

The ameliorative effect of dates (*Phoenix dactylifera* L.) on ethanol-induced gastric ulcer in rats

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Abstract

The present work aimed at testing, in a rat model of ethanol-induced gastric ulceration, a local folk medicinal claim that dates are beneficial in gastric ulcers in humans. Aqueous and ethanolic undialyzed and dialyzed extracts from date fruit and pits were given orally to rats at a dose of 4 ml/kg for 14 consecutive days. On the last day of treatment, rats were fasted for 24 h, and were then given ethanol, 80% (1 ml/rat) by gastric intubation to induce gastric ulcer. Rats were killed after 1 h of ethanol exposure, and the incidence and severity of the ulceration were estimated, as well as the concentrations of gastrin in plasma, and histamine and mucus in the gastric mucosa. A single group of rats that were fasted for 24 h, was administered orally with lansoprazole (30 mg/kg), and was given 80% ethanol as above, 8 h thereafter, served as a positive control.

The results indicated that the aqueous and ethanolic extracts of the date fruit and, to a lesser extent, date pits, were effective in ameliorating the severity of gastric ulceration and mitigating the ethanol-induced increase in histamine and gastrin concentrations, and the decrease in mucin gastric levels. The ethanolic undialyzed extract was more effective than the rest of the other extracts used. It is postulated that the basis of the gastroprotective action of date extracts may be multi-factorial, and may include an anti-oxidant action.

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1. Introduction

Date palms (*Phoenix dactylifera* L., Palmae) have been cultivated in the Middle East over at least 6000 years ago (Copley et al., 2001). For the natives in this region, dates are considered a staple carbohydrate food (Al-Shahib and Marshall, 2003). Date fruits are also used in the production of local beverages and spirits. In local medicinal practices dates are considered a “tonic” and “aphrodisiac”, and in some communities they are thought to be useful against ulcer (Rasheed, personal communication). In fact, Muslims believe that “*He who eats seven dates every morning will not be affected by poison or magic on the day he eats them*” (cited by Miller et al., 2003).

The pollen grains of date palm have been used in Egyptian local practices to improve fertility in women, and in some locations in Arabia date pits are roasted and used in lieu of coffee as a hot beverage.

Relatively few pharmacological studies have been conducted on dates. For example, it has been shown that, depending on the type of extract used, date fruit and pit extracts significantly increase or decrease gastrointestinal transit (GIT) in mice (Al-Qarawi et al., 2003), and that date fruit extract has strong antioxidant and antimutagenic properties (Vayalil, 2002). Date palm kernels have been shown to exhibit antiaging properties and significant reduction in skin wrinkles in women (Bauza, 2002), and natural fats from date palm has been reported to prevent irritant contact dermatitis (Schliemann-Willers et al., 2002). In animals, the pits have been included in the diet of chickens, sheep, fish and rats, and have been shown to enhance

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growth in these species (see Ali et al., 1999 and references therein).

In view of the wide consumption of dates in our region, the fact that dates are anecdotally reputed to be useful against peptic ulcers, and the fact that Muslims customarily consume more of the dates during the fasting month of Ramadan, possibly to protect the gastric mucosa from the damaging effect of gastric acid, and because of the scarcity of information on the pharmacological properties of date fruits and pits, we considered undertaking this study to assess the influence of date extracts on the incidence and severity of ethanol-induced gastric ulceration. In addition, the effect of date extracts on the gastric concentrations of histamine and mucin, and the plasma concentration of the hormone gastrin has also been investigated.

2. Materials and methods

2.1. Animals

Fifty-four adult male Wistar rats weighing between 200 and 250 g were used in this work. They were obtained for the Animal House of King Saud University in Riyadh, and were divided into eight equal groups. The animals were kept at a controlled temperature of $23 \pm 2^\circ\text{C}$, relative humidity of 65–80% and a light regime of 12 h light:12 h dark (lights on at 6:00). Except otherwise mentioned, pelleted Purina chow and water were provided to the rats ad libitum.

2.2. Plant material

Fresh fruits of Sukari dates (*P. dactylifera* L.) were obtained from a local date manufacturing factory. Samples of these dates were kept frozen for future reference.

2.3. Plant preparation and administration

The date fruits were manually separated from the pits and the latter were washed clear of any fruit, dried at room temperature and ground into powder using a stainless-steel blender. The water extract of the date fruit was made by adding distilled water to coarsely pounded date fruit (3:1), and leaving for 48 h in a refrigerator (4°C) with continuous stirring. The aqueous extract was then used daily for 14 consecutive days. To remove sugars from the extract, the aqueous extract was dialyzed. Dialysis was carried out under running tap water for 24 h. The dialyzed water extract was kept refrigerated and used daily for 14 consecutive days.

A soxhlet apparatus was used to obtain an ethanol extract. The ethanol was then evaporated and the residue diluted with water to give the required concentration. The pounded date fruit or pits were added (1:3) to either ethanol or distilled water. Extraction was carried out at 4°C with continuous stirring. The ethanol extracts were then concentrated to dryness

and the residue dissolved in distilled water to the appropriate doses just prior to use.

Dialysis to remove sugars was performed using cellulose tubing (Spectra/Por, width 32 mm, diameter 20.4 mm, volume/length 303 ml, from Spectrum Medical Instruments, Inc., USA).

2.4. Experimental design

Rats were randomly assigned to the following experimental groups:

Group 1: given distilled water (4 ml/kg) orally for 14 consecutive days. On the last day rats were given normal saline (1 ml) 1 h before killing.

Group 2: given distilled water (4 ml/kg) orally for 14 consecutive days. On the last day rats were given 80% ethanol (1 ml) 1 h before killing.

Group 3: Rats were given the aqueous date fruit extract (4 ml/kg) for 14 consecutive days. On the last day rats were given 80% ethanol (1 ml) 1 h before killing.

Group 4: Rats were given the dialyzed aqueous date fruit extract (4 ml/kg) for 14 consecutive days. On the last day rats were given 80% ethanol (1 ml) 1 h before killing.

Group 5: Rats were given the ethanolic date fruit extract (4 ml/kg) for 14 consecutive days. On the last day rats were given 80% ethanol (1 ml) 1 h before killing.

Group 6: Rats were given the aqueous date pit extract (4 ml/kg) for 14 consecutive days. On the last day rats were given 80% ethanol (1 ml) 1 h before killing.

Group 7: Rats were given the dialyzed date pit extract (4 ml/kg) for 14 consecutive days. On the last day rats were given 80% ethanol (1 ml) 1 h before killing.

Group 8: Rats were given the ethanolic date pit extract (4 ml/kg) for 14 consecutive days.

Group 9: Rats were given a single oral dose of lansoprazole (30 mg/kg), and 8 h later was given 80% ethanol as above, 1 h before killing.

2.5. Ethanol-induced gastric lesion

Rats were deprived from food (but not water) on day 14 of the experiment. On the last day of experiment (day 15) rats were given 80% ethanol (1 ml) by gastric intubation 1 h before killing, except for rats in group 1 which was given normal saline (1 ml/rat), and group 9 (positive control) which consisted of six rats that were fasted for 24 h, administered orally with lansoprazole (Sigma, MO, USA) (30 mg/kg), and was given 80% ethanol as above, 8 h thereafter.

The animals were anesthetized with ether, and rapidly decapitated 1 h after ethanol treatment. Blood was collected in heparinized tubes and centrifuged at $900 \times g$ for 15 min at 5°C . The plasma obtained was stored at -20°C pending gastrin assay. The stomach of each animal was excised and opened along the greater curvature. After washing with

normal saline and removal of blood, the gastric lesion was quantified using a stereo-microscope. Gross mucosal damage was assessed in a “blinded” manner by calculation of a lesion index based on the number and severity factor of lesions as described previously (Agrawal et al., 2000). The stomachs were inflated with 10 ml of 1% formalin for 10 min to fix the inner walls. The average number of ulcers per stomach was recorded. The lesion index was calculated as the total number of lesions added to their respective severity factor.

2.6. Measurement of the mucin content in the gastric wall

Gastric mucus was quantitatively measured as described by Corne et al. (1974). The stomachs were removed and were soaked in 0.1% Alcian blue solution for 2 h. The uncomplexed dye was removed by two successive washes at 15 and 45 min in 0.25 M aqueous sucrose solution. Dye complexes with gastric wall mucous were extracted by immersion in 10 ml of 0.5 M MgCl₂ for 2 h. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase was measured at 605 nm by a UV–visible spectrophotometer (B&L 2000). The quantity of mucin was expressed as µg of Alcian blue extracted per weight (g) of stomach.

2.7. Gastrin measurement

The gastrin hormone was assayed in the collected plasma samples using a radioimmunoassay (RIA) technique according to the procedure described by the manufacturer (IBL-Hamburg, Germany, cat. No: MI 131 01).

2.8. Histamine estimation

Histamine in the gastric mucosa was separated by thin layer chromatography according to the modified method of Shalaby (1994). Histamine dihydrochloride (Sigma, MO, USA) was used as standard for identification of the spots in the plates and for spectrophotometric determination (Spectronic 2000, Bausch and Lomb at 570 nm) of samples after elution from the plates. Values were expressed as mg/g of stomach weight.

2.9. Histopathological examination

Gastric tissue samples were fixed in 10% neutral formalin and processed for routine paraffin blocking and H&E staining. These were “blindly” examined under the microscope for histopathological change such as congestion, oedema, erosions, ulcerations and necrosis. The severity of histopathological changes was expressed according to an arbitrary scale (between – to + + + +).

2.10. Statistical analysis

Values reported are expressed as mean ± S.E.M. The significance of differences between the control and ethanol and/or date-treated groups was tested by least squares analysis of variance using the general linear models (GLM) procedures of the statistical analysis system (SAS, 1996).

3. Results

The results of this work are shown in Tables 1–3.

Table 1 shows the effect of treatment with various date extracts on the number, severity and ulcer index. The ethanolic and, to a lesser extent, the aqueous date fruit extract ameliorated the severity of gastric ulceration. The effect of the date pit extract was less evident. The positive control lansoprazole significantly reduced the lesion index.

Table 2 summarizes the histological changes seen after treatment with ethanol and date extracts. Ethanol treatment induced severe necrosis, haemorrhage, congestion and oedema in stomach sections. These actions were markedly ameliorated by lansoprazole, and to a lesser extent by pre-treatment with date fruits and pits. The ethanolic undialyzed date fruit extract was more active in this regard than the other extracts tested.

The concentrations of gastrin in the plasma, and histamine and mucin in the gastric mucosae in control and treated rats with date extracts and lansoprazole are shown in Table 3. Ethanol treatment induced a significant increase in the concentrations of gastrin and histamine, and significantly decreased that of mucin ($P < 0.05$). These effects were significantly antagonized by the pre-treatment of rats with lansoprazole and by date fruit aqueous and ethanolic extracts ($P < 0.05$). Date pits extracts were not significantly effective in antagonizing these effects.

4. Discussion

Rat gastric mucosal damage induced by high concentrations of ethanol has widely been used to investigate gastro-protective effect of medicinal plants (e.g. Zhu et al., 1997). The present results suggest that pretreatment with date fruit (and to lesser extent pit) ethanolic and aqueous extracts for 14 days markedly ameliorated the ulcer index, and some histological and biochemical indices of ethanol-induced gastric ulceration in rats. This lends support to the local folk medicinal claim that dates may be useful to humans with ulcers.

In this work we selected lansoprazole as a reference anti-ulcer drug (rather than a histamine antagonist) because it has been shown that prostaglandins provide a much better anti-ulcer effect on ethanol-induced gastric damage (Cho and Ogle, 1992). Treatment with ethanol-induced the expected actions in the stomach of rats, and those included severe histological damages (necrosis, haemorrhages), and a signi-

Table 1
Effect of date fruit and pits extracts on ethanol-induced gastric ulcers in rats

| Treatment | Number of ulcers/stomach (a) | Severity per stomach (b) | Lesion index (a + b) |
|--|------------------------------|--------------------------|-------------------------|
| Group 1: distilled water + saline | 0 | 0 | No ulcers seen |
| Group 2: distilled water + ethanol | 8.1 ± 0.9 ^a | 8.0 ± 0.9 ^a | 16.1 ± 1.8 ^a |
| Group 3: date fruit aqueous extract + ethanol | 4.2 ± 0.5 ^b | 5.4 ± 0.6 ^b | 9.6 ± 1.1 ^b |
| Group 4: dialyzed date fruit aqueous extract + ethanol | 6.1 ± 0.6 ^c | 6.1 ± 0.5 ^c | 12.2 ± 1.1 ^c |
| Group 5: date fruit ethanolic extract + ethanol | 3.1 ± 0.3 ^d | 4.2 ± 0.4 ^b | 7.3 ± 0.7 ^d |
| Group 6: date pits aqueous extract + ethanol | 7.0 ± 0.6 ^{ac} | 6.1 ± 0.6 ^b | 13.1 ± 1.2 ^c |
| Group 7: dialyzed date pits aqueous extract + ethanol | 6.6 ± 0.6 ^{ac} | 5.4 ± 0.5 ^b | 12.0 ± 1.1 ^c |
| Group 8: date pits ethanolic extract + ethanol | 6.0 ± 0.5 ^{ac} | 4.9 ± 0.5 ^b | 10.9 ± 1.0 ^b |
| Group 9: lansoprazole + ethanol | 1.6 ± 0.3 ^d | 0.9 ± 0.1 ^d | 2.5 ± 0.4 ^d |

Values (means ± S.E.M. from six rats), with different superscripts are significantly different ($P < 0.05$) from the values in the same column. Distilled water (4 ml/kg) or various date extracts were given daily at an oral dose of 4 ml/kg orally for 14 consecutive days; 24 h after the last day of treatment, rats were given 80% ethanol (1 ml) per os 1 h before killing. A single group of rats that were fasted for 24 h, was administered orally with lansoprazole (30 mg/kg), and was given 80% ethanol as above, 8 h thereafter, served as a positive control.

Table 2
Histopathological evaluations of the effects of date aqueous and ethanolic extracts on the induction of gastric lesions in the rats

| Treatment | Necrosis | Haemorrhage | Congestion | Oedema |
|--|----------|-------------|------------|--------|
| Group 1: distilled water + saline | – | – | – | – |
| Group 2: distilled water + ethanol | +++ | ++++ | ++++ | ++++ |
| Group 3: date fruit aqueous extract + ethanol | – | +++ | +++ | +++ |
| Group 4: dialyzed date fruit aqueous extract + ethanol | – | – | + | + |
| Group 5: date fruit ethanolic extract + ethanol | – | + | ++ | ++ |
| Group 6: date pits aqueous extract + ethanol | + | ++ | ++ | ++ |
| Group 7: dialyzed Date pits aqueous extract + ethanol | + | ++ | ++ | + |
| Group 8: date pits ethanolic extract + ethanol | + | + | + | + |
| Group 9: lansoprazole + ethanol | – | – | + | + |

–, normal; +, little effect; ++, appreciable effect; +++, severe effect; +++, intensively severe effect, distilled water (4 ml/kg) or various date extracts were given daily at an oral dose of 4 ml/kg orally for 14 consecutive days; 24 h after the last day of treatment, rats (six in each group) were given 80% ethanol (1 ml) per os 1 h before killing. A single group of rats that were fasted for 24 h, was administered orally with lansoprazole (30 mg/kg), and was given 80% ethanol as above, 8 h thereafter, served as a positive control.

Table 3
Effect of treatment of rats with date fruit and pits extracts on plasma gastrin, gastric histamine and gastric juice mucin activity

| Treatments | Gastrin (pg/ml plasma) | Histamine mg/g stomach weight | Mucin µg/g stomach weight |
|--|---------------------------|-------------------------------|---------------------------|
| Group 1: distilled water + saline | 15.2 ± 1.0 ^a | 22.0 ± 1.5 ^a | 2.4 ± 0.1 ^a |
| Group 2: distilled water + ethanol | 125.2 ± 2.6 ^b | 298.3 ± 18.2 ^b | 0.4 ± 0.3 ^b |
| Group 3: date fruit aqueous extract + ethanol | 107.7 ± 12.3 ^c | 217.3 ± 19.6 ^c | 1.9 ± 0.2 ^c |
| Group 4: dialyzed date fruit aqueous extract + ethanol | 118.5 ± 13.2 ^c | 230.2 ± 24.7 ^c | 2.0 ± 0.2 ^c |
| Group 5: date fruit ethanolic extract + ethanol | 98.5 ± 10.1 ^c | 211.2 ± 3.2 ^c | 2.1 ± 0.2 ^c |
| Group 6: date pits aqueous extract + ethanol | 116.5 ± 12.3 ^c | 228.4 ± 19.8 ^c | 1.6 ± 0.2 ^c |
| Group 7: dialyzed Date pits aqueous extract + ethanol | 113.8 ± 13.1 ^c | 225.8 ± 17.4 ^c | 1.4 ± 0.2 ^c |
| Group 8: date pits ethanolic extract + ethanol | 107.7 ± 11.9 ^c | 205.5 ± 20.2 ^c | 1.2 ± 0.2 ^c |
| Group 9: lansoprazole + ethanol | 34.3 ± 4.3 ^d | 205.5 ± 20.2 ^d | 1.2 ± 0.2 ^d |

Values (means ± S.E.M. from six rats), with different superscripts are significantly different ($P < 0.05$) from the values in the same column. Distilled water (4 ml/kg) or various date extracts were given daily at an oral dose of 4 ml/kg orally for 14 consecutive days; 24 h after the last day of treatment, rats were given 80% ethanol (1 ml) 1 h before killing. A single group of rats that were fasted for 24 h, was administered orally with lansoprazole (30 mg/kg), and was given 80% ethanol as above, 8 h thereafter, served as a positive control.

ficant increase in plasma concentrations of the gastric hormone gastrin, reduction in mucin and an increase in the histamine concentrations in the gastric mucosa. These parameters were selected for study because of their relevance to the pathogenesis of gastric ulceration. For example, gastrin is a gastrointestinal hormone that, among other various functions, regulates gastric acid secretion, releases histamine, and regulates gastric endocrine cell proliferation (Walsh, 1993). Stimulation of the oxyntic cells by histamine is the final common pathway by which neural and endocrine mechanisms act in inducing acid secretion. Histamine is involved in a cycle of

events leading to the production of arteriolar vasodilatation in injured tissues (Black, 1993). Gastric mucus (mucin) is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that cover the entire gastrointestinal mucosa. Moreover, mucin is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals (Repetto and Llesuy, 2002).

In this work we used dialyzed and non-dialyzed extracts. Dialysis was used to remove the sugars from the extracts. Previously dialyzed and non-dialyzed extracts caused opposing

effects on GIT transit (Al-Qarawi et al., 2003). In the present work, non-dialyzed extracts seemed to be more active as gastroprotectants than dialyzed extracts.

Ethanol-induced gastric ulceration is known to be related to an anti-oxidant action, increased lipid peroxidation and generation of free-radicals (Terano et al., 1989). Recently Vayalil (2002) discovered that date extracts possess significant antioxidant action in vitro. This may, at least partially, be one of the possible mechanisms by which date extracts have ameliorated the ethanol-induced gastric ulceration. Recently we have found that dates contain relatively high concentrations of the anti-oxidants melatonin and vitamin E (Al-Qarawi et al., unpublished data). It has been reported that treatment with melatonin prevents gastric ulcerogenesis and decreases ulcer index (Bandyopadhyay et al., 2001; Bubenik, 2002). Vitamin E in palm oil has also been shown to reduce ethanol-induced gastric ulcer (Jarrin et al., 1999). Taken together, these results corroborate our present finding of an ameliorative action of dates on ethanol-induced gastric ulceration, possibly due to its relatively high content of antioxidant substances.

In conclusion, the present work has suggested that date extracts can ameliorate ethanol-induced gastric ulcers, and that, in view of the fact that ethanol induces ulceration by an antioxidant action, and that dates contain relatively high amounts of anti-oxidant substances, it is possible that the mechanism of the gastroprotective action is via an oxidant action, however, other mechanisms cannot be excluded. Pending further pharmacological and toxicological studies to delineate the mechanism(s) of action of dates as gastroprotective agent, and their toxic effects, compounds from dates may potentially be useful in ulcers.

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