pestle; Whatman filter paper size 1; Conical flask; Macerating bottle; Measuring cylinder, Test tubes (Pyrex, France); Syringes (1 mL and 5 mL); Weighing balance (Lab tech. BL 20001 and Mettler P162, USA); Microdynamometer (Ugo Basile, Italy); Water bath (HH-S Digital thermostatic water bath, China); Dissecting kit (Gold Cross Dissecting Set, Malaysia); Plastic ruler and Stop watch.

2.4. Drugs and chemicals

Acetylcholine (Ach) and histamine (H) were purchased from Sigma-Aldrich Inc., 3050 Spruce Street, St. Louis, USA. Castor oil (Bell, Sons and Co Ltd, Southport PR9 9 AL, England); Loperamide (Imodium®– Janssen Pharmaceutical, Pakistan); Medicinal charcoal (Ultracarbon® tablets–Merck KGaA, Darmstadt, Germany); Carboxymethylcellulose.

2.5. Plant extraction and partitioning

The stem-bark of *T. avicennioides* was air-dried at room temperature to a constant weight. The dried plant part was made into powdered form using wooden mortar and pestle. One thousand five hundred grams of the powdered stem-bark of the plant were extracted by maceration in a macerating bottle using 4.5 litres of methanol (98%) as solvent at room temperature. The process was repeated twice and the extracts were pooled together. The liquid extract was concentrated *in vacuo* using rotary evaporator at 40 °C. The crude methanol extract was dissolved in water and serially partitioned with *n*-hexane and ethyl acetate in a separating funnel. Similarly, the fractions obtained were concentrated *in vacuo* at 40 °C. All the fractions were weighed, labeled and stored in an air tight container at 4 °C until required.

2.6. Phytochemical screening

The fractions were screened to detect the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids/triterpenes and tannins using standard test methods [14].

2.7. In vitro studies

2.7.1. Effect of fractions of T. Avicennioides on isolated rabbit jejunum

The rabbits were deprived of food but not water for 18 h before the study. Each rabbit was sacrificed by cervical dislocation and exsanguinated. Their abdomens were cut open and segments of the jejunum (2 cm) were cut and dissected from the adhering mesentery. Each tissue was suspended in a 25 mL organ bath containing Tyrode's solution, aerated with air and allowed to stabilize for 30 min to acclimatize. The effect of different concentrations of acetylcholine (Ach) (1.0×10^{-6} mg/mL–8.0×10^{-6} mg/mL) and the fractions of *T. avicennioides* (1.0×10^{-3} mg/mL–8.0×10^{-3} mg/mL) were tested on both spontaneous and Ach-induced contractions of the isolated tissue. The contact time for each tested fraction on the tissue was 30 s, which was followed by washing the tissue three times with Tyrode's solution. The tissue was allowed to rest for a period of 15 min before the next addition of drug or extract. Changes in tension produced by the test agent were recorded with a microdynamometer (sensitivity of 3.0 mV and speed of 24 mm/min coupled to an isotonic transducer [12]

2.7.2. Effect of fractions of T. avicennioides on isolated guinea pig ileum

Similarly, the method described above for the effect of stem-bark extracts of *T. avicennioides* on isolated rabbit jejunum was used. The effects of histamine (1.0×10^{-3} mg/mL–8.0×10^{-3} mg/mL) and fractions of *T. avicennioides* (1.0×10^{-3} mg/mL–8.0×10^{-3} mg/mL) against histamine-induced contraction of the tissue were also tested.

2.8. Acute toxicity test

The median lethal dose (LD_{50}) as an indication of the acute toxicity of the extract was determined by the method described by Lorke [15]. The test was carried out in two phases. All the animals were fasted for 12 h prior to oral administration of the AMF. In phase one, nine apparently healthy swiss albino mice were randomly divided into three groups of three mice. Mice in groups one, two and three received AMF orally at 10, 100 and 1000 mg/kg, respectively. The mice were observed over a period of 48 h for signs of toxicity and mortality. In the second phase, three mice were randomly assigned into three groups of one mouse each. Animals in groups one, two and three were treated with AMF orally at 1600, 2900 and 5000 mg/kg, respectively. Similarly, the animals were observed for 48 h for any signs of toxicity or mortality. The obtained results were recorded accordingly.

2.9. In vivo studies

2.9.1. Effect of the aqueous methanol fraction on gastro-intestinal transit time in mice

The method described by Vogel and Vogel [16] was used. Twenty-five mice were deprived of feed for 12 h and randomly allotted into five groups of 5 mice each. Animals in groups one and two were administered with distilled water (5 mL/kg) and loperamide (5 mg/kg) and served as untreated and treated control groups, respectively. Similarly, mice in groups three, four and five