



Protective role of *Phoenix dactylifera* fruit against ethanol-induced gastric ulcer in Wistar rats

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Background: Peptic ulcers are gastrointestinal lesions that occur in the stomach and duodenum as a result of ethanol consumption, *Helicobacter pylori* infection and use of non-steroidal anti-inflammatory drugs.

Methods: Twenty Wistar rats were divided into four groups and fasted for 18 hours. Rats in groups 1, 2, 3 and 4 were pretreated with normal saline, gestid suspension and *Phoenix dactylifera* (*P. dactylifera*) extract at 250 & 500 mg/kg respectively, they were given 80% ethanol after 30 minutes. They were all sacrificed after an hour and the stomachs were dissected and opened, pH of stomach content was determined. The stomachs were examined using hand lens for macroscopic lesions, they were fixed in 10% formalin and processed for light microscopic study.

Results: There was significant decrease in pH of stomach content of rats pre-treated with *P. dactylifera* extract as compared to those of rats pre-treated with normal saline, the ulcer index (UI) decrease significantly in rats pre-treated with *P. dactylifera* extract as compared to those of rats pre-treated with normal saline, but a significant increase compare to those of rats pre-treated with gestid suspension. The gastric mucosa of rats pre-treated with normal saline is full of lesions while those of rats pre-treated with gestid suspension and *P. dactylifera* extract showed few mild and moderate lesions. Photomicrograph of stomach of rats pre-treated with normal saline showed distortion of submucosa, loss of luminal epithelium and weakness of muscularis mucosa, those of rats pre-treated with gestid suspension and *P. dactylifera* extract at 250 mg/kg showed normal muscularis mucosa and blood vessels while those of rats pre-treated with *P. dactylifera* extract at 500 mg/kg showed distortion of mucosal layers within the gastric pits.

Conclusions: *P. dactylifera* extract was able to protect the stomach from ethanol-induced gastric ulceration in Wistar rats.

Keywords: Ethanol; gastric mucosa; peptic ulcer; *Phoenix dactylifera* (*P. dactylifera*); ulcer index (UI)

Received: 12 August 2017; Accepted: 25 September 2017; Published: 09 November 2017.

doi: 10.21037/arh.2017.10.01

View this article at: <http://dx.doi.org/10.21037/arh.2017.10.01>

Introduction

Peptic ulcers are pathological gastrointestinal lesions that occur in the stomach and duodenum as a result of imbalance between the defensive gastric mucosa and aggressive factors such as gastric acids and pepsin, free

radicals, leukotrienes, ethanol, *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drugs (NSAIDs) etc. (1-3). Gastric ulcer is characterized by inflammation, hemorrhagic erosions, necrosis, infiltration of neutrophils, reduction of blood flow, reactive oxygen species (ROS) and oxidative stress (4,5). A major source of ROS in ethanol-induced

gastric mucosal damage is the infiltration of neutrophils (2). Neutrophils adhere to the vascular endothelium and subsequently release oxygen-derived free radicals and proteolytic enzymes that are implicated in the pathogenesis of various forms of gastrointestinal ulceration including gastric mucosal damage associated with oral ethanol consumption or administration (6-8). Hydrogen receptor antagonist, proton pump inhibitors, antacids, histamine receptor antagonist are the commonly used antiulcer drugs (9,10). However, most of these drugs result in undesirable adverse side effects; hypergastrinemia and high rate of ulcer recurrence due to long term use (11-14). Therefore, there is a need for a natural and readily available antiulcer drug with little or no side effect. *Phoenix dactylifera* (*P. dactylifera*), commonly known as date palm, is a flowering plant species in the family *Arecaceae*, cultivated for its edible sweet fruit; the plant is widely cultivated and distributed in many tropical and subtropical regions worldwide (15). Studies have shown that date extract enhances antioxidant processes and prevent oxidative stress the antioxidant potential was attributed to the wide range of phenolic compounds, flavonoids, tannins and saponins and vitamin C (16,17). Date palm fruit extracts were reported to exhibit gastrointestinal protection against different ulcer inducing agents (18,19). It also possesses antioxidant, hepatoprotective, anti-ulcerative, anti-inflammatory, anti-proliferative, anti-mutagenic, antibacterial, antifungal and antiviral potentials (20,21). *P. dactylifera* extracts was proven to exhibit antagonist effects to oxidative stress induced by opioid (22). The aim of the present study was to evaluate the protective role of *P. dactylifera* extract against ethanol-induced gastric mucosal damage by evaluating the antioxidant defensive mechanism along with morphological and histopathological changes.

Methods

Plant material

P. dactylifera fruits were purchased from a local salesman in Maiduguri central market and were authenticated by a botanist in University of Maiduguri. The fruit were separated from the pit, washed, air dried and grounded with mortar and pestle. Twenty grams of the powdered fruit was soaked in 1,000 mL of distilled water for 24 hours, filtered with a filter paper and allowed to settle down, it was decanted and oven dried at 50 °C.

Animal Husbandry

Twenty healthy and pathogen free male Wistar Rats were purchased from the Department of Biochemistry Animal House, University of Maiduguri. They were fed with grower mash (Vital Feed, Jos, Nigeria) and water ad libitum. The rats were kept in the Department of Biochemistry animal House for 2 weeks to acclimatize with the animal house condition before the experiment. The research was approved by the Department of Human Anatomy, University of Maiduguri and was given the code number UM/HA/UGP16.17-004, it was conducted in accordance with the guidelines of University of Maiduguri Research and Ethical Committee, the ARRIVE guidelines by National Center for the Replacement Refinement and Reduction of Animals in Research (NC3Rs) and Helsinki Declaration as revised in 2013.

Experimental design

The rats were randomly divided into four groups and fasted for 18 hours. Rats in groups 1 and 2 were pretreated with 1 mL normal saline and gestid suspension (Ranbaxy Pharmaceuticals Inc., Princeton, NJ, USA) respectively while those in groups 3 and 4 were pretreated with aqueous extract of *P. dactylifera* fruit at 250 and 500 mg/kg respectively 30 minutes before administration of 1 mL of 80% ethanol. The LD 50 of *P. dactylifera* fruit was reported to be greater than 3,000 mg/kg (23) 17% of the LD 50 was chosen as the high dose. All the rats were sacrificed after 1 hour by ketamine injection and the stomachs were dissected out and cut opened, the stomach content were deposited in a beaker and 3–5 drops of pH meter was added to determine the pH. The stomachs were washed and examined using a hand lens for macroscopic lesions in the glandular part. The severity of macroscopic lesions formed were estimated using an ulcer index (UI) as reported by (24) using the following scale: 0 for normal mucosa; 1 for vascular congestions; 2 for one or two lesions; 3 for severe lesions; 4 for very severe lesions and 5 for mucosa full of lesions. The total and ulcerated area of each stomach was measured and ulcer indices (UIs), ulcer inhibition rates (UIRs) and total stomach area (TSA) were calculated using the formulae; $UI = UA/TSA \times 100$ (24,25), $UIR (\%) = UI (\text{control} - \text{pretreated}) / UI \text{ control} \times 100$. $TSA = \pi r^2$ where, UA = ulcerated area, $\pi = 3.14$ and r = diameter of stomach/2 (25,26).

Table 1 Effect of aqueous extract of *Phoenix dactylifera* on the pH of stomach content of Wistar rats

Groups	Pre-treatment	Post-treatment	pH
1	Normal saline (1 mL)	80% ethanol	7.90±0.60*
2	GS (1 mL)	80% ethanol	8.50±0.27 ^b
3	PD (250 mg/kg)	80% ethanol	5.70±0.83 ^c
4	PD (500 mg/kg)	80% ethanol	5.50±0.67 ^d

All values are expressed as mean ± SEM, values in the column with different superscripts (*, b, c, and d) are significantly different at P<0.05. SEM, standard error of the mean; PD, *Phoenix dactylifera*; GS, gestid suspension.

Table 2 Effect of aqueous extract of *Phoenix dactylifera* on UI and UIR

Groups	Pre-treatment	Post-treatment	Ulcer index	UIR
1	Normal saline (1 mL)	80% ethanol	55.91±6.94*	–
2	GS (1 mL)	80% ethanol	4.80±3.06 ^b	92.49±4.67*
3	PD (250 mg/kg)	80% ethanol	6.68±2.83 ^d	88.54±4.52 ^b
4	PD (500 mg/kg)	80% ethanol	9.40±3.00 ^d	77.99±11.29 ^d

All values are expressed as mean ± SEM, values in the same column with different superscripts (*, b, c, and d) are significantly different at P<0.05. UI, ulcer index; UIR, ulcer inhibition rate; SEM, standard error of the mean; PD, *Phoenix dactylifera*; GS, gestid suspension.

Histopathology

The stomach of each rat was fixed in 10% formalin, and processed for light microscopic study; they were sectioned at 5 µm and stained with hematoxylin and eosin (H & E) (27).

Statistical analysis

One way analysis of variance (ANOVA) was used to compare the differences between the groups using InStat statistics 3.1 (Graphpad Software, San Diego, CA, USA). All the data were expressed as mean ± SEM and a P value less than 0.05 was considered as the significant level.

Results

There was significant decrease in the pH of rats pre-treatments with 250 mg/kg (5.70±0.83) and 500 mg/kg (5.50±0.67) aqueous extracts of *P. dactylifera* as compared with that of rats pre-treated with normal saline (7.90±0.60) and gestid suspension (8.50±0.27) at P<0.05 (Table 1). The pH of stomach content of rats pre-treated with normal saline and gestid suspension were alkaline while those of rats pre-treated with aqueous extract of *P. dactylifera* at 250 and 500 mg/kg were acidic.

From Table 2, the UI of rats pre-treatments with 250 mg/kg (6.68±2.83) and 500 mg/kg (9.40±3.00) of *P. dactylifera* as was significantly lower than those of rats

pre-treated with normal saline (55.91±6.94) at P<0.05, there was dose dependent increase in the UI of rats pre-treatments with aqueous extracts of *P. dactylifera* at 250 mg/kg (6.68±2.83) and 500 mg/kg (9.40±3.00) respectively with significant decrease in UI of rats pre-treated with gestid suspension (4.80±3.06) as compared with rats pretreated with 250 and 500 mg/kg of *P. dactylifera* extract. There was a decrease in the UIR of rats pre-treated with 250 mg/kg (88.54±4.52) and 500 mg/kg (77.99±11.29) aqueous extracts of *P. dactylifera* as compared with the rats pre-treated with gestid suspension (92.49±4.67) at P<0.05. Rats pre-treated with aqueous extract of *P. dactylifera* at 250 mg/kg showed lower UI (6.68±2.83) than those of rats pre-treated with aqueous extract of *P. dactylifera* at 500 mg/kg (9.40±3.00) and normal saline (55.91±6.94), but has a little higher UI than rats pre-treated with gestid suspension (4.80±3.06), the UIR of rats pre-treated with aqueous extract of *P. dactylifera* at 250 mg/kg (88.54±4.52) is higher than those of rats pre-treated with the extract at 500 mg/kg (77.99±11.29) but lower than those of rats pre-treated with gestid suspension (92.49±4.67) (Table 2).

The gross anatomy of the stomach of rats pre-treated with normal saline showed mucosa full of lesions while those of rats pre-treated with gestid suspension showed few vascular congestions, the gross anatomy of the stomach of rats pre-treated

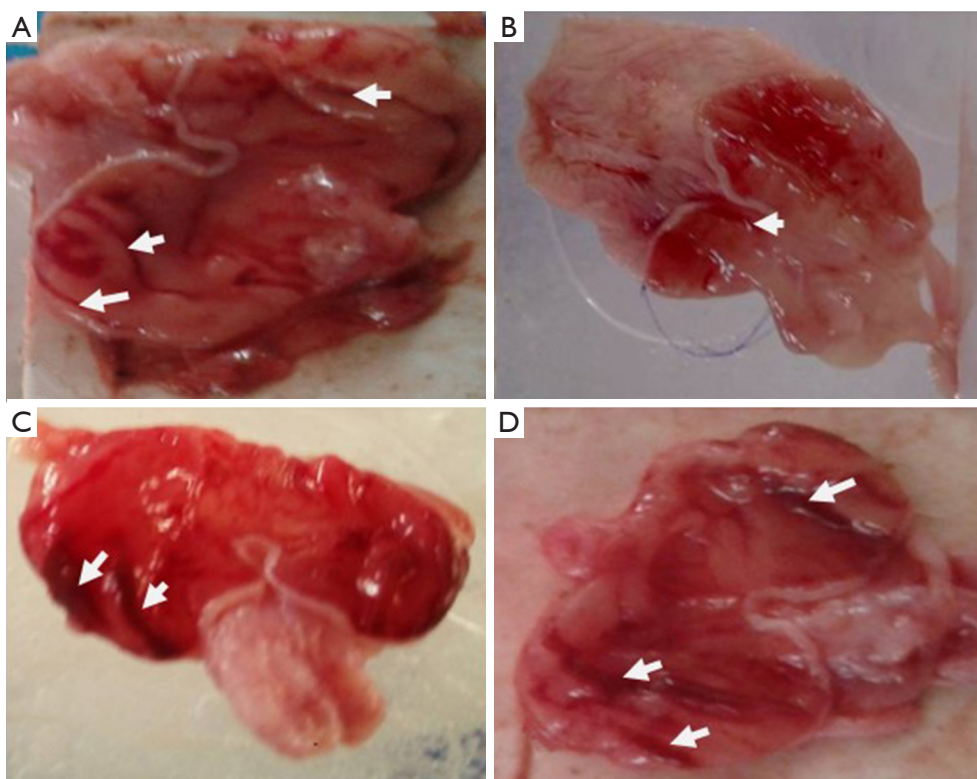


Figure 1 Showing the photograph of gross anatomy of stomach of Wistar rats after post-treatment with 1 mL 80 % ethanol. (A) Pre-treated with normal saline; (B) pre-treated with gestid suspension; (C) pre-treated with 250 mg/kg PD; (D) pre-treated with 500 mg/kg PD. PD, *Phoenix dactylifera*.

with aqueous extract of *P. dactylifera* at 250 mg/kg showed few mild lesions while those of rats pre-treated with and aqueous extract of *P. dactylifera* at 500 mg/kg showed few mild lesions few severe lesions (Figure 1).

Photomicrograph of stomach of rats pre-treated with normal saline showed loss of luminal epithelium, distortion of submucosal layer and weakness of muscularis mucosa with encroaching blood vessels (Figure 2A). Photomicrograph of stomach of rats pre-treated with gestid suspension showed distortion of submucosal layer, normal muscularis mucosa, normal blood vessels within sub-mucosal layer and loss of luminal mucosal epithelium (Figure 2B). Photomicrograph of stomach of rats pre-treated with aqueous extract of *P. dactylifera* at 250 mg/kg showing distortion of submucosal layer, normal muscularis mucosa with some blood vessel within the mucosal layer and a little distortion of luminal mucosal layer (Figure 2C). Photomicrograph of stomach of rats pre-treated with aqueous extract of *P. dactylifera* at 500 mg/kg showing normal muscularis mucosa, distortion mucosal layer

around the gastric pits (black arrows) and loss of luminal mucosal epithelium (Figure 2D).

Discussion

The slightly alkaline pH of stomach content of rats pre-treated with normal saline and gestid suspension showed that normal saline does not contain any substance that will require the secretion of gastric juice while protecting the gastric mucosa by either preventing gastric acid secretion or neutralizing the effect of the acid secreted by gastric glands. The acidic pH of stomach content of rats pre-treated with aqueous extract of *P. dactylifera* at 250 and 500 mg/kg showed that the extract does not protect the stomach mucosa by either preventing gastric acid secretion or neutralizing the gastric acid secreted by the stomach glands, the preventive mechanism might be due to the presence of flavonoids, tannins, saponins and vitamin c that serve as antioxidants to clear the oxygen-derived free radicals and proteolytic enzymes that are implicated

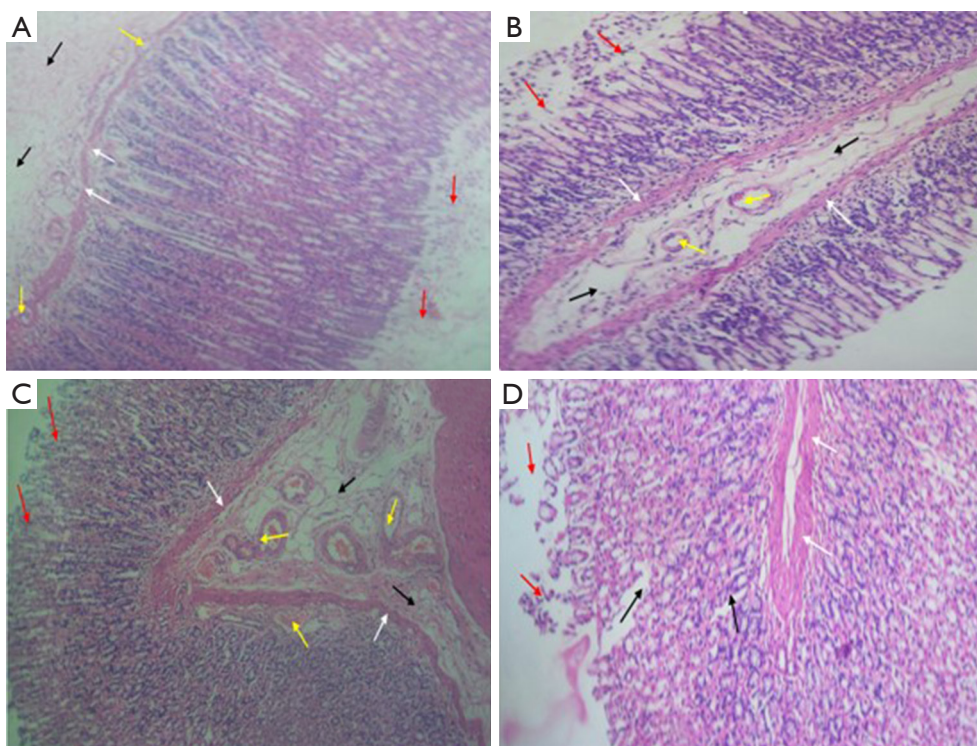


Figure 2 Photomicrograph of stomach of rats (A) pre-treated with normal saline showing distorted submucosal layer (black arrows), blood vessels (yellow arrows) within the muscularis mucosa (white arrows) and loss of luminal mucosal epithelium (red arrows); (B) pre-treated with gestid suspension showing distorted submucosal layer (black arrows), normal muscularis mucosa (white arrows), normal blood vessels within sub-mucosal layer and loss of luminal mucosal epithelium (red arrows); (C) pre-treated with aqueous extract of *Phoenix dactylifera* at 250 mg/kg showing distorted submucosal layer (black arrows), normal muscularis mucosa (white arrows), and normal blood vessels within sub-mucosal layer, some blood vessel within the mucosal layer and a little distortion of luminal mucosal layer (red arrows); (D) pre-treated with aqueous extract of *Phoenix dactylifera* at 500 mg/kg showing normal muscularis mucosa (white arrows), distortion mucosal layer around the gastric pits (black arrows) and loss of luminal mucosal epithelium (red arrows). H&E $\times 100$.

in the parthenogenesis of ethanol induced gastric ulcers (6,7). Because of the high nutrient content of *P. dactylifera* extract (28,29), there is need for gastric secretion to create a favourable acidic pH that will initiate digestion.

The significant decrease in the UI of rats pre-treated with *P. dactylifera* extract at 250 and 500 mg/kg showed that the extract can protect the stomach mucosa from damage due to ethanol consumption, even though gestid suspension offers better protection as having lower UI as compared with that of *P. dactylifera* extract. *P. dactylifera* extract is most effective at 250 mg/kg and because it is a natural product with many medicinal properties (30), it is likely to present little or no side effects.

The high UIR of *P. dactylifera* extract showed that the extract is not only effective in reducing UI but is also effective in inhibiting the rate of ulcer formation and progression,

making the extract better than some ulcer drugs that results in high rate of ulcer recurrence with long term use (11,12).

The extract of *P. dactylifera* at 250 mg/kg was shown to reduce gastric mucosal epithelial damage, distortion of submucosal layer and maintaining endothelial cell integrity, this might be due to the fact that *P. dactylifera* extract contains phenolic compounds and has antioxidant property (30) but at a higher dose of 500 mg/kg, the resulted mucosal damage might be due to the presence of saponin in the extract that was proven to create pores and exert a lytic action on some cell membranes leading to cell damage (31,32).

Conclusions

Aqueous extract of *P. dactylifera* was able to protect the

stomach against ethanol-induced gastric ulcer by its antioxidant property of scavenging free radicals, reducing UI, increasing UIR, reducing gastric lesions, reducing gastric mucosal damage and maintaining the integrity of endothelial epithelium.

Acknowledgments

We wish to acknowledge the effort and contributions of Mustapha Ali, Freda Nathan Abwage, Johnson Adamu, Ladi Yahi Mingila, Mustapha Ahmed Bukar, Moloh Immaculate, Martha Orendu Attah and Helga Bedan Ishaya.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The research was conducted in accordance with the University of Maiduguri Research and Ethical Committee guide for the care and use of laboratory animals, the ARRIVE guidelines by the National Center for the Replacement Refinement and Reduction of Animals in Research and the provisions in accordance with the Helsinki Declaration as revised in 2013.

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doi: 10.21037/arh.2017.10.01

Cite this article as: Musa MA, Dibal NI, Chiroma MS, Makena W. Protective role of *Phoenix dactylifera* fruit against ethanol-induced gastric ulcer in Wistar rats. Ann Res Hosp 2017;1:46.