Evaluation of Anti-inflammatory and Anti-diarrhoeal Activity of Leaf Aqueous Extracts of *Zizyphus Lotus* (L) in Albino Wistar Rats

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Abstract

The present study was carried out to investigate the phytochemical screening, the acute toxicity, *in vivo* anti-inflammatory and anti-diarrheal activities of the *Zizyphus lotus* (*Z lotus*) leaf's aqueous extract. The extract was subjected to phytochemical analysis, acute toxicity study, anti-inflammatory evaluation using carrageenan induced paw edema and the anti-diarrheal activity was assessed by the castor oil induced diarrhea inhibition method in laboratory rats. The preliminary phytochemical screening of the extract revealed the presence of saponins, flavonoids, and triterpenoids. The extract at the doses used caused a significant ($P < 0.05$) reduction in the wet feces dumped by the rat with the castor oil-induced diarrhea, and decreased the distance travelled by the charcoal meal. The results showed that the extract of *Zizyphus lotus* has a significant antidiarrheal and anti-inflammatory activity which supports its use in traditional herbal medicine practice.

Keywords: Acute toxicity; leaf aqueous extracts; anti-diarrhoeal and anti-inflammatory activity; phytochemical; *Zizyphus lotus*

Introduction

Zizyphus, known as jujube, (commonly called in Algeria sedra), belongs to the family of Rhamnaceae. As a tropical and subtropical plant, *Z lotus* usually grows in arid and semi-arid countries and is widespread in Africa, especially in Algeria.

Several parts of *Zizyphus* plants are widely used in traditional medicine for the treatment of many diseases (Baba, 1999), such as gastrointestinal and liver disorders, urinary tract and skin infections, and diabetes (Glombitza *et al.*, 1994; Renault *et al.*, 1997; Croueour *et al.*, 2002). In the

recent years, several scientific reports have been made on the presence of numerous biologically active molecules of *Z lotus*, which could have beneficial effects on nutrition, health, and human and animal diseases (Chouaibi *et al.*, 2012a). In herbal medicine, the properties of bioactive compounds depend on all parts of the plant (root, leaf stem, pulp or fruit) and the type of extract used. *Z lotus* is known for its high content of polyphenols with antioxidant and antimicrobial immuno-modulatory properties (Ghazghazi *et al.*, 2014; Abdoul-Azize *et al.*, 2013). In addition to other biologically active molecules, we mentioned cyclopeptide alkaloids, known as lotusins (Ghedira *et al.*, 1993), saponins, dammarane and various flavonoids (Borgi *et al.*, 2008) which were isolated from this shrub, as well as polyunsaturated fatty acids (oleic and linoleic acid). These latter are characterized by a high carbohydrate content and fibers, rich in seed extracts and endowed with anti-ulcerogenic properties and antioxidant effects (Chouaibi *et al.*, 2012b; Abdeddaim *et al.*, 2014). Hence, the present study aims to identify the phytochemical constituents, the acute toxic effects, and to demonstrate that the *Z lotus* leaf extract can be used as a traditional anti-diarrheal and anti-inflammatory remedy.

Materials and methods

Fresh green *Z lotus* were collected in Summer 2014 at the Interior town Djelfa, located in, north-central Algeria, Djelfa has no shores, from the Oulad Nail Mountains at an elevation of 3.734 feet (1.138 meters). It is situated between the towns of Bou Saâda and Laghouat. A taxonomist from Botany Higher National Agronomic School Departments, Algiers, authenticated the leaves. A voucher specimen was deposited at the Giffen Herbarium of Higher National Veterinary School, Algiers for future reference.

The aqueous extracts were obtained by using an adaptation of the method developed by Guede-Guina *et al.* (1995). Thus, 50 g of leaf powder were put with 500 mL of distilled water to macerate) on a magnetic agitator for 72 h at room temperature. The homogenate was then filtered using clean cotton wool and Whatman paper N°1. This filtrate underwent evaporation under reduced pressure using a Rotavapor at 40°C then was lyophilized for 12 hours and the collected product was preserved in a refrigerator at 4°C for further use. Lyophilized leaves were then a dried form.

Experimental animals

The study was performed on adult Wistar rats of both sexes with an average weight of 200±20g, obtained from the Pasteur Institute of Algiers. The control animals and treated animals were maintained under standard environmental conditions (temperature of 22 ± 3°C, relative humidity: 55-65% and 12 h light/ dark cycle) and had free access to food and water ad libitum.

Guideline for the Care and Use

All studies were conducted in accordance with Guide for the Care and Use of Laboratory Animals and approved by the Laboratory Research Council of Higher National Veterinary School, Algiers Algeria. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research. (Agreement Number 45/DGLPAG/DVA.SDA.14).

Preliminary phytochemical screening

The preliminary phytochemical screening was performed qualitatively using several chemical tests to detect the presence or absence of various classes of phytoconstituents the *Z lotus* extract solution. Phytoconstituents like flavonoids, alkaloids, saponins, tannins, sterols, and terpenes were identified based on the changes in color of the different reagents that were used in accordance with the standard procedures described by Harborne (1973) and Evans (1989).

Acute toxicity study

The Acute toxicity test was performed as per OECD guideline 423 for chemicals testing (2001). Five rats per sex were administered a single oral dose of 2000 mg/kg body weight while the control group received water vehicle. The rats were under continuous observation individually for 30 min, then for the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days in order to record any mortalities or/ and the appearance of general signs and symptoms of toxicity.

Anti-inflammatory activities of the extract; Carrageenan-induced paw Oedema; Anti-inflammatory activity

The anti-inflammatory activity of the *Z lotus* aqueous leaf extracts was investigated in a Carrageenan-induced inflammatory model. Acute inflammation was induced in rats by the method of Winter *et al.*, (1962). The control group was administered a saline solution only, while the

third group was treated with diclofenac sodium. Diclofenac sodium was the preferred positive control substance in studies to compare the results of the test with the known anti-inflammatory activity of the drug 10 mg/kg p.o. The fourth, fifth and sixth groups were administered the leaf aqueous extracts (100, 200 and 300 mg/kg/day p.o. respectively).

The acute inflammatory Oedema was produced by sub-plantar injection of 0.1 ml 1% w/v suspension of Carrageenan in normal saline, in the right hind paw of the rats, 1 h after the oral administration of the doses of the *Z lotus* aqueous leaf extract, positive (Diclofenac) and negative control (carrageenan) substances to the overnight fasted rats. The thickness (mm) of the paw was measured immediately and at 60 minutes interval for four hours after the Carrageenan injection, using vernier calliper (Vasudevan *et al.*, 2006)

The perimeter of paw was measured by using vernier callipers. Measurements were taken at 0–4 h after the administration of the carrageenan.

The anti-inflammatory activity was calculated by using the relation T, Thickness of paw in control group; T₀, Thickness of paw edema in the test compound treated group.

$$\% \text{ inhibition of edema} = (T - T_0 / T) \times 100$$

Antidiarrhoeal activity; Castor oil induced diarrhoea in rats.

Castor oil was used to induce diarrhoea according to the method described by Awouters *et al.* (1978). Male rats (200–220 g) were fasted for 18 h. They were divided into five groups (n=6). Being the control group, group I rats were orally administered normal saline solution (2 mL/kg). The second group received standard drug, loperamide (2 mg/kg) orally as suspension. Doses of 100, 200 and 300 mg/kg body weight of *Z lotus* aqueous extract were orally administered to the groups III, IV and V respectively. After 60 min of the drug treatment, the animals of each group orally received 1 mL of castor oil. The watery faecal material and the number of defecations were noted for up to 4 h in the transparent metabolic cages with filter papers at the base. The Weight of the paper before and after the defecation was noted.

The total diarrhoea faeces for the control group were considered 100%. The results were

expressed as a percentage of inhibition of diarrhoea. The percentage of defecation inhibition was calculated as follows:

$$\% \text{ of Defecation Inhibition of defecation} = \frac{A - B}{A} \times 100$$

A indicates the average number of defecations caused by castor oil;

B (indicates the average number of defecations after administration of the drug and *z lotus* extract).

Gastrointestinal motility assay

Gastrointestinal motility assay was performed according to the method described by Chitme *et al.*, (2004). The experimental rats were completely randomized into five groups of six animals each. The negative control group orally received saline (2 mL/kg) as the control group. The positive control group II orally received the standard drug, loperamide (5 mg/kg body weight). Doses of 100, 200 and 300 mg/kg body weight of *Z lotus* aqueous extract were orally administered to the groups III, IV and V respectively. Thirty minutes after the drug administration, 1 mL of charcoal meal (5%) was orally administered to all animals and 30 min later, all the rats were euthanized by cervical dislocation and their abdomens were opened to measure the distance travelled by the activated charcoal. The results were expressed as percentage of the total length of the intestine from the pylorus to the caecum:

$$\text{Inhibition (\%)} = \frac{D_{\text{control}} - D_{\text{treated}}}{D_{\text{control}}} \times 100$$

D control = weight of intestinal content in control group;

D treated = weight of intestinal content in treated group.

Statistical analysis

Statistical analysis was performed using STATISTICA (Version 10, Stat Soft France, 2003). All the values were expressed as mean $\pm$ SD. The data were assessed using one-way analysis of variance (ANOVA). Statistical significance was accepted at $p < 0.05$.

Results and discussions

Phytochemical screening

During this phytochemical study, the screening allowed us to characterize the different families of existing chemical compounds in the *Z lotus* leaves' aqueous extract. The results of the phytochemical tests performed on the extracts from the leaves, reveal the presence of all the elements analyzed: Flavonoids, alkaloids, saponins, tannins, sterols, and terpenes. The phytochemical screening results are illustrated in Table 1.

Acute toxicity

Limit test was performed at 2000 mg/kg as single dose; the animals did not develop any visible signs of toxicity, and no mortality at the dose (2000 mg / kg). Therefore, the LD50 of the aqueous extract of *Z lotus* could be classified in Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS), hazard category 5 with a LD50 ranging from 2000 mg / kg and 5000 mg / kg orally in rats

Anti-inflammatory activity. Effect of the of the *Z lotus* leaf's aqueous extract on carrageenan induced rat paw Oedema.

The results of the anti-inflammatory activity of the *Z lotus* leaf's aqueous extract at the doses of 100, 200 and 300 mg/kg against paw Oedema induced by carrageenan are shown in Table 2. The Maximum edematous inflammation was obtained 3 h after the administration. The volume of paw oedema varies with time. After one hour of carrageenan injection. The anti-inflammatory activity data indicated that all the test concentrations (100, 200 and 300 mg/kg) were able to significantly reduce the carrageenan-induced oedema at different times after the injection of the phlogistic agent, in comparison to

control ($p < 0.05$) increased activity after 2 hours, the reduction of the oedema by the aqueous extract of the *Z lotus* leaf at the dose of 300 mg/kg was similar to the standard used (diclofenac) throughout the entire period of the observation, No significant difference was observed in oedema inhibition in the standard group (diclofenac 10mg/kg) and the group of the *Z lotus* leaf's aqueous extract at the doses of 100, 200 and 300 mg/kg against paw oedema induced by carrageenan (Table 2).

Determination of anti-diarrhoeal activity.

Effect of castor-oil-induced diarrhoeal

In the castor oil-induced diarrhoea experiment, the aqueous extract of *Z lotus* leaves produced a marked antidiarrheal effect in the rats as shown in table 3, Diarrhoea was clinically apparent in all the animals of the control group 45 min after administration of castor oil for the next 4 hours. A significant ($p < 0.05$) reduction in the number of defecations over the length of the four hours was achieved with the aqueous extract of the *Z lotus* leaves when compared to the control. Generally, all doses of the plant extracts had practically reacted like loperamide, Highest inhibition percentage of defecation in the extract treated groups was observed at 300mg/kg (73.40. %), while the Loperamide retained the maximum percentage inhibition of defecation (80.25%).

Effect of castor oil-induced gastrointestinal motility

The results illustrated in table 4 from the gastrointestinal motility tests showed that the speed of intestinal transit followed the same pattern as in the castor-oil-induced diarrhoeal, and were as follows: the average distance moved by the charcoal marker was greatest for the control group

Table1. Phytochemical constituents in the *Z lotus* leaf's aqueous extract

Phytochemical constituents	Relative presence
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Sterols	+
Terpenes	+

+: presence of specific phytoconstituents; -: absence of specific phytoconstituents.

(group I). The aqueous extract of the *Z lotus* leaves significantly ($p < 0.05$) decreased propulsion of the charcoal meal in the rat gastrointestinal tract and was based on the three doses of extract (100, 100 and 300 mg/kg (p.o), compared with the control group that received normal saline (2ml/kg) (Table 4). The effect is comparable to that of the standard drug loperamide (5 mg/kg) which markedly better reduced the propulsion of charcoal meal through gastrointestinal tract.

Preliminary phytochemical screening of aqueous leaf extract of *Z lotus* revealed the presence

of various chemical constituents. This result was in agreement with earlier findings where the presence of phytochemicals like flavonoids, tannins, saponins, cardiac glycosides, triterpenoids and alkaloids were detected in the plant (Slimani *et al.*, 2017). These phytochemical constituents are physiologically active compounds possessing great potential for therapeutic and prophylactic uses (Jivad *et al.*, 2016). Some of these molecules have been documented to possess interesting anti-inflammatory (Perez, 2001; Han and Bakovic, 2015; Patil *et al.*, 2019) and antidiarrheal proper-

Table 2. Anti-inflammatory effect of the *Z lotus* leaf's aqueous extract on carrageenan-induced rats (mean $\pm$ SD)

Groups	Dose (mg/kg) p.o.	Edema Size Means (mm) $\pm$ SD (%Inhibition)				% inhibition
		1 st h	2 st h	3 st h	4 st h	
Control Carrageenan		5.96 $\pm$ 0.04	5.98 $\pm$ 0.03	6.54 $\pm$ 0.006	6.85 $\pm$ 0.06	-
Carrageenan + diclofenac	10	4.04 $\pm$ 0.21 * 32.21	3.03 $\pm$ 0.24 * 49.33	2.79 $\pm$ 0.23 * 57.33	2.44 $\pm$ 0.23 * 64.37	50.81
Carrageenan + aqueous extract	100	4.40 $\pm$ 0.34 * 26.17	3.85 $\pm$ 0.11 * 35.61	3.14 $\pm$ 0.21 * 51.98	3.08 $\pm$ 0.10 * 55.03	42.19
Carrageenan + aqueous extract	200	4.25 $\pm$ 0.21 * 28.69	3.69 $\pm$ 0.32 * 38.29	3.42 $\pm$ 0.31 * 47.70	3.02 $\pm$ 0.22 * 55.91	42.64
Carrageenan + aqueous extract	300	4.23 $\pm$ 0.13 * 29.02	3.24 $\pm$ 0.32 * 45.81	2.78 $\pm$ 0.24 * 57.49	2.67 $\pm$ 0.22 * 61.10	48.35

*P < 0.05 - significant compared to carragenan treated group.

Table 3. Effect of *Z lotus* leaf aqueous extract on castor oil-induced diarrhoea in rats

Group	Treatment Dose (mg/kg)	Mean number of defecation after 4 h	% Inhibition of defaecation
G1	2 mL normal saline+CO	6.28 $\pm$ 0.18	-
G2	5 mg/kg loperamide+CO	1.24 $\pm$ 0.23 *	80.25
G3	100 mg/kg extract+CO	2.42 $\pm$ 0.13 *	61.46
G4	200 mg/kg extract+CO	2.39 $\pm$ 0.21 *	61.94
G5	300 mg/kg extract+CO	1.67 $\pm$ 0.27 *	73.40

*Statistically significant $p < 0.05$

Table 4. Effect of *Z lotus* leaf aqueous extract on charcoal gastrointestinal transit in albino rats

Treatment mg/kg /Group	Length of small intestine (cm)	Distance travelled by charcoal meal (cm)	Percentage intestinal transit	Percentage inhibition (%)
Group1 (Control)+CH	110.60±2.48	68.73±4.75	62.14	-
Group 2 (loperamide) +CH	107.45±3.42	31.27±1.18	29.10*	54.50
Group 3 (100) +CH	107.18±3.32	46.65±2.3	43.52*	32.12
Group 4 (200) +CH	104.85±4.28	42.10±3.42	40.15*	38.74
Group 5 (300) +CH	106.23±4.52	36.12±3.18	34*	47.44

*Statistically significant $P < 0.05$ compared to the control, CH=charcoal.

ties (Rahman *et al.*, 2018). These might be responsible for the activities of the plant extract seen in this study.

We estimated that the LD50 is higher than 2g / kg since we have not observed mortality or signs of toxicity for 14 days, according to the GHS classification, our product belong to the category 5 product slightly or not toxic.

Carrageenan Induced hind paw edema is the standard experimental model of acute inflammation used to assess the anti-inflammatory activity of several natural and synthetic compounds (Vogel, 2002; Boominathan *et al.*, 2004). It is the distinctive model of the acute inflammation exhibiting a high degree of reproducibility (Panthong, 2007). Diclofenac (10mg/kg) was taken as a standard drug to test anti-inflammatory activity of aqueous leaf extract of *Z lotus*. Mean increase in paw volume and percentage of inhibition of carrageenan induced paw edema were taken as parameters for assessing anti-inflammatory effect of the test drug.

The development of edema in the paw of the rat after the injection of carrageenan has been described as biphasic event. The initial phase which occurred between 0 and 2.5 h has been attributed to the action of histamine, serotonin and bradykinin on the vascular permeability (Vinger, 1987). The oedema volume reached its maximum proximately 3h post treatment and then began to decline (Vinegar *et al.*, 1969; Rosa and Willoughby, 1971).

Based on the result of the present study, the aqueous extracts of *Z lotus* significantly decreased antioedematogenic effect in both phases of carrageenan-induced acute inflammation. The

extract was found to be comparable to Diclofenac in activity, especially at higher doses. Therefore, it can be inferred that the possible inhibitory effect of aqueous extract of *Z lotus* in carrageenan induced inflammation may be due to inhibition of cyclooxygenase leading to inhibition of prostaglandin synthesis. This suggests secondary metabolites present in the plant which may suppress both phases of acute inflammation by interfering with the release and/or activity of the chemical mediators, such as histamine, bradykinin, and serotonin in the first phase. Several studies also reported that the extracts of *Z lotus* exhibited anti-inflammatory properties (El Hachimi *et al.*, 2017; Zhang *et al.*, 2017).

Aqueous leaf extract of *Z lotus* was evaluated for its antidiarrheal potential against castor oil induced diarrhea and antimotility effect in charcoal meal test in Wistar rat. In the castor oil induced diarrhea model in rat, castor oil induces diarrhea through its active metabolite, ricinoleic acid which causes the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE2 α) release. The prostaglandins released in the small intestines prevent the reabsorption of sodium chloride and water, Therefore, inhibition of prostaglandins biosynthesis delays castor oil-induced diarrhea (Rahman *et al.*, 2015), like that of non-steroidal anti-inflammatory drugs. Castor oil induced diarrhea is related to stimulation of prostaglandins biosynthesis (Kaur *et al.*, 2014). Thereby, a previous study suggested that the colon anti-inflammatory status activities demonstrated by *Z lotus* leaf extract improved the inflammatory status.

The charcoal meal test was carried out to determine the effect of aqueous leaf extract of *Z lotus* on gut motility. Similarly, to the findings in the castor oil-induced diarrhea model, all doses of the plant extract showed a significant reduction in gastrointestinal transit using charcoal meal. This activity was comparable to that of loperamide used here as reference drug and acts by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids. It also buttresses the earlier postulation in the castor oil model that this plant extract reduces diarrhea by inhibition of both motility and secretion (Han *et al.*, 2017). So, the antidiarrheal effect of the plant extract may be attributed to the role of bioactive constituents such as tannins and flavonoids in the extract, which have already been reported for their antidiarrheal activity (Zaouani *et al.*, 2018; Mehesare *et al.*, 2019).

The result indicated that aqueous leaf extract possessed significant antidiarrheal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion.

Conclusion

Existing results of this study confirmed that the aqueous leaf extract of *Z lotus* showed significant anti-inflammatory and antidiarrheal activities. Our present study also reported the presence of flavonoids, alkaloids, tannins, glycosides. Flavonoids and alkaloids were responsible for the activities of this plant as traditional medicine and acclaimed effectiveness in treating several painful, inflammatory, and diarrheal problems. Moreover, it could be a potential source for anti-inflammatory and antidiarrheal drug development. It may be concluded that the present study supported the traditional use of *Z lotus* by medical practitioners in the cure of inflammatory, diarrheal and associated disorders.

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