



## Acute Toxicity, Phytochemistry and Anti-diarrheal Effects of *Celtis integrifolia* Lam. Aqueous Leaf Extract in Wistar Albino Rats

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors MBM and BU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript.

Authors NAO, ASS and DY managed the analyses of the study and also participated in manuscript draft. Authors RMI and PPM managed the literature searches and carried out experiment. All authors read and approved the final manuscript.

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### ABSTRACT

*Celtis integrifolia* Lam. plants also known as hackberries or nettle trees are widely spread in warm temperate region of African, Latin America and Asia. The aqueous leaf extract of *Celtis integrifolia* was obtained by soxhlet extraction using distilled water as a solvent. The aqueous leaf extract was then evaluated for its acute toxicity, phytochemical compounds and anti-diarrheal potential using standard protocol. The results showed that the LD<sub>50</sub> of aqueous leaf extract of *Celtis integrifolia* was greater than 3000 mg/kg following up and down procedure, an indication of low toxicity. The phytochemical analysis of the leaf extract indicates the presence of saponins, reducing sugar, tannins, flavonoids, carbohydrate and cardiac glycoside. The leave extract significantly ( $p < 0.01$ )

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reduced the number of unformed faeces in castor oil induced diarrhea in rats. It also significantly ( $p < 0.01$ ; 0.001) reduced the gastrointestinal transit of activated charcoal. Therefore, the aqueous leaf extract of *Celtis integrifolia* is relatively safe and possesses an anti-diarrheal activity.

**Keywords:** Phytochemistry; *Celtis integrifolia*; aqueous leaf extract; diarrhea; rats.

## 1. INTRODUCTION

*Celtis integrifolia* commonly known as hackberries or nettle trees, is a genus of about 60-70 species of deciduous trees wide spread in warm temperate regions of Northern Hemisphere, Southern Europe, Southern and Eastern Asia, Southern and Central North America, South to Central Africa, Northern and South America [1]. *Celtis integrifolia* is used traditionally in Nigeria and other parts of the globe. The medicinal value includes used as pain killer, treatment of chicken pox, measles, gout, epilepsy, ebolics, analgesic and treatment of diarrhea [2].

In recognition of the increased value of medicinal plant in primary healthcare, the World Health Organization (WHO) has advocated for the proper identification, sustainable exploration, scientific development and appropriate utilization of herbal medicine which provide safe and effective remedies in Medicare [3]. Today medicinal plant plays a key role in the advancement of modern studies of biological activities which is also used as a starting point for the development of drugs [4].

In Africa, up to 80% of the population uses traditional medicine for primary health care, and the global market for herbal medicine, currently stands at over 60 million US dollars annually and is growing steadily [5]. In Nigeria, although there are professional traditional medical practitioners such as village elders and herds men who are experienced in diagnosing and treating man and animal diseases [6], in addition the current economic hardship makes it mandatory to explore all the local resources to meet the necessities of life. These includes the herbs and decoction from *Celtis integrifolia* widely used in folk medicine for the control of diarrhea especially in the Northern part of Nigeria even though its efficacy and toxicity have not been fully evaluated and documented.

This study aims at evaluating the toxicity, phytochemistry and anti-diarrheal effects of the aqueous leaf extract of *Celtis integrifolia*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection and Identification

Fresh leaves of *Celtis integrifolia* were collected between 9:00-10:00am, in June 2015 from Mafa local government area of Borno state, Nigeria and identified by a botanist at the Department of Biological sciences, University of Maiduguri and a Voucher specimen was deposited at the Department of Veterinary Physiology, Pharmacology and Biochemistry herbarium, University of Maiduguri.

### 2.2 Extract Preparation

The leaves of *Celtis integrifolia* were cut into pieces and air dried in the laboratory under room temperature for seven days. The dried samples were grounded into powder using pestle and mortar. The powdered sample was exhaustively extracted with water using reflux method. The reflux apparatus comprises of a round bottom flask fixed to a condenser that has an inlet and outlet called water in and out.

Six hundred grams of the powder was put into the flask and water was added. The mixture was heated for three hours and filtered. The procedure was repeated twice by adding water heated for 1 hour and filtered. A total of 1.5 litres of water was used for the extraction. The filtrate was then put into a beaker and heated on a heating mantle. The procedure allowed the evaporation of water until the filtrate became very thick. The thick liquid was then allowed for complete evaporation of the water. A spatula was used to scrap the extract from the metal tray.

### 2.3 Experimental Animals

Wister Albino rats of both sexes were used for the experiment. They were kept in plastic cages and allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. They were fed with growers mash (Saunders Nigeria Ltd, Jos) and provided water *ad libitum*.

## 2.4 Acute Toxicity

The up and down procedure as described by [7] and modified by [8,9] was used to evaluate the oral acute toxicity of aqueous leaf extract of *Celtis integrifolia*. Five adult rats, weighing between 130 to 180 g were randomly selected for the experiment. They were housed in individual cages for two weeks prior to the treatment to allow them acclimatize to the laboratory condition. The rats were fasted overnight, but allow free access to water and were given aqueous leaf extract of *Celtis integrifolia* orally at a dose of 3000 mg/kg (limit dose) and were observed for 48 hours after administration of the extract for signs of toxicity or death. All procedures involving laboratory animals complied with National Academy of Science guidelines on handling of experimental animals [10], and ethical approval for this study was obtained from animal ethics committee of University of Maiduguri, Faculty of Veterinary Medicine.

## 2.5 Phytochemical Screening

The aqueous leaf extract of *Celtis integrifolia* was subjected to phytochemical screening for identification of various classes of chemical compounds such as sterol, triterpenes, alkaloids, flavonoids, carbohydrates, tannins, emodols, anthracenosides, flavone aglycone, saponin, cardiac glycosides, polyuronides as described by [11,12].

## 2.6 Assessment of Anti-diarrheic Effect of the Extract

### 2.6.1 Effect of the aqueous leaf extract on castor oil induced diarrhea in rats

Twenty five rats weighing between 110 to 180 g were used for this study. The method of [13] was used. The rats were deprived of feed for 12 hours, before the commencement of the experiment, but were allowed free access to water. They were separated into 5 groups of five rats each. Rats in groups A, B, C respectively received 250 mg/kg, 500 mg/kg, and 750 mg/kg dose of aqueous leaf extract of *Celtis integrifolia* orally respectively. Those in group D received 2 ml of normal saline orally, while those in group E were given diphenoxylate HCL 5 mg /kg body weight intraperitoneally (I.P). The rats were housed singly in a cage lined with white blotting paper. One hour after extract treatment, the rats were given each 1 ml of castor oil orally. The rats

were observed for 6 hours for watery (wet) or unformed faeces. The watery faeces from each rat were counted at the end of the experiment and a group mean obtained.

### 2.6.2 Effect of aqueous leaf extract of *Celtis integrifolia* on gastrointestinal transit of activated charcoal

Twenty five rats weighing between 120 to 170 g were used for this experiment. The method of [14] was used. The animals were deprived of feed 18 hours to experiment, and thereafter divided into 5 groups of 5 rats each. The rats were allowed free access to water. Group 1 received 2 ml of normal saline orally and Group 2 received intraperitoneally (I.P) 3 mg/kg of atropine sulphate. Groups 3, 4 and 5 were treated orally with 250 mg/kg, 500 mg/kg and 750 mg/kg doses of aqueous leaf extract of *Celtis integrifolia* respectively. Ten minutes after the drug and extract administration, 1 ml of 5% activated charcoal suspension in 10% aqueous solution of *Celtis integrifolia* was given orally to each rat. The rats were sacrificed 30 minutes later and the abdomen opened to assess the intestines. The distance travelled by charcoal meal from pylorus was measured and expressed as percentage of the total length of the intestine from pylorus to the caecum [15].

## 2.7 Data Analyses

Data collected were expressed as Means  $\pm$  Standard Deviations (S.D) while one way analysis of variance (ANOVA) was used to analyze the extent of variation between groups and p values equal to or less than ( $p < 0.01$  or  $p < 0.001$ ) were reported as moderate and highly significant [16]. The computer software Graph pad instant 3.0 for windows USA<sup>®</sup> was used to analyze the data.

## 3. RESULTS

### 3.1 Phytochemical Test

The results of the phytochemical analysis of the aqueous leaf extract of *Celtis integrifolia* are shown (Table 1). The result showed that the color of the extract was green and the  $p^H$  was 5.4. The phytochemical test revealed the presence of carbohydrate, tannins, saponins, cardiac glycosides, flavonoids, and reducing sugar. While, anthraquinine, alkaloids and soluble starch were absent.

**Table 1. Phytochemical screening of the aqueous leaf extract of *Celtis integrifolia***

Chemical components	Aqueous leaf extract of <i>Celtis integrifolia</i>
Tannins	+
Saponins	+
Cardiac glycosides	+
Flavonoids aglycones	+
Reducing sugar	+
Alkaloids	ND
Anthraquinine	ND
Soluble starch	ND
Carbohydrate	+

Key: Qualitatively + = presence ND = not detected

### 3.2 Acute Toxicity Study

The administration of aqueous leaf extract of *Celtis integrifolia* at a dose rate of 3000 mg/kg body weight to Wistar albino rats orally, did not produce any mortality in the treated rats. The oral LD<sub>50</sub> using the up and down procedure therefore could not be calculated. However, clinical signs of depression and anorexia were noticed few hours following the extract administration.

### 3.3 Effect of Aqueous Leaf Extract of *Celtis integrifolia* on Castor Oil Induced Diarrhea

The result of the effect of extract on castor oil induced diarrhea is shown in Table 2, aqueous leaf extract of *Celtis integrifolia* (250-750 mg per os) and diphenoxylate 3 mg/kg body weight, significantly ( $p < 0.001$ ) protected rats against castor oil induced diarrhea in dose dependant manner when compared with control (Table 2).

### 3.4 The Effects of Aqueous Leaf Extract of *Celtis integrifolia* on Gastrointestinal Transit of Activated Charcoal

The distance travelled by the charcoal meal in the control group in comparison to the entire length of the small intestine from the pyloric sphincter to the ileum-caecum junction was used as an index of gastrointestinal motility in rats. The aqueous leaf extract of *Celtis integrifolia* (250-750 mg/kg) given orally, significantly ( $p < 0.01$  or  $0.001$ ) reduced the gastrointestinal distance travelled by the charcoal meal in rats compared with the normal saline group (Table 3). The extract produced dose dependent decrease of gastrointestinal transit of charcoal meal in rats.

The gastrointestinal transit of charcoal meal produced by atropine (3 mg/kg) was similar to that of *Celtis integrifolia* aqueous leaf extract at 750 mg/kg.

## 4. DISCUSSION

Acute toxicity of aqueous leaf extract of *Celtis integrifolia* at the dose of 3000 mg/kg did not produce any mortality in the treated rats. The administration of such high dose to animals without death or serious toxicity may be an indication of relative safety of the extract. According to [17], substances with the LD<sub>50</sub> of 50-500 mg/kg body weight are regarded as being highly toxic, while those above 500 mg/kg body weight but not more than 1000 mg/kg are moderately toxic and those substances whose LD<sub>50</sub> are above 1000 mg/kg body weight are regarded as being relatively safe (low toxicity).

The phytochemical test of aqueous leaf extract of *Celtis integrifolia* Lam indicated the presences of saponins, tannins, reducing sugar, flavonoids and cardiac glycosides all in moderate concentrations. These chemical constituents have been shown to have therapeutic values and anti-microbial activity against both gram positive and gram negative organisms also reported to have neoplastic activity [18,19]. Also, [20] and [21] reported that tannins are known to inhibit microbial proliferation by denaturation of enzymes involved in microbial metabolism and their astringent properties are also important in wound healing.

Previous experiments have shown that flavonoids possess anti-allergic, anti-inflammatory and anti-oxidant properties [22]. Glycosides present in low concentration in *Celtis integrifolia* aqueous leaf extract are known to exact physiological action, with diuretic and anti-microbial activities [23,24].

The inhibition of diarrhea and reduction in faecal output by a substance are the basis for the pharmacological evaluation of a potential anti diarrheal agent. Many anti diarrheal agents act by reducing gastrointestinal motility, the *Celtis integrifolia* aqueous leaf extract was observed to reduce diarrhea produced by castor oil. Castor oil is a triglyceride of fatty acids. It contains ricinoleic acid, which when released induces irritation of the gastrointestinal mucosa, induces inflammation, may cause increased fluid secretion and enhance motility of the

**Table 2. Effect of aqueous leaf extract of *Celtis integrifolia* on castor oil induced diarrhea in Wistar albino rats**

Treatment (mg/kg)	No. of rats	Mean no. of unformed faeces	Percent protection (%)
Control (CO + Normal saline)	5	14.8±1.6	0.000
250 mg/kg + CO	5	7.2±1.8	51.4 <sup>a</sup>
500 mg/kg + CO	5	5.2±4.7	64.9 <sup>a</sup>
750 mg/kg + CO	5	3.4±1.5	77.0 <sup>a</sup>
Diphenoxylate (3 mg/kg) + CO	5	1.6±0.5	89.2 <sup>a</sup>

Mean ± Standard deviation based on five observations; CO = Castor oil; <sup>a</sup>p<0.001 (significantly different from the control)

**Table 3. Effect of aqueous leaf extract of *Celtis integrifolia* on mean gastrointestinal transit of activated charcoal in rats**

Treatment	Total length of the intestine (cm)	Total movement of charcoal (cm)	Percentage (%) distance travelled by activated charcoal
Distilled H <sub>2</sub> O	75.25±5	52.66±3.65	0.000
Atropine SO <sub>4</sub> (3 mg/kg)	74.50±5	20.26±2.27	60.52 <sup>a</sup>
Extract 250 mg/kg	96.25±5	34.5±10.68	34.48 <sup>b</sup>
Extract 500 mg/kg	83.00±5	29.26±7.75	44.45 <sup>a</sup>
Extract 750 mg/kg	80.50±5	22.36±2.73	57.53 <sup>a</sup>

Mean ± Standard deviation based on five observations.

<sup>a</sup>p<0.001 ( Highly significant different from the control).

<sup>b</sup>p<0.01 ( Moderate significant different from the control)

gastrointestinal tract resulting in diarrhea [21]. Since the extract has the ability to inhibit the castor oil induced diarrhea, the anti-diarrheal effect may include decreased gastrointestinal secretion and/or inhibition of gastrointestinal motility. Diphenoxylate (Standard drug) an opiate used in this study is known to inhibit gastrointestinal secretion and motility. From this study, it is likely that the extract may mediate its effect through similar mechanism.

Flavonoids present in the aqueous leaf extract of *Celtis integrifolia* used in this study have been demonstrated to inhibit contraction induced by spasmogens [25,21]. The ability of the extract to inhibit gastrointestinal transit in rats suggests that the aqueous leaf extract of *Celtis integrifolia* may possess antispasmodic agents. Properties such as these may be responsible for the anti-diarrhea effect of the water extract of this plant. The results provided some justification for the use of these plants as natural anti-diarrheal agents as well as remedy against colic.

The extract was also observed to have dose dependently prolonged gastrointestinal transit period of the charcoal. The passage of a charcoal meal through the gastrointestinal tract in

rats is used as a parameter for gastrointestinal motility and to study the laxative effect as well as the inhibition of intestinal motility [25,21]. The effect on the gastrointestinal transit revealed that the extract at 750 mg/kg reduced the distance travelled by 57.5%. While, Atropine (3 mg/kg) an anti-muscarinic drug reduced the transit distance by 60.5%.

## 5. CONCLUSION

Conclusively, phytochemical screening revealed the presence of saponins, tannins, alkaloid, flavonoids, cardiac glycosides, carbohydrate and reducing sugars. The extract has low acute toxicity, and has anti-diarrhea effect, since it inhibited castor oil induced diarrhea and decreased the gastrointestinal transit of charcoal in rats.

## CONSENT

It is not applicable.

## ETHICAL ISSUES

All procedures involving laboratory animals complied with National Academy of Science

guidelines on handling of experimental animals [15] and ethical approval for this study was obtained from animal ethics committee of the Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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