

## • Research Article

# Antidiarrheal activity of hexane extract of *Citrus limon* peel in an experimental animal model

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### ABSTRACT

**OBJECTIVE:** Acute diarrhea is one of the major illnesses that cause death in children, despite clinical interventions and the use of oral rehydration therapy. Thus, there is need to discover other effective, affordable and accessible treatments for this disease. Therefore, this study was carried out to investigate the effects of hexane extract of *Citrus limon* peel (HECLP) on castor oil-induced diarrhea in rats.

**METHODS:** Diarrhea was induced in male albino Wistar rats weighing 100–150 g. The antidiarrheal activity of HECLP at different oral dosages (5, 10 and 20 mg/kg) was investigated by counting the number of wet fecal pellets. Animals were further treated with propranolol, prazosin, nifedipine and atropine to assess the effects of receptor blockers on the activities of the extract. The effects of HECLP on castor oil-induced enteropooling and the intestinal transit time of activated charcoal were also evaluated.

**RESULTS:** Each of the 3 doses of *C. limon* significantly reduced ( $P < 0.05$ ) the number of wet fecal pellets produced by animals, with 20 mg/kg HECLP producing the highest percentage inhibition (34.2%). Wet fecal pellet inhibition by the standard drug loperamide (3 mg/kg p.o.) was 68.4% relative to the negative control. Blockage of  $\beta$  adrenergic receptors by propranolol abolished the antidiarrheal effects of HECLP. Intestinal fluid accumulation was inhibited by 68.7% and 78.5% by 20 mg/kg HECLP and loperamide respectively. Furthermore, 20 mg/kg HECLP significantly ( $P < 0.05$ ) reduced the percentage intestinal transit time ( $21.4\% \pm 1.42\%$ ), relative to the control ( $34.2\% \pm 4.29\%$ ); atropine (5 mg/kg, intraperitoneal injection) significantly ( $P < 0.001$ ) reduced the percentage intestinal transit time to  $11.2\% \pm 0.85\%$ .

**CONCLUSION:** These results suggest that *C. limon* peel possesses antidiarrheal effects through antisecretory and antimotility mechanisms that act through the  $\beta$  adrenergic system.

**Keywords:** antimotility; antisecretory; diarrhea; lemon; peel

**Citation:** Adeniyi OS, Omale J, Omeje SC, Edino VO. Antidiarrheal activity of hexane extract of *Citrus limon* peel in an experimental animal model. *J Integr Med*. 2017; 15(2): 158–164.

[http://dx.doi.org/10.1016/S2095-4964\(17\)60327-3](http://dx.doi.org/10.1016/S2095-4964(17)60327-3)

Received October 9, 2016; accepted December 23, 2016.

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## 1 Introduction

Diarrhea is defined as a disease condition in which a person passes loose or watery stool for three or more times during a 24-hour period.<sup>[1]</sup> Statistics show that diarrhea kills about 2 195 children every day, a number greater than the deaths caused by malaria, acquired immune deficiency syndrome and measles combined.<sup>[2]</sup> The frequency and severity of this disease are worsened by lack of access to clean water and hygienic disposal of human waste, malnutrition, poor hand-washing technique, dirty environmental condition and lack of access to affordable health care.<sup>[3]</sup> The greatest proportion of deaths from diarrhea occur in Africa and South Asia.<sup>[4]</sup> However, it is both preventable and treatable.

*Citrus limon*, commonly known as lemon, belongs to the family Rutaceae. *Citrus* fruits are mainly used by juice processing industries, where the peels are generally thrown away. However, several reports have shown that these peels have important medicinal benefits. Lemon peels contain pectin, which is capable of reducing plasma and liver cholesterol.<sup>[5]</sup> Lemon peel has also been reported to be effective in curing kidney stone disease and can be used to prevent the disease and its recurrence.<sup>[6]</sup> Literature shows that the hexane extract of lemon peel has antidiabetic activity.<sup>[7]</sup> Furthermore, two of the active constituents of lemon peel, limonene and salvestrol Q40, possess anticancer properties.<sup>[8]</sup>

Other research has shown that *C. limon* peel exhibited highly significant antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.<sup>[9]</sup> In developing countries, enterotoxins that are produced and secreted by bacterial organisms like *Vibrio cholerae*, *Salmonella*, *Shigella* and *E. coli* are the principal causes of diarrhea.<sup>[10]</sup> Thus, the use of *C. limon* peel might prove effective in treating diarrhea. Diarrhea is also characterized by increased secretion of fluid into the intestine, reduced absorption of fluid from the intestine and alterations in intestinal motility, usually accompanied by increased propulsion.<sup>[11]</sup> No studies were found that reported traditional medicinal uses of lemon peel in the treatment of diarrhea. Furthermore, there were no reports on the use of *C. limon* in the treatment of diarrhea. Therefore, with the aforementioned medicinal benefits of lemon peel, the authors of this research decided to investigate the effects of hexane extract of *C. limon* peel (HECLP) on castor oil-induced diarrhea in rats by evaluating the frequency of defecating wet feces, intestinal motility and gastric enteropooling.

## 2 Materials and methods

### 2.1 Animals

Healthy male albino Wistar rats (100–150 g) were used. The animals were housed under standard controlled environmental conditions, with a 12-hour light/dark cycle. They were kept in wire meshed cages and fed with standard pellet diet (Vital Feeds, Grand Cereals LTD, Jos, Nigeria) and water *ad libitum*. The rats were allowed to acclimatize for two weeks before the experiments. This study was given ethical clearance by the Institutional Research and Ethics Committee of Kogi State University, Anyigba, Nigeria (KSU/CHS/REC/002/VOL2). Animals were handled as per the guidelines of the Committee, which is also in accordance with the internationally accepted principles for laboratory animal use.

Plastic experimental cages were prepared as follows: white blotting paper was used to line the floor of the cages and wire gauze was placed about 2 cm above the floor. Rats were placed in these cages 2 h daily for one week, to familiarize them with the experimental environment. For all the experiments, the animals were fasted for 18 h, but they had access to water.

### 2.2 Preparation of hexane extract

Fresh *C. limon* (lemon) was bought from Railway Market, Makurdi, Benue State, Nigeria. The peels were removed and cut into small pieces with a knife, dried completely under shade and ground with an electric grinder into coarse powder.<sup>[7]</sup>

The coarse powder was soaked in hexane in a 500 mL flat bottom reagent bottle. It was kept at room temperature and allowed to stand for 10 d with occasional shaking and stirring. After this, the liquid hexane was filtered through cotton wool and then through filter paper. Then hexane was allowed to evaporate at temperature 40–50 °C yielding the extract of peel of *C. limon*.<sup>[7]</sup>

### 2.3 Number of wet fecal pellets

In this experiment, oral administration of castor oil was used to induce diarrhea. A pretest for castor oil-induced diarrhea in rats was conducted and all rats responded by passing wet feces. Thus, the antidiarrheal activity of *C. limon* peel was evaluated according to the method previously described by Teke et al.<sup>[12]</sup> The animals were fasted for 18 h and divided into 5 groups of 5 animals each. Each rat was put in a separate cage and given 1 mL castor oil. Unless otherwise noted, all treatments in this experiment were administered orally. After 30 min, animals in the different groups were treated as follows: group I received the vehicle alone (0.4 mL of 1% Tween 80); group II, loperamide (3 mg/kg); group III, HECLP (5 mg/kg);

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