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(RESEARCH ARTICLE)

Evaluation of laxative activity of aqueous extract of C*itrullus colocynthis* (L.) Schrad. fruits on mice

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Abstract

Constipation is a common gastrointestinal disorder with a well-recognized tendency to cause discomfort which also affects the quality of life. Medicinal plant is defined as any substance with one or more of its organs containing substances that can be used for therapeutic purposes or which can be used as precursors for synthesis of drugs. *Citrullus colocynthis* (L.) Schrad. (Bitter apple), a valuable plant from Cucurbitaceae family is a non-hardy, herbaceous perennial vine, branched from the base with leafy and stiff-haired stems. This study evaluated the laxative activity of aqueous extract of *Citrullus colocynthis* fruits on mice. Bisacodyl (25 mg/kg), a standard drug. served as positive control while aqueous extract of *Citrullus colocynthis* fruits was administered at the doses of 20 and 50 mg/kg orally. Sixteen (16) animals were used and placed in metabolic cages designed specially to separate urine and fecal materials. The fecal outputs were collected and weighed at every 8 and 16 hours for 5 days. The results revealed the extract to have time and dose dependent activity patterns. Higher activity of the extract was recorded in 50 mg/kg up to 8 hours throughout the period of experiment. This showed that the efficacy of the doses could not last for 16 hours since treatment was only given once per day. This result also showed higher activity when compared with the standard drug, Bisacodyl. This study supported the folkloric laxative use of *Citrullus colocynthis* fruit.

Keywords: Constipation; Medicinal Plants; *Citrullus colocynthis*; Laxative; Cucurbitaceae.

1. Introduction

Constipation is a common gastrointestinal problem, which causes many expenses for the community with an estimation prevalence of 1-80% worldwide (Sanchez and Bercik, 2011), where the condition is characterized by a wide geographical variation and correlated with the patient quality of life. Generally, a person is considered to be constipated when bowel movements result in the passage of small amounts of hard and dry stools, usually fewer than three times a week (Longstreth *et al.* 2006). It is considered a common side effect of several medications such as analgesics, anticholinergics and cation-containing agents (Branch and Butt, 2009). A number of factors are also involved in functional disorders of defecation and defined in patients with constipation including rectal hyposensitivity, altered rectoanal reflex activity, increased rectal duct capacity and rectal motor dysfunction (Scott *et al.,* 2011). Constipation increases with aging and can be a chronic condition requiring the use of laxatives over the long-term. Laxatives are drugs that loosen stools and enhance bowel movements. They are used to treat and prevent constipation. These laxatives significantly enhance the motility and secretion of the intestine by regulating electrolyte transport by the intestinal mucosa (Schiller, 2001). Lifestyle and dietary modifications are considered as first steps in the treatment of chronic constipation (Schuster *et al.,* 2015), reassurance of their concept of a healthy or regular bowel movement and biofeedback. Identification of patients that need psychological support should be undertaken because constipation may be aggravated by stress or may be a manifestation of emotional disturbance. Laxatives pharmaceutical products available without prescription are generally referred to as over-the-counter laxatives. In managing constipation, over-

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the-counter laxatives may be used in two ways; either for treatment or for prevention of constipation. Treatment of constipation refers to the use of laxatives to relieve constipation symptoms while prevention refers to the use of laxatives to prevent the symptoms of constipation from occurring (Werth *et al.,* 2015). Medications to treat and prevent constipation can be classified into bulk-forming laxatives, emollients and stool softeners, osmotic agents, stimulants and prokinetic agents. Latest discoveries and development in therapeutic and aromatic plants have shown the way to the improvement of health care and humanity (Maver *et al.* 2015). Many people are turning to natural foods and herbs with laxative properties instead of over-the-counter and/or prescription laxative drugs because they are considered readily available, less expensive, less or no side effect and provide other medicinal benefits attributable to the plants.

Citrullus colocynthis (L. Schrad. (Figure 1), a valuable plant from Cucurbitaceae family, widely distributed in the barren region, is a non-hardy, herbaceous perennial vine, branched from the base (Rahimi *et al.* 2012). Its common names include bitter apple, colocynth, vine of Sodom in English, Egunsi-baara in Yoruba, Elili/egwusi in Ibo and Egbsi/guna in Hausa. *C. colocynthis* grows in Africa and Asia, including the Southeast, East, Southwest and Central area of Iran (Rahimi *et al.,* 2012). It grows well in desert regions, especially in alkaline soils and shows high resistance to water stress and salinity (Abdulridha *et al.,* 2020). The leaves are acutely divided, lobes slender, thick and almost 5-10 cm in length with approximately 3-7 lobes and are alternate on its lengthy petioles (Amamou *et al.* 2011). The flowers are mendacious with male and female both separate. Its yellow flowers individually grow on the leaf axils (Savithrama *et al.* , 2007). The fruits are bulbous or ovoid, indehiscent berry and 5-7. 5 cm in width. The fruits are smooth, extremely bitter, yellow and dry. After ripening, the fruits have coriaceous peel, filled with a spongy flesh containing several white and brownish ovate seeds, oily, shiny and compressed without an edge. The seeds of the fruits have the length and width of 0. 75 cm and 0. 5 cm respectively (Fleming, 2000). The seeds are oblong-shaped and are situated on the parietal placenta. The roots are large perpetual long, bifurcate and rocky vine-like while the stems spread on the soil and have affinity to mount over herbs and shrubs by their axillary branching tendrils.

In folk medicine, *Citrullus colocynthis* is widely used by rural inhabitants as a purgative, anti-diabetic, anti-neoplastic, anti-rheumatic and anti-allergic agent (Tannin-Spitz *et al.,* 2007). A decoction of the entire plant, made with the juice of funnel is used as the indurations of the liver. Traditionally, this plant is used in the treatment of diseases like cancer, leukoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia and throat diseases while fruit pulp acts as an antiepileptic, purgative, diuretic and used against gonorrhea (Uma and Sekar, 2014). The fruit is pungent, cooling laxative, antipyretic, anthelmintic and carminative. The leaves are diuretics and utilized as a part of the treatment of jaundice and asthma. The root is utilized as a laxative and for the treatment of rheumatism, urinary diseases, jaundice and in snake poison (Uma and Sekar, 2014). Due to the principal secondary metabolite, cucurbitacin glycoside, the drug is extremely toxic and strongly affects the mucous membrane by irritation and pain. Overdose leads to vomiting, bloody diarrhea, colic, kidney irritation and sometimes excessive production of urine which results to failure of kidney to produce urine. Lethal dosages usually associated with convulsions, paralysis and circulatory collapse, following the intake of doses at 2g (Fleming, 2000). The seeds of *Citrullus colocynthis* contain edible oil; 56% of it contained linoleic acids while 25% contained oleic acids (Sawaya *et al.,* 1983). Palmitic and stearic acids are the main fatty acids found with concentrations ranging from 8. 1 to 17. 3% and 6. 1 to 10. 5% respectively. High content of essential mono saturated fat linoleic acid (50. 6-60. 1%) in the oil of seed acts as a vital component for the restorative activities. Fat profile of unsaturated fatty acids exposes that the class of linoleic-oleic acid bear a close resemblance to few other vegetable oils. So, it is most likely going to have potential uses for cooking like some other cucurbit seed oils (Dhakad *et al.,* 2017). The seeds also contain 13. 19 g of protein, 18. 59 g of fat, 4. 91 g of moisture and 2 mg/100 g of ash. Minerals present in the seeds contain 569 mg/100 g of calcium, 465 mg/100 g of potassium, 210 mg/100 g of magnesium, 30 mg/100 g of phosphorus, 11. 9 mg/100 g of sodium, 11. 6 mg/100 g of iron, 5. 1 mg/100 g of copper and 1. 1 mg/100 g of zinc (Hussain *et al.,* 2014).

In *Citrullus colocynthis,* various important chemical constituents derived from the entire plant and documented in literature are carbohydrates, proteins, amino acids, alkaloids, flavonoids, terpenoids etc. The well-known bioactive compounds of the fruits imparting medicinal value are a group of cucurbitacins (A, B, C, D, E, I, J, K and L) with some other components like alkaloids, terpenoids, tannins, saponins, anthranol, caffeic acid and cardiac glycoloids (Uma and Sekar, 2014). Cucurbitacin E was richly isolated from methanol extract of the fruit pulp while colocynthoside A and colocynthoside B were isolated from the fruits. Hydromethanolic extracts were reported to show the presence of alkaloids, tannins, saponins, flavonoids, unsaturated sterols, terpenes, sterols and steroids from fruits, leaves and roots and the amount of alkaloids, tannins, saponins and flavonoids are found to be of very low quantity from leaves (Nora *et al.* , 2015). Ursolic acids, cucurbitacin E 2-O-β-D glycopyranoside and 4- methylquinoline were obtained from methanolic extract of the fruits. The hydromethanolic extract, ether extract, chloroform to methanol (1:1) extracts and butanol extract of fruits were reported to contain 3-O-methylether and cucurbitacin glycoside (Delazer *et al.* , 2006). Other compounds of cucurbitacins obtained from butanolic extract were cucurbitacin L 2-O-β-D glycopyranoside, hexanocucurbitacin I 2-O-β-D glucopyranoside, cucurbitacin K 2-O-β-D glucopyranoside, khekadaengoside E,

cucurbitacin J 2-O-β-D glucopyranoside and cucurbitacin I 2-O-β-D glucopyranoside. The butanol fraction of extract of whole plant parts of methanol revealed the presence of certain flavonoid glycoside compounds such as isoorientin 3'-O-methylether, isovitexin and isosaponarin along two cucurbitacin glycoside compounds such as 2-O-β-D glucopyranosyl cucurbitacin L and 2-O-β-D glucopyranosyl cucurbitacin I (Dhakad *et al.* , 2017).

Figure 1*Citrullus colocynthis* (L.) Schrad

2. Material and methods

2.1 Plant specimen

Fruit of *Citrullus colocynthis* was collected at Ikare Akoko (Akoko North East Local Government Area), Ondo State, Nigeria. Identification and authentication of the specimen were done by Dr. O. A. Obembe, a taxonomist, at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The Voucher specimen was preserved at the Herbarium of Department of Plant Science and Biotechnology, AAUA for future reference. Fruit of *Citrullus colocynthis* weighing 440 g was blended with a mechanical grinder and dissolved in 400 ml of distilled water. The filtrate was collected, freeze-dried and made into concentrations of 20 and 50 mg/kg b. w for the analysis.

2.2 Experimental animals

Sixteen (16) healthy male Swiss albino mice weighing between 18 and 38 g were obtained from the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria and housed in animal house of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. They were acclimatized for a period of one week before the commencement of the experiment and were kept in a cage under controlled environmental conditions of temperature, relative humidity and 12 hours light/dark/cycle. They were fed *ad libitum* everyday with standard diets and water and were handled in compliance with National Institute of Health Guidance (NIH Publication, 1985). Animals were divided into four groups of four animals each based on their body weight which was used to calculate the amount of the treatment administered.

2.3 Experimental design and treatment administration

Laxative activity was performed according to the method reported by Capasso *et al.* (1986) with slight modification. The experiment lasted for 5 days. Every day, the animals were deprived of food and water for 8 hours prior to the treatment. Group 1 which served as the negative control received normal saline (25 ml/kg b. w.), group 2 which served as the positive control received bisacodyl (25 mg/kg b. w.), the reference standard drug for laxative while groups 4 and 5 received the doses of the extract in 20 and 50 mg/kg b. w. respectively. All drugs were administered orally using oral cannula. Immediately after administration, the animals were placed in metabolic cages specially designed to separate urine and feces. The fecal outputs were collected and weighed after a period of 8 and 16 hrs.

2.4 Statistical analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) to generate the mean and standard error. Mean generated were separated and compared by Duncan's New Multiple Range Test (DNMRT).

3. Results and discussion

Constipation is difficulty in defecation that can be treated or managed by the over-the-counter laxatives to soften stools and enhance bowel movement. Fecal consistency is correlated to the ratio of the water-holding capacity of the insoluble solids, such as those that are derived from dietary fiber and to the total water in the lumen (Fine *et al.* , 1998). Many over-the-counter laxatives affect water absorption and/or secretion in the gut. In this study, an attempt was made in evaluating the laxative activity of *Citrullus colocynthis* fruits on mice (Table 1). The result revealed significant laxative activity of the fruit extract when compared to control (negative) and Bisacodyl groups (Positive control) atboth 8 and 16 hours of treatments for five (5) days. The result of the study showed that the groups treated with 20 and 50 mg/kg body weight of aqueous extract of *Citrullus colocynthis* fruit gave dose dependent increase at 8 hours as well as 16 hours in Day 1 and dose dependent decrease at 16 hours from days 2 to 5. This showed that the efficacy of the doses could not last for 16 hours when compared to the control since treatment was only given once per day. There were statistical differences (P<0. 05) in all the results oftreated groups when compared to the standard drug (positive control) and negative control groups but negative control and 20 mg /kg of *C. colocynthis* were not significantly different (P>0. 05) at 16 hrs in Day 5. The result of this study was in agreement with the findings of Tambe and Bhambar (2016) that methanol extract of *Hibiscus tiliaceus* Linn. bark produced significant laxative activity, in a dose dependent manner up to 8 hours of drug administration. Sengottuvelu *et al.* (2014) also demonstrated the laxative activity of *Polyalthia longifolia* in rats. The results showed that an oral administration of the bark extract of *Polyalthia longifolia* produced significant and dose dependent increase in fecal output of rats. Also, the laxative activity of *Argemone mexicana* was studied in rats where oral administration of the extract showed significant and dose dependent increase in fecal output of rats in regards to the accumulation of water in the intestine (Shripad *et al.* , 2017). The presence of phyto-constituents like terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids can be responsible for the laxative effects of plant material (Longanga *et al.,* 2000). Also, anthraquinones have been reported to exhibit laxative activity due to their ability to increase intestinal motility and secretion (Malik and Muller, 2016). Therefore, presence of these phytochemicals in *Citrullus colocynthis* may be responsible for its laxative activity.

Table 1 Laxative activity of the fruit of *Citrullus colocynthis* aqueous extract on fecal weight of mice

Treatments					
Days	**Time (hrs)**	**Negative control**	**Bisacodyl (25 mg/kg)**	**20 mg /kg *C. colocynthis***	**50 mg/kg *C. colocynthis***
1	8	0. 16±0. 00^{a}	0. 27±0. 01^{c}	0. 24±0. 00^{b}	0. 51±0. 00^{d}
	16	1. 11±0. 01^{a}	1. 83±0. 01^{b}	3. 15±0. 01^{c}	4. 13±0. 03^{d}
2	8	0. 27±0. 01^{a}	0. 41±0. 01^{b}	0. 51±0. 01^{c}	0. 53±0. 00^{c}
	16	1. 87±0. 03^{a}	4. 15±0. 01^{b}	6. 48±0. 01^{d}	4. 29±0. 00^{c}
3	8	0. 37±0. 01^{b}	0. 18±0. 00^{a}	0. 41±0. 00^{c}	0. 84±0. 00^{d}
	16	3. 23±0. 03^{d}	1. 57±0. 01^{a}	2. 99±0. 00^{c}	2. 63±0. 03^{b}
4	8	0. 21±0. 00^{b}	0. 16±0. 01^{a}	0. 30±0. 00^{c}	0. 32±0. 00^{d}
	16	2. 94±0. 02^{c}	0. 37±0. 01^{a}	3. 61±0. 04^{d}	2. 65±0. 01^{b}
5	8	0. 57±0. 01^{c}	0. 09±0. 00^{a}	0. 40±0. 00^{b}	0. 45±0. 07^{d}
	16	2. 61±0. 01^{c}	0. 88±0. 03^{a}	2. 56±0. 00^{c}	2. 16±0. 04^{b}

Note: Data are represented as mean ± standard error. Mean values with the same superscript alphabets in the rows are not significantly different from each other (P>0. 05).

4. Conclusion

The aqueous extract of *Citrullus colocynthis* fruit possessed a laxative activity more than the reference drug, Bisacodyl. This was based on the fact that *C. colocynthis* aqueous extract showed prominent increase in intestinal motility and secretion, thus, enhancing bowel movement. Although, a large number of potentially active compounds have been found in *C. colocynthis,* further studies are needed for the isolation, identification, structural elucidation and screening of any of the above mentioned active principles with regard to specific medicinal uses and gastrointestinal effects of the extract are recommended.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

This research was approved by the Research Ethics Committee on animal use of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

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