Gastro-protective, Anti-\textit{Helicobacter pylori} and, Antioxidant Properties of Moroccan \textit{Zizyphus lotus} L.

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**ABSTRACT**

\textit{Zizyphus lotus} L. is a common medicinal plant used in Moroccan folk medicine to treat gastrointestinal disorders such as ulcers and diarrhea. In this paper, we aimed to evaluate the anti-ulcer properties of \textit{Z. lotus} (fruits) methanol extract (ZLM) in various experimental models, as well as its anti-\textit{Helicobacter pylori} and anti-scavenging properties. Anti-ulcer studies were performed in three ulcerogenic induced models (HCl/Ethanol, pylorus ligation and aspirin) in Wistar rats. Up to 500 mg/kg body weight, ZLM produced a non-significant inhibition in the acute ulcer induced by HCl/EtOH solution and a significant effect on the aspirin model (46.2%). The anti-ulcer effect was lower, for both models, compared to cimetidine and omeprazole used as positive controls. ZLM showed a significant reduction of gastric juice secretion and total acidity and an increase in pH value in pylorus-ligation model similarly to positive controls. ZLM inhibited, at 128µg/ml, three \textit{H. pylori} clinical strains among which two were resistant to metronidazole and clarithromycin. ZLM showed a moderate scavenging capacity in DPPH assay ($IC_{50} = 477.6 \pm 47.6 \mu g/ml$). ZLM extract act essentially as antioxidant agent, which support the use of this plant in the traditional Moroccan medicine to cure gastrointestinal disorders.

**INTRODUCTION**

Most of the drugs from plants which have become important in modern medicine had a folklore origin and are traditionally used in some systems of medicine (Borgi \textit{et al}., 2007). \textit{Zizyphus} spp. is used in folk medicine to treat diarrhea, ulcers, and fevers, and also as a sedative (Abalaka \textit{et al}., 2010). \textit{Zizyphus lotus} L. (Desf) is abundantly present in the Mediterranean region, throughout Libya to Morocco, Algeria and southern European countries like Spain, Sicily, Greece and Cyprus (Benammar \textit{et al}., 2010). The \textit{Z. lotus} seeds have a real food value by containing water (6%), protein (19%), total carbohydrate (41%) and oil (33%), as well as essential minerals such as calcium, potassium and magnesium (Chouaib, 2012). In fact, \textit{Z. lotus} L. is very reputed in Moroccan medicinal therapy where the fruits are prescribed in convalescences as febrifuge and invigorating. Also, fruits powder moistened with sour milk or water are applied as plasters against the furuncles and abscess (Bellakhdar, 1997). Moreover, fruits are described as demulcent and included into the treatment of throat and bronchopulmonary irritations as well as ulcer (Le-Floc’h, 1983, Borgi \textit{et al}., 2007). From the roots bark of \textit{Z. lotus} four dammarane saponins were isolated (Renault \textit{et al}., 1997) and new cyclopeptides alkaloids, lotusine A and D, lotusine B, C, E and F were reported by Ghedira \textit{et al}., (1993, 1995).

Borgi \textit{et al}., (2007), found that root barks of \textit{Z. lotus}, given intra-peritoneally, showed a significant and dose-dependent anti-inflammatory and analgesic activity in carrageenan-induced paw oedema in rat. Hence, the presence of flavonoids in the \textit{Zizyphus} extracts was supposed to be responsible for these beneficial effects. Previous studies have shown that \textit{Z. lotus} fruits possess antifungal and molluscsidal activity (Lahlou \textit{et al}., 2002). Gastric ulcer is among the most serious diseases in the world (Repello and Llesuy, 2002).
The pathogenesis of gastric ulcers is often depicted as an imbalance between mucosal integrity and aggressive factors. Factors that impair mucosal defense are HCl, gastrin, histamine, Helicobacter pylori, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), ethanol, caffeine and stress, while factors that promote mucosal integrity are gastric mucus and bicarbonate, gastric mucosal barrier, prostaglandins (PGs) and mucosal blood flow (Brunton, 1996; Friedman and Peterson, 1998). Helicobacter pylori, is an ancient infection agent present in human stomach for thousands of years (Blaser, 1999). In fact, chronic infection with Helicobacter pylori has been demonstrated to be one of the major causes of gastritis resulting in various disease states including peptic ulcers, gastric adenocarcinoma, and mucosal-associated lymphoid tissue (MAL T) lymphoma (Williams and Pounder, 1999). During the last decade, many medicinal plants continue to provide valuable therapeutic agents for the treatment of ulcers both in modern medicine and by the traditional system throughout the world (Jainu et al., 2006). Different therapeutic agents, including plant extracts, are used to inhibit gastric acid secretion or to boost mucosal defense mechanisms by increasing mucus production, stabilizing surface epithelial cells, or interfering with prostaglandin (PG) synthesis (Lewis and Hanson, 1991). Several studies have suggested that the phytochemical content and antioxidant/free radical scavenging effect of fruits and vegetables contribute to their protective effect against chronic and degenerative diseases (Seifried, 2007; Goodman et al., 2011). Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical induced tissue injury (Mukhopadhyay, 2012). In fact, free radicals play a role in the pathogenesis of chronic degenerative diseases including cancer, autoimmune, inflammatory, cardiovascular and neurodegenerative diseases and aging generally (Surh et al., 2001; Vaya and Aviram, 2001; Aruoma, 2003; Seifried, 2007). Owing to these facts, synthetic and natural compounds with potential antioxidant activity are receiving increased attention in biological research, medicine and pharmacy (Seifried, 2007; Goodman et al., 2011). In the present study we investigated the gastro-protective, anti-Helicobacter pylori and antioxidant properties of Z. lotus L. methanol extract (ZLM). Three experimental gastric ulcer models were assayed: pyloric ligation, (HCl/EtOH) and aspirin-induced gastric ulcer. The obtained results were compared to cimetidine and omeprazole, drugs commonly used in the treatment of ulcers.

MATERIEL AND METHODS

Plant material and preparation of Z. lotus methanol extract

Z. lotus fruits were collected in Essaouira Region (Morocco). The taxonomic identification of the plant material was confirmed by a plant taxonomist (Prof. A. Ouhammou) in the Laboratory of Plant Ecology of the Faculty of Sciences-Semlalia, Marrakesh (Cadi Ayyad University), where a voucher specimen was deposited (ZLI026). Z. lotus L. fruits were dried in the shade and thereafter ground to a powder. 150 g was exhaustively extracted with methanol-water mixture (70:30) in a Soxhlet extractor. The aqueous methanol extract (ZLM) was filtered and then concentrated to dryness under vacuum. The residue (yield=46 % w/w) was stored in a refrigerator at 4°C until the time of extract use.

Phytochemical screening

Preliminary phytochemical screening of Z. lotus extract involved qualitative determination of the following substances: alkaloids, saponins, terpenes, tannins, quinones, and flavonoids. Determinations were carried out in accordance with procedures described by Harborne (1991).

Anti-ulcerogenic essay

Animals

Wistar rats of both sexes, weighing 150-200 g were used for the anti-ulcerogenic essay. The animals were supplied by the Animal Care Facility of the Faculty of Sciences Semlalia, Cadi Ayyad University of Marrakech (Morocco). They were randomly assigned to different groups and a period of 5 days was allowed for adaptation on each experiment. Animals were kept under standard environmental conditions (25 ± 2° C; 12/12 h light/dark cycle). They were provided with standard rodent pellets diet and had free access to tap water. Before testing, the animals were fasted for 48 h with access to water ad libitum. All studies were authorized by the Ethical Committee for Animal Care of the Faculty of Sciences Semlalia, University CAdi Ayyad, Marrakech, Morocco, in accordance with the National Institute of Health “Guide for the Care and Use of Laboratory Animals” (NIH publication no. 85-23, 1985).

Dose preparation and route of administration

The doses of ZLM, aspirin, cimetidine and omeprazole were selected based on literature survey (Jorge et al., 2004; Ajaikumar et al., 2005). ZLM extract was dissolved in distilled water. Two concentrations, 25 and 50 mg/ml were prepared in order to obtain the doses: 250 and 500 mg/kg bodyweight respectively. Aspirin, cimetidine and omeprazole were dissolved in distilled water. Solutions were prepared in order to obtain the administered doses which were 400, 100, and 20 mg/kg bodyweight respectively. HCl/ EtOH solution (0.3 M/60 %, v/v) was used as necrotizing agent to induce gastric ulcers (Sannomiya et al., 2005). All solutions were administered orally through gastric intubation in all experiments.

Gastric lesions induced by HCl/ Ethanol

The animals were divided into six groups of six rats each. Group I: normal control (NC) was given 1 ml distilled water orally. Group II through Group VI received 1 ml of HCl/EtOH solution orally to induce gastric ulcers (Sannomiya et al., 2005). Group II served as experimental control (EC), Groups III and IV (ZLM250 and ZLM500) were administered ZLM at doses of 250 and 500 mg/kg respectively, Group V (CIM) received cimetidine at a dose of 100 mg/kg, and Group VI (OMP) received...
omeprazole at a dose of 20 mg/kg. In treated groups, ZLM, cimetidine and omeprazole were administered orally 1 hour before HCl/EtOH, and 60 minutes after necrotizing agent administration, the rats were sacrificed with an overdose of urethane (i.p). Their stomach was excised and opened along the greater curvature. The mucosal surface was observed with the help of a magnifying lens and the extent of gastric damage was scored according to the method described by Main and Whittle (1975) using the following scale: 0 = normal mucosa, 1= patches length was less or equal to 1 mm, 2= patches length was between 1 and 2 mm, 3= patches length was over than 2 mm. The Ulcer index (UI) was calculated by using the formula:

\[ UI = [1 \times (\text{number of lesions of grade 1}) + 2 \times (\text{number of lesions of grade 2}) + 3 \times (\text{number of lesions of grade 3})] \times 10^{-1} \]

(Main and Whittle, 1975; Ajaikumar et al., 2005).

Pylorus-ligated rats (anti-secretory studies)

In pylorus ligation model, gastric secretion parameter was measured following the method of Shay et al. (1945). All groups of rats were fasted for 48 hours, with free access to water. One hour prior pylorus ligation, ZLM (250 and 500 mg/kg), cimetidine (100 mg/kg), omeprazole (20 mg/kg) and distilled water were administered orally to rats. The animals were anaesthetized with urethane (0.5-1.5 g/kg ip); then a small excision was done on the abdomen in order to make a ligature on the pylorus area. Four hours later, animals were sacrificed with an overdose of urethane. The abdomen was opened and another ligature was placed around the esophagus close to the diaphragm. The stomach was removed and the gastric content collected in tubes to determine the total amount of gastric juice (in ml) and its pH values. Ulcer index was calculated as described above. The total acid content of the gastric secretions which is the total amount of acid in the solution was determined by titration to pH 7.0 with 0.01 N of NaOH and calculated using the following formula (Gill et al., 2012).

\[ \text{Total acid content of the gastric secretion} = \frac{V_{\text{NaOH}} \times \text{normality} \times 100 \text{ (meq/L/100g)}}{0.1} \]

Gastric lesions induced by Non-steroidal Anti-Inflammatory Drug (Aspirin)

The experiment was performed according to the method reported by De Andrade et al., (2007). After 12 h of fasting, the rats were randomly divided into five groups of six animals each. The first group was given 1 ml of vehicle (distilled water); the second and the third groups were treated respectively with cimetidine (100 mg/kg) and omeprazole (20 mg/kg). The remaining two groups received 250 and 500 mg/kg of ZLM. All the treatments were administered orally. One hour after treatment, all the rats received aspirin (400 mg/kg) to induce gastric ulcer. Four hours later, animals were sacrificed by an intraperitoneal overdose of urethane. The stomachs were removed, and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots; the ulcerative index was calculated as described above.

Drugs and chemicals

Hydrochloride acid, ethanol, methanol, urethane, sodium hydroxide and formalin were of analytical grade and were purchased from Sigma-Aldrich (Germany). Cimetidine was obtained from “Promopharm Pharmaceuticals” Casablanca (Morocco). Omeprazole was obtained from “Cooper Maroc” Casablanca (Morocco). Aspirin was obtained from “Bayer Maroc” Casablanca (Morocco).

Statistical analysis

Values were expressed as mean ± S.E.M. The statistical difference between the treated groups and the negative control was calculated by using the Student’s t-test. Results were considered significant if \( p < 0.05 \), and highly significant if \( p < 0.001 \).

In vitro evaluation of ZLM activity against \( H. pylori \)

Bacterial strains

Twenty-two clinical strains of \( H. pylori \) as well as \( H. pylori \) J99 reference strain were used in this study. The clinical strains were obtained from gastric biopsy specimens of patients from the Pellegrin Hospital, Bordeaux (France) and suffering from chronic gastritis and peptic ulcer. Strains were preserved in the Laboratory of Bacteriology (Centre National de Reference des Campylobacters et Helicobacters, Bordeaux, France). After undergoing the strains to different identification tests (fresh examination, catalase, oxidase, and urease tests), they were stored at -80°C in peptone glycerol solution until use.

Drugs and chemicals

Methanol and DMSO were purchased from Sigma (Germany). Sterile water (VERSOL) was obtained from Aguettant Laboratory (France). Clarithromycin and metronidazole were obtained from Abbot France and Rhône-Poulenc Rorer, Montrouge, (France) respectively. Defibrinated sheep blood and poly-vitex were obtained from bioMerieux Laboratory (France). Mueller Hinton Agar was purchased from Bio-Rad Laboratories (France).

Preparation of test solutions

Stock solutions (2 mg/ml) of ZLM, clarithromycin and metronidazole were prepared with DMSO (2%), methanol and sterile water, respectively. They were sterilized by filtration through a 0.45 µm Millipore filter (Millex HA). Two-fold serial dilutions were then performed in sterile water to give final concentrations in Petri dishes ranging from 128 to 0.5µg/ml.

Preparation of inocula

The inocula of bacterial strains were prepared from 72 h-old culture and suspensions were adjusted to 2-3 McFarland standard turbidity using sterile Brucella broth.
Detection of inhibitory activity

Minimal inhibitory concentrations (MIC) were determined by agar dilution method at concentrations ranging from 0.5 to 128 µg/ml. Antibiotics were used at the same concentrations. To each plate, 3.6 ml defibrinated sheep blood supplemented with poly-vitex, 3.6 ml of extracts or antibiotics, and 28.8 ml of growth medium (Mueller Hinton II Agar, BBL) at pH 7.3, were added, respectively. Final inoculums of 2-3 McFarland standards were spotted at the end with multipoint inoculators (Steers apparatus). The solvent and blank controls contained in addition to the growth medium (28.8 ml) and defibrinated sheep blood (3.6 ml), 3.6 ml of 2% DMSO or sterile distilled water respectively. The plates were then incubated at 37°C for 48 hours in a microaerobic atmosphere consisting of 80% N₂, 15% CO₂ and 5% O₂ (Concept 300, anaerobic work station). The MIC was defined as the lowest concentration at which no visible growth was observed.

Determination of total phenolics compounds

Total phenol content was determined using the Folin-Ciocalteu reagent (Singleton et al., 1999). 500 mg of methanol dry extract was dissolved in distilled water (100 ml). 50 µl of each solution were taken and to which 2 ml of distilled water and 0.25 ml of the reagent of Folin-Ciocalteu are added. After agitation with the vortex, 0, 75 ml of a sodium carbonate solution to 20% were added and the volume was made up to 5.0 ml with distilled water. The tubes are then well agitated and incubated at temperature of 40°C during 30 minutes. The optical density is read at 760 nm. A range standard is carried out in parallel with p-coumaric acid with 20 mg/100 ml. The contents of phenolic compounds are expressed of p-coumaric (µg of acid per gram of dry matter).

DPPH free radical scavenging assay

The antiradical activities of ZLM was estimated using the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) (SIGMA) according to the method of Brand-Williams et al. (1995). Stock solutions of DPPH were prepared in Methanol of analytical grade. Four concentrations (100, 200, 400 and 800 µg/ml) of ZLM extract were prepared with methanol. Quercetin (SIGMA) and vitamin E (alpha-tocopherol) (SIGMA) were used as positive controls, they were dissolved in methanol to obtain final concentrations ranging from 100 to 1.56 µg/ml. 0.75 ml of ZLM methanol solution at different concentrations was mixed with 1.5 ml of DPPH methanol solution (20 mg/l; 0.05 mM). The controls contained all the reagents except the extract or positive controls. The tubes are placed in darkness and the DO was read after 30 minutes. The initial absorbance of the DPPH is measured by using a solution containing 0.75 ml of MeOH and 1.5 ml of solution of DPPH (blank). The values are presented as the mean of triplicate. A linear regression curve was established in order to calculate the IC₅₀ (µg/ml) which the amount of sample necessary to decrease by 50% the absorbance of DPPH. Inhibition of free radical by DPPH in percent (%) was calculated with the following equation:

\[
\% \text{Inhibition} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100
\]

where \(A_{\text{sample}}\) and \(A_{\text{blank}}\) are the absorbance of the sample and the blank, respectively.

RESULTS AND DISCUSSION

Anti-ulcer activity

Results are reported in Table 1. Administration of HCl/EtOH solution to the control group caused a significant increase in the number of lesions in the gastric mucosa visible as thick reddish-black lines in comparison to treated and positive control groups. At 250 and 500 mg/kg, ZLM extract did not significantly prevent the formation of gastric lesions induced by HCl/EtOH, whereas, cimetidine and omeprazole treated groups showed a significant reduction (p<0.001 and p< 0.05 respectively) in ulcer index (Table 1). In pylorus ligated rats, ulcer index and volume of gastric secretion in treated groups with both tested doses of ZLM has been significantly reduced (p<0.001) in comparison to the control group. A significant increase in pH values was noted at 500 mg/ml (p<0.001). While, total acidity was decreased significantly at 500 mg/kg (p< 0.001). On aspirin induced ulcer model, ZLM exhibited at 500 mg/kg a significant reduction in ulcer index (p< 0.05) This gastro-protective effect was less important than that of reference drugs which showed higher inhibitory effects (p< 0.001).

Anti-Helicobacter pylori activity

The results of the anti-Helicobacter pylori essay (Table 2) showed that, ZLM did not exert any inhibitory effect on the reference strain J99 and showed a relatively weak antibacterial inhibitory activity against the twenty two clinical strains with MIC ₅₀ and MIC ₉₀ greater than 128µg/ml. Nevertheless, ZLM has inhibited, at 128µg/ml, two clinical strains resistant to metronidazole and one resistant strain to clarythromycine. Peptic ulcers are due to overproduction of gastric acid and/or decrease in gastric mucosal protection mechanisms. That is why; the anti-ulcer potential and ulcer healing drugs are known to possess the property of decreasing offensive factors or increasing the defensive factors (Pimple et al., 2012). Ethanol is one of the ulcerogenic agents that induces intense damages in gastric mucosa by promoting disturbances of mucosal microcirculation, ischemia and appearance of free radicals, endothelin release, degranulation of mast cells, inhibition of prostaglandins and decrease of gastric mucus production (Hiruma-Lima et al., 2009; Liu et al., 2012). It has been shown that the administration of HCl/EtOH causes injury by attacking the protein in the gastric mucosa leading to a reduction in the protein level (Asai et al., 2011). HCl/EtOH induced gastric ulcers also promotes status in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissues injury (Konturec et al., 1998). Results showed a weak gastro-protective ability of ZLM while cimetidine and omeprazole showed a significant reduction of ulcer index. In pylorus ligation model, ulcers are resulted due to an accumulation of gastric acid and pepsin which leads to the auto-digestion of
gastric mucosa (Bansal and Goel, 2012). Oxidative stress plays an important role in the pathogenesis of more than 100 diseases (Popovic et al., 2009). Moreover, experimental studies have demonstrated that oxygen-generated free radicals and lipid peroxidation are involved in the pathogenesis of acute gastric lesions induced by ethanol, nonsteroidal anti-inflammatory drugs or \textit{H. pylori} (Das et al., 1998; Popovic et al., 2009; Bansal and Goel, 2012). In this model, ZLM reduced the ulcer formation and increased the pH and decreased total acidity values comparatively to omeprazole. These results clearly show anti-secretory activity of ZLM rather than gastro-protective ability. To confirm the effect of ZLM against gastric ulcer we evaluated its efficacy in aspirin-induced-ulcer model. In fact, NSAIDs like aspirin can cause damage to the gastro-duodenal mucosa via several mechanisms, including their topical irritant effect on the epithelium, impairment of the mucosal barrier function, suppression of gastric prostaglandins synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury. The presence of acid in the lumen also contributes to the pathogenesis of NSAIDs-induced ulcers and bleeding by impairing the restitution process, interfering with haemostasis and inactivating several growth factors that are important in mucosal defense and repair (Toma et al., 2005; Whittle, 2003). It’s also important to note that NSAIDs can increase acid secretion, through prostaglandin inhibitory effect on parietal cells (Ligumsky et al., 1983; Soll., 1986). As mentioned previously, ZLM inhibited significantly the ulcer formation in NSAID’s model. This finding suggests that ZLM can act as a stimulator of PGs synthesis. \textit{H. pylori} is known to play an important role in various human upper gastrointestinal tract disorders such as chronic gastritis, peptic ulcer disease and gastric cancer (Ustün, 1986). As recognized, \textit{H. pylori} act essentially by neutralizing gastric acidity by its enzyme urease. In fact, urease converts urea into ammonia, which then counters the stomach acid. This creates a neutralizing environment for protecting \textit{H. pylori} from acid in the stomach (Lin et al., 2005). Our results revealed that ZLM inhibited two metronidazole resistant strains and one strain resistant to clarythromycine at 128 µg/ml. According to Aligiannis et al. (2001), a plant extract with MIC < 500mg/ml is considered as a strong inhibitor. Based on our results, we can compare the ZLM effect on gastric contents to the effect of omeprazol which act essentially as an inhibitor of proton pump H+, K+/ATPase as well as an inhibitor of acid secretion. Cimetidine belongs to a class of medications called histamine H$_2$-antagonists. Histamine is a natural chemical that stimulates stomach cells to produce acid. Histamine H$_2$-antagonists inhibit the action of histamine on the acid-producing cells of the stomach and reduce stomach acid (http://www.medicinenet.com/cimetidine/article.htm).

\textbf{Antioxidant activity}

The antioxidant property of ZLM was evaluated using a free radical (DPPH) scavenging test and total phenol content was evaluated using Folin-Ciocalteteu protocol. In this study the investigation of the antioxidant activity of ZLM is justified by the fact that its anti-ulcer effect might be related to its antioxidant activity. Results showed that ZLM hold a total phenol content of 1200 µg equivalent coumaric acid per gram of dry extract. Whereas the IC$_{50}$, which is, the concentration of the test material causing 50% decrease in initial DPPH, was 477.6±47.6mg/ml. This value is much higher than positive control quercetine and \&-tocopherol.

ZLM exerted a weak but concentration-dependent free radical-scavenging activity in comparison with quercetine used as positive control. Although, further studies are needed to understand the mechanisms of activity of \textit{Z. lotus}, its reported anti-inflammatory activity (Borgi et al., 2007), may be involved since the ulcer infection is in fact an inflammatory reaction.

\textbf{Phytochemical screening}

The phytochemical analysis of methanol extract of \textit{Z. lotus} fruits revealed the presence of saponins, terpenes, sterols, and tannins and traces of flavonoids. Coumarins and alkaloids were not detected. Benammar et al. (2010) showed that \textit{z. lotus} l. fruit seeds are richer in fatty acids than other parts of the plant. Besides, the \textit{Zizyphus} plant seems to be a good source of saturated, monounsaturated and polyunsaturated fatty acids. As far as the essential fatty acids are concerned, linoleic acid was present in all parts of the plant, where the fruit pulp was found to be the richest source, hence Moroccan nomads uses \textit{Z. lotus} fruit in their travel as a source of energy; moreover it allow them to feel satiated (Bellakhder, 1997). Arachidonic acid was detected only in leaves of the \textit{Zizyphus}. Other fatty acids were present principally in fruit seed and leaves. In the same paper, results revealed that concentration of vitamin A and C was higher in fruit pulp than those of the leaves, root and stem of the \textit{Z. lotus} L. (Desf.). Interestingly, the root bark is known for its antidiabetic activity (Glombitza et al., 1994). Basing on these bibliographic data and our finding, we can suggest that the antacid property of \textit{Z. lotus} fruit is mainly due to its richness in chemical compounds known for their biological activities as anti-inflammatory or antioxidant potencies. The Phytochemical screening shows that ZLM contain tannins and saponins as the major compounds and traces of flavonoids and terpenes & sterols. Tannins are one of most important botanical compounds with anti-ulcer and gastro-protective activities (Bansal and Goel, 2012). Also being an astringent, tannins may have precipitated microproteins on the site of ulcer thereby forming an impervious protective pellicle over the living to prevent absorption of toxic substances and resist the attack of proteolytic enzymes (Vasconcelos et al., 2000).

Pharmacologically, tannins are known to possess anti-ulcer activity (Al-Rehailly et al., 2002). They react with the outermost layer of mucosa and make it less permeable and more resistant to chemical and mechanical injury or irritation (Nwafor et al., 1996; 2000). Saponins exhibit, like tannins, a gastroprotective effect (Asl and Hosseinzadeh, 2008). The reported antioxidant ability of both (Francis et al., 2002; Amarovicz, 2007; Asl and Hosseinzadeh, 2008) would explain, to some extent, the observed antiulcer effect.
Table 1: Effects of Z. Lotus against lesions induced by HCl/EtOH, pylorus ligation and, aspirin in rats.

<table>
<thead>
<tr>
<th>HCl/EtOH</th>
<th>Pylorus ligation</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>OMP</td>
</tr>
<tr>
<td>pH</td>
<td>UI</td>
<td>UI</td>
</tr>
<tr>
<td>Acidity (mEq/l/100g)</td>
<td>36.0 ± 0.2</td>
<td>23.0 ± 0.2</td>
</tr>
<tr>
<td>UI</td>
<td>1.3 ± 0.2</td>
<td>0.2 ± 0.1***</td>
</tr>
<tr>
<td>Control</td>
<td>2.3 ± 0.4</td>
<td>82.6</td>
</tr>
<tr>
<td>pH</td>
<td>2.7 ± 0.3</td>
<td>4.2 ± 0.8**</td>
</tr>
<tr>
<td>V (ml)</td>
<td>2.8 ± 0.2</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.9 ± 0.1**</td>
<td>0.8 ± 0.1***</td>
</tr>
</tbody>
</table>

Data are represented as mean ±SEM. Statistical analysis was done by Student test. * = P < 0.05; ** = P < 0.01 and *** = P < 0.001 as compared to control. (n = 6 rats per group).

Table 2: Polyphenols content and antioxidant effects of Z. lotus methanol extract (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Polyphenols (µg CAE/g)</th>
<th>IC50 (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td>ZLM</td>
<td>1200</td>
</tr>
<tr>
<td>Quercetine</td>
<td>-</td>
</tr>
<tr>
<td>VitE</td>
<td>-</td>
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</table>

CAE: Coumaric acid equivalent. IC50: Inhibitory concentration that causes 50% decolourization of DPPH. ZLM: Zizyphus lotus Methanol extract.

CONCLUSION

Based on our results, we conclude that the efficiency of ZLM in ulcer models may be due mainly to its antacid effect in comparison to its gastro-protective effect. These findings can support the use of Z. lotus fruits in Moroccan folk medicine for the treatment of gastric ulcer.

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REFERENCES


Williams MP, Founder RE. Helicobacter pylori: from the benign to the malignant. Am J Gastroenterol, 1999; 94:11-16.

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