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Evaluation of gastroprotective effect of jujube honey in ethanol-induced stomach ulcer in mice

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Honey has been widely documented to have therapeutic benefits, especially on the healing of wounds, digestive disorders, and as an antimicrobial agent. It's known that stomach ulcers are one of the most prevalent diseases that affect humans with no effective treatment. The objective of this study was to investigate the gastroprotective effects of jujube honey against ethanol-induced gastric ulcer in mice and to explore possible mechanism of its action on the gastric mucosa. Stomach's tissues were examined for ulcerative lesions and white blood cell count (WBC), plasma C-reactive protein (CRP) and nitric oxide (NO) for clinical inflammation evaluation. The antioxidant enzyme activities were investigated using the measurement of superoxide dismutase (SOD) and catalase. Treatment with jujube honey reduced the ulcer index and preserved gastric tissue. It also decreased the inflammatory response by reducing WBC and plasma levels of CRP and NO in ulcerative group. In addition, jujube honey-treated group showed increased antioxidant enzyme activities of catalase and SOD. Furthermore, diluted jujube honey is more effective in preserving mucosal gastric integrity. In conclusion, these findings suggest that jujube honey, especially with dilution, may exert a protective effect against ethanol-induced gastric ulcer, through the improvement of antioxidant system and suppression of the inflammatory response via its bioactive compounds.

Keywords: Anti inflammatory, Antioxidant, Bioactive compounds, Gastroprotective, Jujube honey, Stomach ulcer

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Gastric ulcer is very prevalent digestive tract disease in human and is considered to be a global health problem[1]. The pathogenesis of gastric ulcer is a multifactorial in nature and is caused by an imbalance between the aggressive factors destroying the gastric mucosa and the protective agents of the stomach wall[2]. Many factors can increase the incidence of gastric ulcer, including alcohol consumption, stomach acid, pepsin production, bile acids, generation of reactive oxygen species (ROS), *Helicobacter pylori* infection and chronic ingestion of non steroidal anti-inflammatory (NSAIDs) drugs[3]. Several new treatments and natural compounds are investigated for their gastroprotective effects through using ethanol-induced gastric ulcer model[4]. Ethanol causes a rapid influx of neutrophils into the damaged area, which is essentially an acute inflammatory process. Reactive oxygen species (ROS) are then formed in greater amounts, which contributes to oxidative stress in the cellular components including nucleic acids, lipids and proteins[5]. However, defensive mechanisms manifest through the activation of various mechanisms including gastric mucosal integrity, appropriate tissue microcirculation, normal stomach motility, mucus secretion, gastric mucosal integrity, bicarbonate production, nitric oxide (NO), prostaglandin biosynthesis, surface phospholipids, cell renewal, growth factors and antioxidant enzymes[6].

In addition, the antioxidant capacity and other biological properties are related to bioactive compounds found in natural products.They possess several medicinal properties for their potent

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antioxidant activity against diseases related to oxidative stress[7].

Historical evidence demonstrates that honey has been used for medicinal applications for thousands of years, by ancient generations, traditions and civilizations[8]. In Islamic traditional medicine, honey was considered as a detergent drug for treating ulcers and removing moistures and putrefaction from the body[9]. Ibn Hamadush the great Algerian scientist and traditional indigenous doctor, almost in 18th century, had considered honey as a drink, food and drug[10]. Moreover, he recommended an infused rose honey as a gastroprotective agent. Recent findings have shown that honey has several therapeutic effects, especially for gastrointestinal diseases and wound healing[11]. In addition, the stomach-protective properties of various types of honeybee such as Manuka, Kelult Malaysian and Turkish honeys against gastric ulcer were demonstrated in previous studies[12,14].

Interestingly, jujube honey largely used in Algeria and worldwide Jujube honey, which is commonly used in Algeria and around the world, was characterized by its high pH, electrical conductivity, potassium, calcium, and high total phenolic content and other molecules[15,16]. It is derived from the Jujube lotus tree, *Ziziphus lotus* (L.) Lam., commonly called "Sedra or Sidr" belonging to the family *Rhamnaceae* occurring across the Mediterranean Basin, North Africa and the Sahara and the Arabian peninsula[17]. Thus, previous studies have demonstrated that the extracts from root barks, fruits and leaves of *Ziziphus lotus* possessed gastroprotective and antiulcerogenic effects in experimental gastric ulcer models[18].

Traditionally, the jujube fruit was considered as valuable in treating diarrhea and gastric ulcer whereas the jujube leaves were used for the treatment of asthma and lung diseases[9,10]. Despite lacking in documentation on the use of jujube honey as traditional medicine, currently it was recommended by local traditional healers for the treatment of digestive and intestinal problem, skin diseases, cough, imbalances of lungs, and anemia.

It's known that water is essential for life and performs crucial functions, including solubility and efficacy of some drugs. In this regard, mixture of honey with water strengthens the stomach and increases appetite[9]. Moreover, honey was also recommended to be taken with warm water early in the morning on an empty stomach[19]. Recently it was suggested that therapeutic effect and antioxidant activity properties of honey increased with the solubility of its bioactive compounds in water[20].

Considering that honey has multiple biological activities, the present study was undertaken to evaluate physicochemical parameters of jujube honey and to estimate its gastroprotective effect against ethanol-induced gastric ulcer in mice, with examining the hypothesis that diluted jujube honey may be more effective in preventing stomach ulcer. Accordingly, the fundamental mechanisms by which jujube honey displays its efficiency were explained in terms of oxidative stress assessment and inflammatory response evaluation.

Methodology

Honey sample and chemical agents

Unifloral *Ziziphus lotus* honey (Sidr honey) was collected from beekeepers in Laghouat region (Algeria) during season of summer. The honey was collected from the hives and kept in the dark at a temperature of -4°C in hermetically sealed glass jars. Omeprazole (Antag® 20 mg) was obtained from Biocare Laboratories (Algiers) to use as a reference standard medication for gastric ulcers. All the other chemical products employed in the current investigation were of the best quality and analytical grade.

Physicochemical analyses

Physicochemical analysis for measurement of moisture content, electrical conductivity, specific gravity and pH of jujube honey were determined according to the instructions of the International Honey Commission[21]. All the procedures were performed in triplicate.

Moisture content

Moisture is an essential factor in evaluating honey quality. Environmental conditions, level of honey maturity obtained in the hive and handling during collecting, processing and storage all determine the proportion of water content in honey[22]. Moisture content in jujube honey was evaluated using a refractometer (Carl-Zeiss Jena, Germany), by determining the refractive indices at 20°C. The Wedmore table was used to calculate the moisture level and the results were expressed as percentages.

Electrical conductivity

Electrical conductivity was evaluated at 20°C in a 20% (w/v) jujube honey solution of 20 g of honey in 100 mL by a conductimeter in CO_2-free deionized distilled water (Mettler Toledo, Greifensee, Switzerland), according to

the harmonized methods of the European Honey Commission[23]. The results were expressed as μS/cm.

Specific gravity

According to Nandaa *et al*.[24] the specific gravity of jujube honey was determined by dividing the weight of a glass beaker (50 mL) filled with honey by the weight of the same beaker filled with water, the specific gravity of jujube honey was calculated.

pH measurement

pH was determined by pH-meter (Mettler Toledo, Greifensee, Switzerland) in a solution containing 10 g of jujube honey in 75 mL of distilled water[23].

Determination of jujube honey antioxidant activity *in vitro*

Determination of total phenolic content

A modified version of the Folin-Ciocalteu method was used to measure the total phenolic content of the jujube honey[25]. Briefly, 1 g of jujube honey was diluted in 10 mL of distilled water and filtered through Whatman No: 1. Subsequently, 100 μL of Folin-Ciocalteu reagent (50%) were added to 100 μL of honey solution at different concentrations following dilution in distilled water (0.1, 0.5 and 1 g/mL), The flask was stoppered and let to stand for 3 min at room temperature. Thereafter, 2 mL of sodium carbonate (20%, Na_2CO_3) were added. Following 1h of incubation at room temperature in the dark, the absorbance was measured at 760 nm against a distilled water blank using a UV-VIS Spectrophotometer (Hitachi U-1900, Japan). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100 g of honey.

Determination of total flavonoid content

Measurement of total flavonoid content was measured according to the aluminum chloride colorimetric method[26]. 1 mL of distilled water was used to dissolve 1 g of jujube honey and then sonicated for 20 min. Thereafter, 0.1, 0.5, and 1 g/mL of honey solution diluted in distilled water was mixed with 0.3 mL of 5% sodium nitrite, 0.3 mL of 10% aluminium chloride. Optical density reading at 510 nm of samples was taken after 10 min of incubation in the dark, with respect to a distilled water control. For each 100 g of honey, the data were reported as mg of quercetin equivalent (QE).

Determination of free radical-scavenging ability by the use of the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical assay

The DPPH• radical scavenging capacity of the jujube honey samples was determined as previously described by Brand-Williams *et al*. with slight modification[27]. In brief, a 1 mL extemporaneously prepared methanolic solution of DPPH• radical (6×10^{-5} M) was mixed with 500 μL of jujube honey at various concentrations after dilution in distilled water (0.05, 0.1 and 0.2 g/mL). The contents were vigorously agitated, then kept at room temperature in the dark for 20 min before reading the optical density at 517 nm. In each test, the examined sample alone in methanol was used as a blank, and the DPPH• radical alone in methanol was used as a control. The percentage of radical scavenging capacity (RSC) of the investigated samples was estimated using the following equation:RSC (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$, where $A_{control}$ and A_{sample} are the absorbance values of the control and the tested samples, respectively. Furthermore, the IC50 value, showing the concentration that caused 50% DPPH• radical scavenging, was determined from the graph plotted RSC percentage against sample concentration in order to compare the radical scavenging capacity of the samples.

Evaluation of gastroprotective activity

Animals and treatment

Thirty, Balb/c male mice aged 6 weeks weighing between 25 and 30 g were used in this study obtained from Pasteur Institute of Algiers. The animals were housed in the animal facility at Faculty of Science, University M'hamed BOUGARA, Boumerdes, Algeria, at a temperature of 25°C and relative humidity ranging from 60 to 70%, under a 12 h light/dark cycle during all the experiment. The animals were given free access to a standard commercial pellet diet and water *ad libitum*. To avoid coprophagy, each animal was maintained in a wire mesh cage. The experimental design was approved by the Houari Boumediene University of Sciences and Technology Ethics committee and is in accordance with the guidelines of the Algerian Association of Experimental Animal Sciences (N 45/DGLPAG/DVA.SDA.14).

Treatment groups

Mice were randomly separated into 5 groups (6 animals in each) prior to 5 days of acclimatization to laboratory conditions. All treatments were administered intra-gastrically (gavage) once daily for 7 days at a volume of 200 μL/mice, excepting that ethanol treatment was performed on day 7:

– Control: mice in this group received saline solution (0.9%).

- Ethanol (ulcer control group): mice in this group received absolute ethanol (99.9%).
- Omeprazole: mice in this group received omeprazole (40 mg/kg) dissolved in distilled water[12].
- Jujube honey: mice in this group received 5 g/kg b.w. of jujube honey.
- Diluted jujube honey: mice in this group received 2.5 g/kg b.w. of jujube honey diluted in sterilized distilled water (v/v).

Induction of ulcer

Absolute ethanol was adminiseterd intragastrially to induce an ulcer as described elsewhere with slight modification, by giving 200 µL of absolute ethanol for one hour[28]. On day 7 of experiment, all pre-treated animals were fasted for 12 h (water was allowed) and received intra-gastrically 200 µL/mouse of absolute ethanol. The same volume of saline (0.9% NaCl) instead of ethanol was given to the control group. Then, one hour after ethanol administration, all animals were euthanized by cervical dislocation under 25% urethane anesthesia and blood samples were collected in heparinized tubes for biochemical analyses. Plasma samples were collected by centrifugation at 3000 rpm for 10 min at 4°C and then analyzed within 24 h. Also the stomachs were removed and opened along the greater curvature and washed in 0.9% physiological saline solution before being fixed on cardboard to eliminate the gross gastric lesion, to clean away the blood clots and to remove stomach contents.

Gross examination of gastric mucosa

Using an illuminated magnifying lens, the stomach mucosal layer was carefully examined for occurrence of ulcers, which were then measured for their individual regions. The severity of mucosal ulcer was assessed by measuring the area in cm^2 of each lesion. The ulcer area (cm^2) was measured using AxioVisio software (Carl Zeis AxioVision REL. 4.6., Germany) and the percentage of inhibition was calculated by the following formula according to the recommendation of Wasman *et al*., with slight modification[29]:

Percentage of inhibition (%) = [Injury area (ulcer control) - Injury area (treatment or reference)] / [Injury area (ulcer control)] × 100%.

Histopathological study

Tissue samples were taken from the stomach, fixed in 10% formalin for 24 h and processed for paraffin embedding. Paraffin sections (4 µm) were prepared and stained with hematoxylin and eosin (H&E) for histological examination through the light microscope.

Measurement of some biochemical indices

Antioxidant activity

Superoxide dismutase (SOD) activity was measured by the nitroblue tetrazolium (NBT) reduction method[30]. The amount of enzyme activity that results in a 50% inhibition of NBT to formazan was used to define one unit of SOD activity.

Activity of catalase (CAT) was evaluated as the rate of H_2O_2 reduction at 37°C at 240 nm using a spectrophotometer based on the method of Aebi *et al.*[31]. At 25°C and pH 7.0, one unit of CAT activity is the quantity of enzyme that catalyzes 1 mol H_2O_2 in 1 min.

Nitric oxide assay

The level of nitric oxide in the plasma was evaluated using the modified Griess method[32]. The values were expressed as µmol/L.

C-Reactive protein plasma measurement

In order to study the inflammatory status, C-Reactive Protein (CRP) plasma concentration was determined by Spinreact kit according to the manufacturer's instructions (Spinreact, S.A./S.A.U. Santa Coloma, Spain), and the values were expressed as µg/L.

Analysis of leukocyte counts

Whole blood sample was analyzed for the counts of total leukocyte using the routine hematology laboratory method (Abacus 380 Automated Hematology Analyzer, Diatron, Budapest, Hungary).

Statistical analysis

The results were presented as mean ± standard error of the mean (SEM). The variations in means were determined by one-way ANOVA, followed by Bonferroni's multiple comparison test using GraphPad Prism 7.0 (GraphPad Software Inc., La Jolla, CA, USA). The significance level for the null hypothesis rejection was set at 5% ($p<0.05$).

Results

Physicochemical parameters

The results of the physicochemical parameters of the jujube honey are shown in Table 1. Jujube honey was characterized by high values of pH, electrical conductivity, specific gravity, phenolic compounds and antioxidant capacity whereas its moisture content

Table 1 — Physiochemical parameters, total phenolic and total flavonoid contents of jujube honey

pH	Moisture content (%)	Electrical conductivity (µS cm^{-1})	Specific gravity (g/cm^3)	Total phenolic content (GAE mg/100 g)	Total flavonoid content (QE mg/100 g)	Radical scavenging capacity (%)
5.2±0.057	14.57±0.57	648.33±22.83	1.36±0.02	159.78±16.24	3.52±0.24	33.8±5.39

Data are expressed as mean ± SEM (n = 3)
GAE : Gallic Acid Equivalent; QE : Quercetin Equivalent; µS: micro Siemens

Table 2 — Effect of jujube honey on % body weight gain and food and water consumption in mice

Treatment regimen	% b.w. gain	Food consumption (g/day/100 g b.w.)	Amount of water ingested (mL/day/100 g b.w.)
Control	4. 30±1.36	48.03± 6.81	52.87±1.93
Omeprazole (40 mg/kg)	-9.82±2.54	38.59±2.57	41.01±8.22
Jujube honey (5 g/kg)	-4.26±5.58	32.35± 5.02	60.2±10.30
Diluted jujube honey (2.5 g/kg)	-7.62±4.60	36.85±3.48	20.42±11.55 [a, b2]

Data are expressed as mean ± SEM (n = 6).
Units are as given in the text. One way ANOVA followed by Bonferroni's multiple comparison test.
[a]p<0.05: vs control group, [b2]p<0.01: Jujube honey vs diluted jujube honey group. b.w.: body weight.

showed a lower mean. Furthermore, the honey concentration needed to scavenge 50% DPPH (IC50) was 14.531.58 mg/mL.

The effect of jujube honey on body weight gain and food and water intake

Mice treatment with jujube and diluted jujube honey for 7 days caused a non significant change in % body weight gain and daily food consumption as compared to the control group (Table 2). However, the daily water intake value showed a significant decrease in the diluted jujube honey group compared to control group (20.42±11.55 vs 52.87±1.93 mL/day/100 g b.w.; p<0.05).

Effect of jujube honey on stomach lesion severity

In the present study, treatment of mice with omeprazole (40 mg/kg) resulted a significant reduction in the ulcer area in comparison the ethanol group (p<0.001) (Table 3). The jujube honey or diluted jujube honey pretreatment in ethanol group significantly decreased the ulcer area as compared to the ethanol group (p<0.001) (Table 3). However, Percentage of ulcer inhibition in jujube honey and diluted jujube honey treated mice was 55.55% and 60.57%, respectively, whereas that in omeprazole-treated mice was 80.64%.

The effect of different treatments on macroscopic study examined in ethanol-induced gastric ulceration in mice is shown in Fig. 1. Ethanol treatment caused severe lesions in mice, including extensive visible hemorrhagic necrosis of the gastric mucosa (Fig. 1b). Omeprazole pretreatment of ethanol-ingested mice reduced stomach mucosal lesions compared to the ethanol group (Fig. 1c). Pretreatment with jujube honey showed less protection in ethanol-ingested mice (Fig. 1d). In contrast, the gastric mucosal was significantly protected from ethanol-induced lesions after pretreatment with diluted jujube honey (Fig. 1e).

Table 3 — Effects of jujube honey on gastric pH and ulcer area in mice

Animal's group	Gastric pH	Ulcer area (cm^2)
Ethanol (Ulcer control)	4.97±0.20	0.93±0.05
Omeprazole (40 mg/kg)	6±0.25	0.18±0.02[c1]
Jujube Honey (5 g/kg)	6.41±0.20	0.41±0.14[c1]
Diluted Jujube Honey (2.5 g/kg)	6.41±0.77	0.36±0.03[c1]

Data are expressed as mean ± SEM (n = 6)
Units are as given in the text. One way ANOVA followed by Bonferroni's multiple comparison test.
[c1]p<0.001: vs ethanol group.

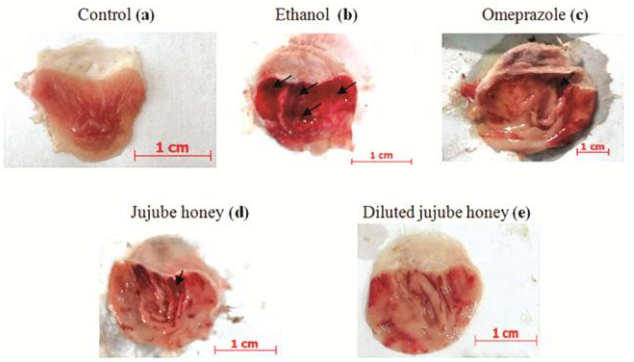

Fig. 1 — Effect of jujube honey on gross lesions examined in ethanol-induced gastric ulcer in mice. (a) Control: has no injury of gastric mucosa tissues; (b) ethanol (ulcer): displays sever lesions with extensive visible haemorrhagic necrosis of gastric mucosa; Arrows indicate the area of hemorrhagic lesions in the inner surface of the stomach; (c) omeprazole: mild lesions of gastric mucosa are observed compared to the lesions in ethanol (ulcer); (d) Jujube honey: slight injuries with visible haemorrhagic of gastric mucosa; (e) diluted jujube honey: nearly normal gastric mucosa tissues. Scale bar =1 cm

Histological studies of the control group's gastric mucosa stained with H&E revealed intact surface epithelium (Fig. 2a). In contrast, mucosal injury was observed in ethanol-treated mice in the form of epithelium damage, erosion areas and ulcerations (Fig. 2b). However, pretreatment of ethanol ingested mice with omeprazole showed mild lesions of gastric mucosa (Fig. 2c). Pretreatment of ethanol-ingested mice with jujube honey showed slight injuries of gastric mucosa (Fig. 2d). On the other hand, pretreatment with diluted jujube honey showed normal gastric mucosa tissue and has no disruption to the surface epithelium (Fig. 2e).

Table 3 shows the effects of jujube honey on ethanol-induced stomach pH changes. The pretreated groups with raw or diluted jujube honey showed no significant increase in pH value of stomach contents compared to the ethanol group (6.41 vs 4.97; p>0.05). Similarly, the omeprazole group also did not show any significant increase in the pH of stomach contents compared with ethanol-ingested mice (6 vs 4.97; p>0.05).

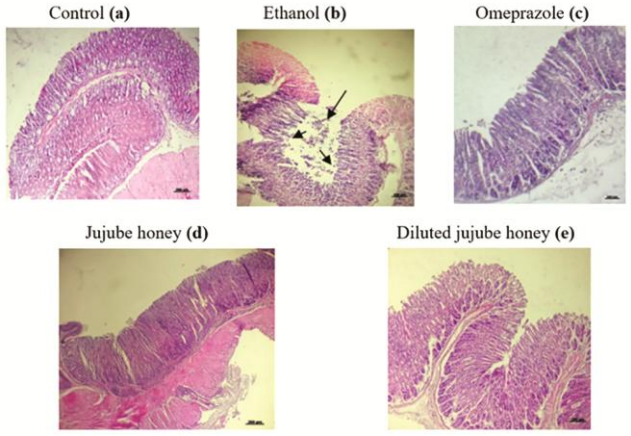

Fig. 2 — Histopathological study of the effect of jujube honey on gastric injuries in ethanol-induced mice. Control: gastric mucosa showed intact tissue; (b) ethanol (ulcer): displays sever disruption to the surface epithelium and necrotic lesion of gastric mucosa; Arrows indicate the area of lesions in the inner surface of the stomach; (c) omeprazole: mild lesions of gastric mucosa but deep mucosal damage is absent; (d) Jujube honey: slight injuries of gastric mucosa; (e) diluted jujube honey: normal gastric mucosa tissue and has no disruption to the surface epithelium. Histopathological tissue sections were stained with H&E. Scale bare =200 µm

Effect of jujube honey on some biochemical parameters

Effect of jujube honey on the activities of plasma Superoxide Dismutase (SOD) and Catalase (CAT)

Table 4 shows the results of the enzymatic antioxidant analyses. Ethanol treatment of mice induced a significant decrease in both SOD and catalase activities as compared to the control mice (p<0.01). Pretreatment of ethanol-ingested mice with both omeprazole and diluted jujube honey significantly increased plasma SOD (3.25- and 4.25-fold, respectively) and catalase enzyme activity (2.79- and 2.71-fold, respectively) compared to the ethanol group.

Effect of jujube honey on plasma C-reactive protein (CRP):The mean of the plasma inflammatory marker CRP was significantly increased in the ethanol group compared to control group (23.08±1.00 vs 5.45±0.33 µg/L; p<0.001). However, daily consumption of jujube honey for 7 days decreased CRP values (17.08±0.71 µg/L) whereas the diluted jujube honey caused remarkable decrease (6.05±0.63 µg/L) compared to the ethanol group (Table 4).

Effect of jujube honey on plasma Nitric Oxide (NO) concentrations: Treatments of mice with ethanol caused a significant increase in NO plasma level as compared to the control group (15.49±1.18 vs 9.96±0.93 µmol/L; p<0.01) (Table 4). Both omeprazole and diluted jujube honey pretreatment reduced significantly plasma NO levels in ulcer-induced mice (11.51±0.50 µmol/L; p<0.05 and 10.02±1.09 µmol/L; p<0.01, respectively).

Effect of jujube honey on white blood cells count

The mice pretreated with diluted jujube honey before ethanol administration showed an obvious

Table 4 — Effect of jujube honey on plasma Superoxide Dismutase (SOD), Catalase activities, CRP and nitric oxide (NO) in ethanol-induced gastric ulceration model in mice

Animal's group	SOD (U/mL)	Catalase (µmol H_2O_2/min/mL)	CRP (µg/L)	NO (µmol/L)
Control	0.34±0.02	3.01±0.31	5.45±0.33	9.96±0.93
Ethanol (Ulcer)	0.04±0.01^{c}	0.87±0.34^{b}	23.08±1.00^{c}	15.49±1.18^{b}
Omeprazole (40 mg/kg)	0.13±0.006	2.43±0.31^{a1}	11.93±0.72^{c1}	11.51±0.50^{a1}
Jujube honey (5 g/kg)	0.07±0.03	1.73±0.35	17.08±0.71^{c1}	13.55±0.51
Diluted jujube honey (2.5 g/kg)	0.17±0.04	2.36±0.25	6.05±0.63^{c1}	10.02±1.09^{b1}

Data are expressed as mean ± SEM (n = 6).
Units are as given in the text. One way ANOVA followed by Bonferroni's multiple comparison test.
^{b}p<0.01: vs control group, ^{c}p<0.001: vs control group; ^{a1}p<0.05: vs ethanol group, ^{b1}p<0.01: vs ethanol group, ^{c1}p<0.001: vs ethanol group. SOD: Super Oxide Dismutase, CRP: C-Reactive Protein, NO: Nitric Oxide.

decrease in the level of leukocytes with 2389± 139 cells/µL, when compared with the ethanol group (4540±188 cells/µL; $p<0.001$). However, pretreatment of ethanol-ingested mice with raw jujube honey caused a no significant decrease with 3638±138 cells/µL ($p>0.05$) as compared to the ulcer-induced mice.

Discussion

This study reveals the protective effect of jujube honey in treating gastric ulcer induced by ethanol. According to the limits defined by the European honey standard, the jujube honey values displayed a high quality[33]. Jujube honey was distinguished than the majority of monofloral honey by higher values of polyphenolics, pH and electrical conductivity and by lower moisture content. Interestingly, the Jujube honey's pH level honey was close to neutral, which is significantly higher than the Indian and Australian honeys pH[34,35]. The moisture content in the investigated honey was lower than 20%, which is well below the imposed limit of European Commission[33]. Regarding the electrical conductivity, the values of jujube honey were higher than found in Chinese jujube honey[16]. Additionally, the phenolic compounds could be considered high when compared with honeydew honeys from Romania[36] and commercial honeys from India[34]. These differences of jujube honey when compared with other honey types were highly related to its darkest colored and could be influenced by environmental conditions in drier regions[15].

Our results are similar with previous research that reported that honey feeding had no effect on body weight gain and food consumption compared with rats fed by Manuka honey[12]. Moreover, diluted jujube honey feeding resulted in a decrease in water intake amounts, compared either with control or with jujube honey groups. In this study, unifloral jujube honey was found to have significant gastroprotective activity against ethanol-induced stomach ulcers under macroscopic and histopthaological examination of gastric mucosal layer. This is consistent with previous works, which have shown the gastroprotective effect of various types of honey in rats, whether unifloral or multifloral and from various botanical origins[37]. Furthermore, our findings are consistent with recent research which demonstrated that the Kelult Malaysian honey and Turkish honey having anti-ulcer properties against ethanol induced gastric ulcer in rats[13,14]. However, when compared to the other groups, diluted jujube honey treated group shows a promising effect in gastroprotective mucosa.

Gastric ulceration is known to occur simultaneously, when the physiological balance between the protective barriers and the ulcerogenic factors is disrupted with mucosal aggression or decrease of stomach epithelium resistance[38]. In this investigation, catalase and SOD enzymes antioxidant activities were also enhanced by jujube honey. Jujube honey's antioxidant capacities and free radical scavengers can be attributed to its antioxidant phenolic and flavonoid contents[15]. The antioxidant properties of flavonoids involve transition metal ion chelation, reactive oxygen species scavenging, decrease of lipid peroxidation and increase of enzymatic and non enzymatic antioxidants[39]. The jujube honey flavonoids content could also contribute to higher antioxidant activity that acts through several chemical mechanisms.

Reduced in CRP plasma level in diluted jujube honey and decrease in white blood cells counts was related to the anti-inflammatory effect of jujube honey. It was reported that diluted natural honey intake leads to a reduction in the concentration plasma levels of prostaglandins such as prostaglandin E2, prostaglandin F2a and thromboxane B2 in normal individuals, thus showing anti-inflammatory effects[40]. It is found that Gelam honey exhibits its inhibitory effects by attenuating NF-κB pathway, with subsquent decrease of inflammatory mediators[41].

Ethanol-induced gastric ulcers are commonly associated with nitric oxide (NO) modulation pathway[12]. In our study, NO levels in jujube honey groups may have regulatory effects on prevention of ulcer. Thus, the gastroprotective mechanism displayed by jujube honey was to maintain the normal physiological levels of NO and to reduce its undesired effects due to tissue damage and inflammation. Its Known that bioactive phenolic compounds comprise an excellent source of antioxidant chemicals and has anti-apoptotic properties via inhibiting ROS generation and NO-induced cell death[42]. There was a clear correlation between phenolic compounds ROS scavenging capacity of jujube honey and NO induced inflammation. Gelam honey's phenolic compounds are recognized for down regulating NF-κB. This, in turn, decreases the iNOS biosynthesis[43], and subsequently, inhibits nitric oxide production. The phenolic compounds in jujube honey may be able to suppress NO by scavenging the

NO radical and inhibiting NF-κB but the exact mechanism remains unclear.

Several reports have shown that omeprazole which is largely used to treat gastrointestinal disorders, has major side effects and causes peripheral neuropathy, abdominal pain, nausea, diarrhea, vomiting, flatulence, headache and fulminant hepatic failure[44]. Omeprazole is known to work by inhibiting adenosine triphosphatase, an essential enzyme in the process of H^+ and K^+ exchange within the gastric parietal cell, in the final steps of the acid secretory process[45]. While the jujube honey pH values were highest compared to other types of honey, there was no significant difference in stomach pH when compared to the omeprazole group. Therefore, jujube honey can be used as a potential new agent that is effective and has no health-associated adverse effects than those of omeprazole widely used to cure gastric ulcer.

Conclusion

Traditionally, the use of honey as an antiulcerogenic agent is one of the most important therapeutic approaches of gastric disorders. The present investigation was designed for the fact that jujube honey exhibited it's gastroprotective effect through antioxidant and anti-inflammatory properties. As a result, we demonstrates that jujube honey treatment, can protect against ethanol-induced gastric-ulceration, probably via activating antioxidant enzymes, by suppressing CRP inflammatory activity and by attenuating the release of nitric oxide. In summary, diluted jujube honey was likely to be more effective and exerts its gastroprotector effects by improvement of antioxidant and anti-inflammatory properties via its potent bioactive compounds.

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Conflict of Interest

The authors declare no conflict of interest.

Author's Contributions

AB: Experimental study, Methodology, Writing, YZ: Data compilation, Statistical analyzer, AB: Experimental study, Methodology, LH & LK: Methodology, SS: Research guidance, NAS : Guidance, Manuscript revision and MK: Writing support, Supervision.

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