https://dx.doi.org/10.4314/jpb.v15i2.3
Vol. 15 no. 2, pp. 117-124 (September 2018)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Effects of methanol extract of *Citrullus lanatus* Thunb. (Curcubitaceae) fruit rind on experimentally-induced diarrhoea in Swiss-albino mice

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Received 20th March 2017; Accepted 14th June 2017

Abstract

This research aims at validating scientifically, the claim on use of *Citrullus lanatus* (watermelon) fruit rind as herbal medicine in treating diarrhoea in Northern Nigeria. Qualitative phytochemical screening was evaluated for presence of chemical constituents in methanol extract of *Citrullus lanatus* fruit rind (MECL). MECL was evaluated for oral acute toxicity in Swiss albino mice and established to be safe up to the highest dose of 5000 mg/kg. Effect(s) of extract was evaluated on models of experimentally induced diarrhoea in mice. Flavonoids, steroids, alkaloids, saponins and triterpenes were present. Mice were grouped into five comprising of five animals each for all models of experimentally-induced diarrhoea; pre-treated group I (negative control) received orally, deionised water (10ml/kg), groups II, III and IV were administered 125, 250 and 500 (mg/kg) of MECL respectively. Group V (positive control) received loperamide (3 mg/kg) for castor oil-induced diarrhoea and fluid accumulation tests, while for charcoal meal test; group V received atropine sulphate (0.1 mg/kg) as positive. A significant ($p \leq 0.01$ and $p \leq 0.001$) non-dose dependent effect was elicited by extract, delaying onset of diarrhoea and reducing severity of diarrhoea respectively on castor oil-induced diarrhoea. MECL delayed propulsive movement of charcoal along intestine, significantly ($p \leq 0.05$) decreasing volume (ml) of intestinal content compared to negative control of deionised water. Conclusively, MECL elicited antidiarrhoeal activity prolonging onset of diarrhoea, reducing severity of wet faeces, delaying movement of charcoal and decreasing volume of intestinal content, confirming the claim by herbal practitioners on its use in treating diarrhoea in Northern Nigeria.

Keywords: *Citrullus lanatus*; Diarrhoea; Flavonoids; Intestinal motility

INTRODUCTION

Diarrhoea continues to be a health problem all over the world and a leading cause of mortality and growth retardation in children, despite efforts of many governments to curb it [1,2]. In developing countries, it has been shown to account for 1.5-2 million deaths in children under the age of five [3]. According to WHO/UNICEF report [4], 15 countries worldwide account for almost 75% of all deaths from diarrhoea among children under the age of five each year, and more than 80% of child deaths due to diarrhoea occur in Sub-Saharan Africa and South Asia [5]. In Nigeria, it kills some 194,000 children under the age of five yearly [6]. Several medicines collected from plants are used to treat various diseases such as diarrhoea, urinary tract

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ISSN 0189-8442

infection, cutaneous abscesses, bronchitis, and parasitic diseases [7]. Diarrhoeal pathogens are becoming distressingly resistant to antimicrobial agents and some of the major treatment of diarrhoea (oral rehydration solutions, loperamide) may not completely reduce the volume of stool or duration of illness [8], leading to increased pill burden and longer duration of therapy. The World Health Organization (WHO) encourages studies for treatment and prevention of diarrhoea, by constituting a Diarrhoeal Diseases Control Programme (DDCP) which includes the study of traditional medical practices, together with evaluation of health education and prevention approaches [9-11].

Citrullus lanatus Thunb. (Curcubitaceae), commonly called watermelon and botanically classified as a fruit [12] is a prostrate or climbing annual plant with several herbaceous, firm and stout stems up to 3 m long. In Nigeria, watermelon is called *"kankana"* (Hausa), *N la/ kekere*, *Egun* (Yoruba), *Ugu* (Igbo) [13]. The plant is traditionally used for centuries in treatment of various health aliments having antioxidant [14], anti-inflammatory [15,16], laxative [17] and analgesic properties [15]. In a watermelon, basically 92% is alkaline water; good for stomach especially for those who are suffering from ulcers [18,19] good for sore eyes as it contains vitamins A and C, skin (as it contains vitamin B_6). Although several uses of *C. lanatus* fruit rind in traditional medicine have been documented, many of these claims are yet to be validated by researchers to establish the affirmation on its use scientifically as having antidiarrhoeal activity on pharmacological basis of diarrhoeal induction in Swiss albino mice.

EXPERIMENTAL

Collection and identification of plant material. Fresh, whole *C. lanatus* fruits and leaves were obtained from a farm in Gundutse–Dan Hassan Village, Kura L.G.A. of Kano State, Nigeria. Botanical identification was carried out by Mallam Namadi Sunusi of Herbarium Unit of Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, where a voucher specimen number 1266 was deposited for future reference.

Experimental animals. One hundred and fifty-four Swiss-albino mice (males and females) weighing 18-25 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Animals were fed normal rat feed and provided water *ad-libitum*. All experiments were conducted during the daytime (08.00-18.00 h). All protocols approved by the Institutional Animal Ethical Committee according to the School's Academic Guidelines for use and care of experimental animals with approval number DAC/IW-IT/0711/16 were followed.

Laboratory equipment. Animal cages (locally made), deionised water, plain paper, water bath (HH-S Digital thermostatic water bath), weighing balance (Lab tech. BL 20001 and Mettler P162, USA), dissecting kit (Gold Cross, Malaysia), syringes, stop watch, ruler

Drugs and chemicals. Methanol (AR JHD UN1230; Guangdong Guanghua Sci-Tech. Co., Ltd., China), Carboxy Methyl Cellulose (Evans Medical Lt Speke, Liverpool), Atropine sulphate vial injection (Gland Pharma Ltd., Ameerpet, Hyderabad, India), Castor oil (Bell, Sons and Co (DRUGGISTS) Ltd, Southport PR9 9 AL, England), Loperamide (Imodium®, Janssen Pharmaceutical, Beerse, Belgium), Chloroform, Medicinal charcoal (Ultracarbon® tablets – Merck KGaA, Darmstadt, Germany), Charcoal meal (5% activated charcoal suspended in 2% carboxymethyl cellulose).

Preparation and extraction of plant material. Fresh *C. lanatus* fruit rind (white and green exocarp) were peeled and sliced

into thin smaller pieces and air dried for twelve days until a constant weight was obtained. Air-dried rinds were reduced to powder form using pestle and mortar and weighed. The powder weighing 1.1 kg was extracted using cold maceration method with 70% methanol solvent (3.5 litres), with occasional shaking for three days. The extract was concentrated in flask evaporator under reduced pressure and then subjected to drying under temperature (45-50^{o}C) over a water bath. The sticky mass (extract) obtained was stored in a labeled airtight container and placed in a desiccator until it was to be used. Percentage yield was calculated as: (weight of sticky extract / weight of dried powder) x 100

Preliminary phytochemical screening. The presence of steroids, phenols, triterpenes, flavonoids, alkaloids, saponins, tannins, glycosides, essential oils, of the methanol extract of *C. lanatus* fruit rind were determined using methods described by Sofowora [20] and Evans [21]. Confirmatory thin layer chromatography was carried out using procedures described by Hess [22].

Acute toxicity study. The median lethal dose (LD_{50}) was determined using Lorke's Method [23]. In the first phase, nine mice randomly divided into three groups of three mice per group were given 10, 100 and 1000 mg extract/kg orally (via cannula), respectively. Mice were observed for 4 h post administration for signs of adverse effects / toxicity and death after 24 h. There was no death recorded after 24 h for the whole groupings; thus, phase two was initiated. Three mice were administered the extract orally at doses of 1600, 2900 and 5000 mg/kg, to each mouse respectively. They were observed for signs of toxicity for the first four hours and mortality after 24 h. The LD_{50} value was determined by calculating the geometric means of the lowest dose that caused death and the highest dose for which the animal survived.

Pharmacological studies:

Castor oil-induced diarrhea. The experiment was conducted according to the method described by Awouters *et al.* [24], which is used to assay for secretory form of diarrhoea. Mice fasted overnight were randomly allocated to five groups of five animals each. Group I received deionised water (10 ml/kg), groups II-IV received MECL fruit rind (125, 250 and 500 mg/kg) respectively. Group V received loperamide (3 mg/ kg) in suspension. All drugs were administered via the oral route and solutions were dissolved in deionised water according to drug concentrations. A numeric score based on the stool consistency was assigned as follows: normal stool = 1, semisolid stool = 2 and watery stool = 3. Percentage protection against diarrhoea was calculated with respect to the number of wet faeces excreted using the formula:

$$\% \text{ inhibition} = [(\text{Number of WFC} - \text{Number of WFT}) / \text{Number of WFC}] \times 100$$

where WFC = wet faeces in negative control group
WFT = wet faeces in test group

Gastrointestinal motility test. The effect of the extract on gastrointestinal motility was evaluated using the castor oil-induced intestinal motility model [25]. Peristaltic index (PI) /Intestinal transit was calculated as a percentage of distance travelled by charcoal meal relative to the whole length of the intestine.

*Castor oil-induced enteropooling (fluid accumulation test)***.** Intestinal fluid accumulation (osmotic diarrhoea) was determined using the method described by Robert *et al.* [26], to test the diarrheagenic property of prostaglandins. Values less than the negative control group were recorded as protection from diarrhoea.

Analysis of data. Data collected were presented as Mean ± SEM. Multiple comparison of data was carried out using the one-way Analysis of Variance (ANOVA), followed by Dunnett's post-hoc test, where

the negative control group was compared with the positive group and test groups, using SPSS Version 20 software; where *p* values ≤ 0.05, were considered statistically significant. Results were presented as graphs and tables where appropriate.

RESULTS

Percentage yield of methanol extract of *C. lanatus* fruit rind. Powdered dried weight of fruit rind of 1.1 kg produced 262.76 g of methanol crude extract, giving a percentage yield of 23.89 %.

Preliminary phytochemical screening. Preliminary phytochemical screening, along with confirmatory thin layer chromatogram of the methanol extract of *C. lanatus* fruit rind revealed the presence of alkaloids, carbohydrates, saponins, cardiac glycosides, flavonoids, steroids and triterpenes. However, anthraquinones and tannins were absent.

Acute toxicity study. Acute toxicity studies showed no lethality or toxic reactions at all doses of the extract used in the study upon oral administration up to the highest dose of 5000 mg/kg in mice.

Castor oil-induced diarrhoea in mice. Pretreatment of mice with methanol extract of *C. lanatus* fruit rind at all doses significantly ($p \leq 0.01$) delayed onset of diarrhoea with the intermediate dose (250 mg/kg) producing a percentage inhibition of 61.21%. A decrease in mean number of diarrhoeal faeces excreted for all extract doses was observed in a non-dose dependent manner when compared to the negative control group of deionised water. The lowest dose of the extract (125 mg/kg) produced the least number of droppings (4.50) (Table 1).

Gastrointestinal transit time model (charcoal meal test). Propulsive movement of charcoal meal after oral treatment along the intestinal length was delayed with increasing doses of the methanol extract of *C. lanatus* fruit rind (250 mg/kg and 500 mg/kg) when compared to the negative control group of deionised water (Table 2). The positive control (atropine sulphate 0.1 mg/kg) produced a statistically significant ($p \leq 0.05$) reduction in distance travelled by meal (35.14 cm) when compared to the negative control (45.86 cm).

Effect of methanol extract of *C. lanatus* fruit rind on castor oil-induced enteropooling in mice. Fluid accumulation caused by castor oil-induced diarrhoea within the lumen of the intestine was reduced significantly ($p \leq 0.05$) with the lowest and highest doses of the extract (125 and 500 mg/kg) pooling 0.58 ml and 0.56 ml when compared to the negative control group (0.77 ml) as shown in Table 3. The standard drug (loperamide 3 mg/kg) significantly ($p \leq 0.001$) reduced the volume of intestinal content (0.40 ml) when compared to the negative control group of deionised water (0.77 ml).

Table 1: Effect of methanol extract of *C. lanatus* fruit rind on castor oil-induced diarrhoea in mice

Treatment Group (mg/kg)	Mean onset of diarrhoea (min) ± SEM	Mean no. of wet faeces ± SEM	% Inhibition of wet faeces	Mean no. dry faeces ± SEM	% Inhibition of dry faeces
Deionised water (10ml/kg)	25.60 ± 1.03	11.60 ± 0.98	-	4.20 ± 0.49	-
MECL (125)	85.67± 14.49	4.50 ± 0.65^{xx}	61.21	4.80 ± 0.58	-14.28
MECL (250)	92.75 ± 14.00^{x}	5.20 ± 0.97^{xx}	55.17	3.80 ± 0.37	9.50
MECL (500)	42.00 ± 5.58	4.60 ±0.51^{xx}	60.34	4.20 ±0.49	0.00
Loperamide (3)	148.67± 30.99	1.50 ±0.29^{xx}	87.07	2.40 ±0.40	42.86

^{x} $p \leq 0.01$, ^{xx} $p \leq 0.001$, using one way ANOVA, followed by Dunnett's (2 sided) Post-Hoc Test, where all groups were compared to the control group (deionised water) MECL- Methanol Extract of *C. lanatus* fruit rind

Table 2: Effect of methanol extract of *C. lanatus* fruit rind on gastrointestinal transit of charcoal meal in mice

Treatment Group (mg/kg)	Mean dist. trav. by charcoal (cm) ± SEM	Mean whole intestinal length (cm) ± SEM	% Peristaltic Index ± SEM	% Inhibition
Deionised water (10ml/kg)	45.86 ± 1.96	51.44 ± 1.53	89.07 ± 2.19	-
MECL (125)	45.90 ± 1.53	59.30 ± 1.28	77.54 ± 3.05	-0.087
MECL (250)	36.50 ± 2.07	52.50 ± 2.46	69.69 ± 3.29^{xx}	20.41
MECL (500)	39.50 ± 1.95	51.90 ± 2.62	76.30 ± 2.77 ^{x}	13.86
Atropine Sulphate (0.1)	35.14 ± 4.37^{x}	55.58 ± 3.99	62.29 ± 4.05^{xx}	23.38

^{x} $p \leq 0.05$, ^{xx} $p \leq 0.001$, using one way ANOVA, followed by Dunnett's (2 sided) Post-Hoc Test, where all groups were compared to the control group (deionised water) MECL- Methanol Extract of *C. lanatus* fruit rind

Table 3: Effect of methanol extract of *C. lanatus* fruit rind on castor oil-induced enteropooling in mice

Treatment Group(mg/kg)	Mean volume of intestinal content (ml) ± SEM	Mean whole length of intestine (cm) ± SEM
Deionised water (10ml/kg)	0.77 ± 0.04	55.10 ± 0.64
MECL (125)	0.58± 0.06^{x}	55.34 ± 1.98
MECL (250)	0.60 ± 0.04	54.12 ± 1.24
MECL (500)	0.56 ± 0.05^{x}	52.90 ± 0.60
Loperamide (3)	0.40 ± 0.04^{xx}	58.38 ± 1.49

^{x} $p \leq 0.05$, ^{xx} $p \leq 0.001$, using one way ANOVA, followed by Dunnett's (2 sided) Post-Hoc Test, where all groups were compared to the control group (deionised water) MECL- Methanol Extract of *C. lanatus* fruit rind

DISCUSSION

Preliminary phytochemical screening on aqueous extract of *C. lanatus* fruit pulp revealed the presence carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins and polyphenols [17]. Moreover, research carried out by Ezeigbo *et al.* [27] on the plant *Acacia occidentale* reported its antidiarrhoeal activity to be due to presence of flavonoids and saponins in the plant. The various phytoconstituents found in methanol extract of *C. lanatus* fruit rind partly describes the probable antidiarrhoeal activity elicited in models of experimentally induced diarrhoea. According to Lorke's classification of chemicals, determination of median lethal dose (LD_{50}) of the methanol extract was found to be safe up to the highest dose of 5000 mg/kg. In experimental studies, LD_{50} determination gives a guide on subsequent doses of the substance to be used for experimental procedures.

Diarrhoea results from an imbalance between absorptive and secretory mechanisms in the intestinal tract accompanied by hypermotility, leading to excessive fluid loss in the faeces [28]. Inhibition of experimental diarrhoea and reduction in faecal output by a substance are the basis for pharmacological evaluation of a potential antidiarrhoeal agent [29]. Results obtained from castor oil-induced diarrhoea model revealed that methanol extract of *Citrullus lanatus* fruit rind significantly reduced severity of diarrhoea induced by castor oil, which was found to be comparable to loperamide; a drug widely employed against diarrhoeal disorders induced by castor oil, prostaglandin and cholera toxin [30]. It is evident, however, that castor oil hydrolyses to ricinoleic acid in the duodenum causing irritation and inflammation of the intestinal mucosal wall, leading to release of prostaglandins (PGE_2) and histamine, which stimulate motility and intestinal secretions [31]. The prostaglandins thus released promote vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines, thereby preventing reabsorption of sodium chloride and water [32]. The inhibitors of prostaglandin biosynthesis are therefore considered to delay castor oil-induced diarrhoea [33]. Extracts of plants that contain flavonoids are known to modify the production of cyclooxygenase 1 and 2 and lipo-oxygenase [34], thereby inhibiting prostaglandin production. The

methanol extract may therefore elicit its inhibitory effect via similar mechanism. A decrease in motility of gastrointestinal muscles prolongs the stay of substances within the intestinal lumen [35], thereby allowing for better absorption of water and other electrolyte molecules. The above result is supported by the findings of Brown and Taylor [36] who stated that castor oil-induced gastrointestinal hypermotility has been suggested to be indirectly mediated by the cholinergic system since it is inhibited by atropine, a known anticholinergic agent. In this study of gastrointestinal transit, atropine sulphate significantly delayed the intestinal transit of charcoal meal. It has been observed from this study that the reduction in propulsive movement of charcoal may be due to antispasmodic and/or anticholinergic properties of *Citrullus lanatus* fruit rind, as anticholinergic agents are known to inhibit gastrointestinal hyper motility [37]. Decrease in gastric motility causes further absorption of water from faeces and may additionally contribute to reducing its watery texture. The MECL fruit rind significantly inhibited castor oil-induced intestinal fluid accumulation exhibiting a non-dose dependent reduction in volume of intestinal content. These observations suggest that the extract inhibits gastrointestinal hyper-secretion and enteropooling by enhancing electrolyte and water reabsorption from the intestinal lumen. Electrolyte absorption, according to studies carried out by Duggan *et al.* [38] determines the efficiency of nutrient absorption by the extract, and that of other intestinal contents. Results obtained from this research showed that antidiarrhoeal activity was well elicited with lower doses of the extract, while higher doses produced sudden contractile effect on the gastrointestinal tissue.

Conclusion. The methanol extract of *Citrullus lanatus* fruit rind elicited antidiarrhoeal activity scientifically, confirming the traditional claim by herbal practitioners of its use in treating diarrhoea in Northern Nigeria.

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