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ORIGINAL ARTICLE

Hyphaene thebaica Mart. Extract Attenuates Oxidative Stress and Bax- and Bcl-2-Mediated Apoptosis in Ethanol-Induced Gastric Ulcers in Rats

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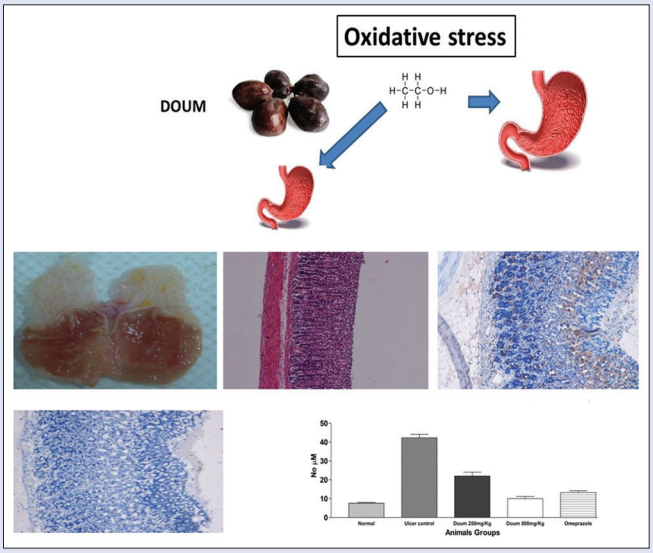
ABSTRACT

Objectives: *Hyphaene thebaica* Mart. (locally known as Doum) is a well-known tropical plant and is traditionally used for the treatment of gastrointestinal tract (GIT) ulcers. The current study aimed to investigate the antioxidative and gastroprotective properties of *H. thebaica* fruit rind extract against experimentally ethanol-induced gastric ulceration in Sprague–Dawley rats. **Materials and Methods:** Two experiments were carried out: first, with 20 animals for dose selection and safety of the extract; then, a second with 30 animals for the gastric ulcer model. The two selected doses of *H. thebaica* extract (250 and 500 mg/kg) and antiulcer drug (omeprazole: 20 mg/kg) were administered through oral gavage for 2 weeks prior to ulcer induction. Acidity, mucus weight, ulcer area, and histopathology were used to assess the gastroprotective effects of *H. thebaica* extract. The antioxidative properties were assessed using the lipid peroxidation assay, non-protein thiol levels, superoxide dismutase activity, nitric oxide assay, and immunohistochemical staining of mitochondria-regulated apoptosis proteins such as Bax and Bcl-2. **Results:** In the current study, no *in vivo* toxicity of *H. thebaica* extract was observed. In the gastric model, preadministration of *H. thebaica* resulted in a significant reduction in ulcer area and mucus weight in a dose-dependent manner. Moreover, gross and histological findings confirmed the gastroprotective properties of *H. thebaica* extract. Quantitative assessment of microscopic lesions revealed a significant difference between the groups. These properties were observed to be mediated through the modulation of oxidative stress. *H. thebaica* modulated the Bcl-2 and Bax proteins and inhibited apoptosis. **Conclusion:** The gastroprotective properties of *H. thebaica* nominate it as a potential nutraceutical candidate.

Key words: Apoptosis, doum palm, gastric ulcer, gastroprotection, *Hyphaene thebaica*, lipid peroxidation

SUMMARY

- The antioxidative and gastroprotective properties of *Hyphaene thebaica* fruit rind extract against experimentally ethanol-induced gastric ulceration in Sprague–Dawley rats are reported.
- No *in vivo* toxicity of *H. thebaica* extract was observed.
- Preadministration of *H. thebaica* resulted in a significant reduction in ulcer area and mucus weight in a dose-dependent manner.
- Gross and histological findings confirmed the gastroprotective properties of *H. thebaica* extract.
- Quantitative assessment of microscopic lesions revealed a significant difference between the groups.
- *H. thebaica* modulated the Bcl-2 and Bax proteins and inhibited apoptosis.



Abbreviations used: ALT: Alanine aminotransferase; ANOVA: one-way analysis of variance; APES: aminopropyltrimethoxysilane; AST: aspartate aminotransferase; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; DAB: 3,3×-diaminobenzidine;

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INTRODUCTION

Hyphaene thebaica Mart. (Doum) is one of the agroforestry trees with significant nutritional, medicinal, environmental, and economic value.[1,2] It is one of the most valuable plants on the globe and is also known as Gingerbread fruit.[2] The geographical distribution of this tree includes tropical Africa, the Middle East, and Western India.[1] *H. thebaica* parts are a precious source for a diversity of products helping many people in medicine, as well as for ropes and baskets. Antioxidants and secondary metabolites such as glycosides, flavonoids, phenols, tannins, steroids,

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saponin, terpenes, and terpenoids have also been detected in this tropical tree.[3] Many researchers investigated the antimicrobial, antioxidant, anti-inflammatory, and anticancer potential of *H. thebaica*.[2,4,5] It is also reported to possess antihypertensive properties through acetylcholine esterase and renin inhibition mechanisms.[2]

Oxidative stress and free radicals are known to interfere with cellular stability and performance.[6,7] Reactive oxygen species (ROS) is a collective term utilized for oxygen-generated free radicals (hydroxyl radical, nitric oxide, and superoxide) and nonradical oxygen derivatives of high reactivity (singlet oxygen, hydrogen peroxide, peroxynitrite, and hypochlorite).[8,9] Various types of these radicals are reported to cause many cytological changes such as inflammation, apoptosis, and microinjury, leading to several diseases and pathological conditions.[10,11] Natural antioxidants are used extensively to counteract the harmful effects of these free radicals,[12] and inhibit apoptosis through intrinsic and extrinsic pathways.[13] Bcl-2 and Bax proteins are key regulators in the intrinsic pathway of apoptosis.

Previous *in vitro* findings demonstrated that the fruit of *H. thebaica* is a source of potent antioxidants, (Elansary, Salem *et al.* 2017)[14] and is traditionally used as a treatment for GIT ulcers.[15,16] Therefore, the current study was designed to investigate the *in vivo* antioxidative and gastroprotective effects of *H. thebaica* fruit rind extract against experimentally ethanol-induced gastric ulceration in Sprague–Dawley rats. The antiapoptotic effects of this plant were investigated through the immunohistochemical staining of Bcl-2 and Bax proteins.

MATERIALS AND METHODS

Chemicals and reagents

Nitro blue tetrazolium was obtained from Thermo Fisher Scientific, USA. An immunohistochemistry kit (LSAB®2 System-Horseradish peroxidase [HRP]) was purchased from DAKO, USA. Ethanol, formaldehyde, sodium hydroxide, paraffin wax, hematoxylin, eosin, hydrogen peroxide, bovine serum albumin, thiobarbituric acid, tricholoroacetic acid, and DTNB (Ellman's Reagent; 5,5-dithio-bis-[2-nitrobenzoic acid]) were procured from Sigma Aldrich, KSA. Omeprazole was obtained from Teaching Hospital Pharmacy, Jazan University, KSA.

Plant materials and extraction

H. thebaica fruits were procured from an exclusive shop in Jeddah, Kingdom of Saudi Arabia (KSA). The plant was identified botanically by Dr. Siddig Ibrahim Abdelwahab, Medical Research Centre. The voucher specimen (2019/2/HT) was deposited at the Medical Research Centre, Jazan University. The thin dried brown rind of the fruits was freed and powdered. Powder was extracted using ethanol (95%) for 72 h three successive times. The solvent was dried completely using a rotary evaporator (GmbH, Germany) under vacuum conditions. Standardized dried extract was kept at − 4°C for further studies. The dried powder of the extract was dissolved in distilled water for oral gavage.

Animals

Two experiments were carried out: first, with 20 animals for dose selection and safety of the extract; then, a second experiment with 30 animals for the gastric ulcer model. Sprague–Dawley rats (150 ± 30 g) were obtained from the Animal Resource Centre, Medical Research Centre (MRC), Jazan University, KSA. All experiments in this study were ethically conducted in accordance with the World Medical Association Declaration of Helsinki. All approvals for this study were obtained from the Institutional Committee of Animal Studies, Jazan University (MRC/1440-5A- December 23, 2018). Animals were left to acclimatize for 2 weeks prior to the initiation of the experiment.

Dose selection study

Rats of both sexes were randomly divided into four equal groups. Animals were fed with a single dose of *H. thebaica* (0, 100, 500, and 2000 mg/kg). Rats were observed for 15 days for any abnormal clinical, physiological, and behavioral changes. Blood samples were collected using cardiac puncture and analyzed for biochemical liver functions using a colorimetric method. Data for these parameters are not shown.

Gastric ulcer model

Thirty male rats were randomly divided into five groups of six each. The ulcer model was based on a previously published method.[13,17] Ulceration was induced using 5 mL/kg of ethanol (95%, Sigma Aldrich, KSA) in overnight-fasted animals in Groups II, III, IV, and V, whereas Group I was left as a normal control. Omeprazole was used as the reference antiulcer drug (20 mg/kg; dissolved in distilled water) in Group III while animals in Group II were left without any treatment. Based on the findings of the dose selection study, two doses of the *H. thebaica* extract (250 and 500 mg/Kg; Groups IV and V, respectively) were used. *H. thebaica* and omeprazole were orally administered for 2 weeks prior to ethanol-induced ulceration. One hour after ethanol administration, all animals were sacrificed by an overdose of diethyl ether. Blood samples were collected immediately using cardiac puncture and the serum was collected after centrifugation. Stomachs were quickly detached and dipped in 10% buffered formalin solution. Liver enzymes were assessed using a colorimetric method as described by the manufacturer's instructions (MyBioSource, Inc., USA).

Macroscopic and biochemical gastric assessments

Gastric content and tissues were obtained for macroscopic and chemicopathological evaluation. Acidity was measured using a pH meter and titration with sodium hydroxide solution and expressed as mEq/L.[18] Mucus weight was measured using a sensitive digital balance. A planimeter and dissecting microscope (×1.8) were used to assess macroscopic gastric lesions in all animal groups. The total ulcerated area was scored as the total number of small squares (2 mm × 2 mm) having ulcers, and the inhibition percentage (I%) was obtained by the standard formula as described earlier with minor modifications.[19]

Histopathological examination

Stomach tissues were fixed in formalin (10%) and embedded in paraffin wax. Gastric microsections (5 µm) were placed on glass slides and stained with hematoxylin and eosin (H&E) for histopathological assessment under a normal light microscope. Slides were assessed by two independent histopathologists. Lesions were scored using an ordinal scale ranging from 1 to 3 as shown in Table 1.[20]

The microscopic evaluation score was based on histological analysis. The scoring of the different parameters allows calculation of the average microscopic score.

Immunohistochemistry

Formalin-fixed and paraffin-embedded gastric tissues were cut to 3–4 µm thickness and mounted on 3-aminopropyltrimethoxysilane (APES)-treated glass slides (DAKO, USA). Antigen retrieval was performed using microwave digestion. Hydrogen peroxide was used to block endogenous peroxide. Tissue sections were washed with Tris-buffered saline (pH 7.6) and blocked with 0.01% d-biotin. Nonspecific antibody binding was avoided using 3% bovine serum

albumin. Monoclonal anti-rat Bax and Bcl-2 antibodies were utilized at concentrations of 1:400 and 1:800, respectively. Immunohistochemical staining was performed using a LSAB®2 System-HRP kit (DAKO, USA) at ambient temperature according to the manufacturer's manual. Peroxidase enzyme was identified after incubating the tissues with 3,3×-diaminobenzidine (DAB) by the presence of a cytoplasmic brown precipitate that is insoluble in alcohol. Finally, the sections were counterstained with H and E. Specificity of staining was controlled by the exclusion of primary antibodies.

Lipid peroxidation assay

A lipid peroxidation assay was conducted using the thiobarbituric acid reactive substance (TBARS) assay[21] on stomach homogenate. The supernatant of the gastric tissue, trichloroacetic acid solution, and thiobarbituric acid were mixed and incubated for 30 min in a water bath (95–100°C). The mixture was then centrifuged and the supernatant collected to measure malondialdehyde (MDA) levels (532 nm). The findings were expressed as MDA (μmol/g tissue) after comparing with an MDA standard curve. The standard curve was established using linear regression with MDA concentration and optical density. The equation coefficient (*R*) was 0.99.

Non-protein thiol levels

Non-protein thiol levels (NPSH) were estimated according to previously described method.[22] In brief, small pieces of tissue from each stomach were homogenized in 4°C tricholoroacetic acid (5%, w/v). The homogenates were centrifuged at 6500 g, and the supernatants were utilized to quantify NPSH levels by reacting with a DTNB substrate (Ellman's Reagent). Absorbance was obtained at 412 nm, and the readings were expressed as nmol NPSH/g tissue. Total protein in stomach samples was determined according to the Lowry method.[23] Bovine albumin was utilized as the standard commercial protein. The standard curve was established using linear regression with bovine albumin concentration and optical density (412 nm). The equation coefficient (*R*) was 0.989.

Superoxide dismutase (SOD) activity

Estimation of superoxide dismutase (SOD) activity was performed by oxidation of NH_2OH in alkaline media, which was accompanied by reduction of nitro blue tetrazolium (NBT).[24] This reaction leads to the production of nitrite, which was detected colorimetrically. SOD activity (units per milligram of protein) was expressed as the quantity of the enzyme required to react with 25 mM NBT.

Nitric oxide (NO) assay

The level of NO in the gastric tissue was evaluated using the Griess assay.[13] The standard curves were plotted using sodium nitrite. Gastric tissues were homogenized in potassium phosphate buffer, and the supernatant was obtained at 4°C. Fifty microliters of Griess solution (0.1% *N*-[1-naphthyl, ethylenediamide dihydrochloride] and 1% sulphanilamide in 5% phosphoric acid) were added to the gastric homogenate and incubated for 15 min at room temperature. A spectrophotometer was used to observe the absorbance at 540 nm to analyze the samples. The findings were reported as μM nitrate/nitrite per gram of protein.

Statistical analysis

All values were reported as means ± SE (standard error of the mean). The one-way analysis of variance (ANOVA) test of inference was used to assess the statistical significance of the differences between the groups, followed by Duncan's *post hoc* analysis. Ordinal data for scoring of the H and E slides were analyzed using nonparametric ANOVA (Kruskal–Wallis test). A value of *P* less than 0.05 was considered statistically significant. All procedures were conducted using Statistical Package for Social Sciences (SPSS) software version 21.0.

RESULTS

H. thebaica extract did not show any abnormal clinical signs, behavioral changes, mortalities, or post-mortem abnormalities for the examined doses at the end of the 15 days of examination in the dose selection experiment (Study one). Hematopathological and biochemical assessment of vital organs and tissues depicted no considerable changes for *H. thebaica*-fed animals. In the gastric model, the liver biochemical tests, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed no significant differences between groups, eliminating the possibility of toxicological effects of *H. thebaica* extract on the liver of the experimental animals [Table 2].

Animals in Group II showed the highest ulcer area, whereas the lowest was observed with animals in Group III. Interestingly, animals treated with *H. thebaica* extract in Groups IV and V showed a significant reduction in the ulceration area, almost similar to that in Group III [Table 3]. In addition, Group II showed the highest content of mucus compared with other groups and *H. thebaica* extract was capable of significantly reducing the mucus content similar to Group III. pH augmentation was only observed in Groups III, IV, and V [Table 2].

Macroscopic examinations of the glandular part of the rat stomachs were carried out to assess the formation of ulcer lesions induced by ethanol. These examinations were performed upon incision of the stomach along the greater curvature. Group II showed the most severe hemorrhagic lesions on the glandular part of the stomach, whereas the treated groups showed the least [Figure 1]. Microscopic examination of gastric tissues stained with H and E for Group II demonstrated extensive and edematous mucosa with penetration of inflammatory leucocytes in the submucosal layer of the stomach wall. Pretreatment with *H. thebaica* extract led to the reduction or absence of submucosal edema and leucocyte infiltration in a dose-dependent manner [Figure 2]. Kruskal–Wallis ANOVA revealed a significant difference between groups, whereby Group II showed the highest mean of the scored lesions as depicted in Figure 3. Immunohistochemical staining of Bcl-2 revealed an intense brownish color in the cytoplasm of gastric cells in the *H. thebaica* extract-treated Groups (IV and V) compared with Group II [Figure 4]. When the Bax antibody was used to stain the cytoplasm of gastric cells in the *H. thebaica* extract-treated

Table 1: Microscopic score evaluation

Microscopic lesions	Score 1	Score 2	Score 3
Depth of the erosion	Up to 1/3 of the total mucosal depth	Up to 2/3 of the total mucosal depth	Total mucosal
Hemorrhage	Focal	Mild	Severe
Inflammation	Light	Mild	Severe

Table 2: The effect of *H. thebaica* extract on liver function tests. *H. thebaica* extract was administrated orally for 15 days prior to ulcer induction

Pretreatment for 15 days*	*n*	AST (IU/L)	ALT (IU/L)
None (Normal)	6	275.5±19[a]	45.6±9.2[a]
Doum (250 mg/kg)	6	285.2±32[a]	50.5±10.7[a]
Doum (500 mg/kg)	6	273.4±27[a]	52.4±6.8[a]

*Groups with same alphabets are statistically indifferent

Table 3: Effects of *H. thebaica* extract and omeprazole on experimental induction of ulceration using ethanol

Pretreatment*	Groups*	*n*	Ulcer area (mm^2)	% of inhibition	Mucus (g)	pH	Malondialdehyde (µmol/g tissue)	SOD activity (U/mg protein)	NPSH level	Total protein
None (Normal)	I	6	-	-	0.122±0.01[a]	7.04±0.53[a]	8.0±0.28[a]	45.2±0.25[a]	2.1±0.21[a]	6.2±1.29[a]
Ethanol (control)	II	6	97.20±19.6[a]	-	0.99±0.04[b]	2.6±0.3[b]	31±2.54[b]	16.2±0.48[b]	0.62±0.01[b]	11.5±0.31[b]
Omeprazole (20 mg/kg)	III	6	15.65±2.5[b]	83.9	0.47±0.01[c]	5.50±0.23[c]	15.1±3.17[c]	26.5±1.71[c]	1.15±0.01[c]	7.18±1.49[a]
H. thebaica 250 mg/kg)	IV	6	28.5±5.6[c]	70.7	0.62±0.12[d]	5.9±0.5[c]	12.3±2.7[c]	22.5±0.38[b]	1.69±0.14[d]	5.6±1.27[a]
H. thebaica (500 mg/kg)	V	6	19.17±3.0[b]	80.3	0.42±0.02[c]	6.9±0.9[a]	9.4±0.83[a]	48.4±0.92[a]	1.9±0.05[a]	5.1±1.12[a]

*Groups with different superscripted alphabets are statistically different ($P<0.05$). ANOVA was used to assess the statistical significance of the differences between the groups followed by Duncan's *post hoc* analysis

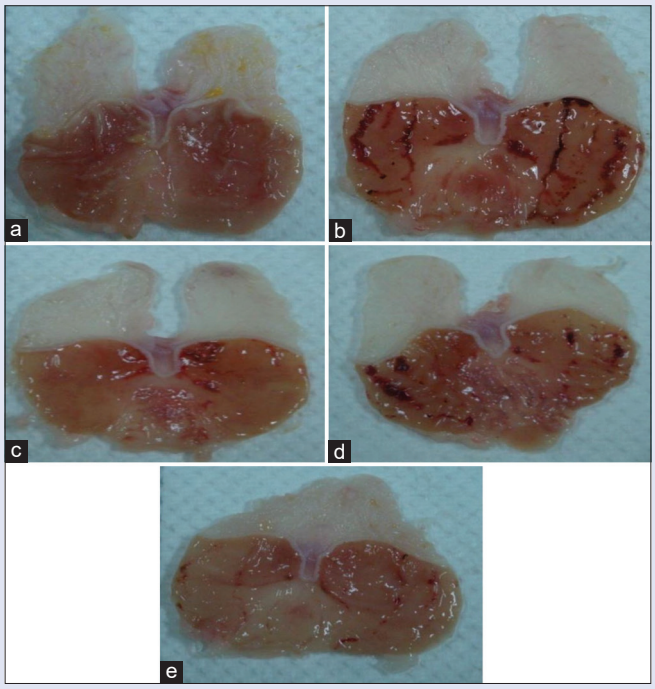

Figure 1: Macroscopic examination of hemorrhagic lesions on the glandular part of the rat stomach. (a-e) are representative photos from Groups I, II, III, IV, and V, respectively. 3B showed the most severe hemorrhagic lesions on the glandular part of the stomach

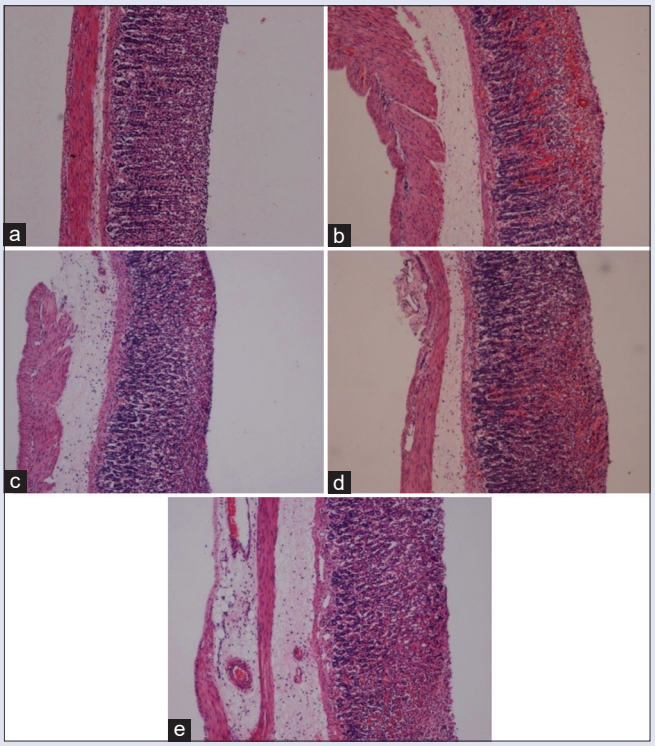

Figure 2: Histopathological examination of the gastric tissues stained with hematoxylin and eosin (H and E). (a-e) are representative photos from Groups I, II, III, IV, and V, respectively. Group II (3B) demonstrated extensive and edematous mucosa with penetration of inflammatory leucocytes in the submucosal layer of the stomach wall

Groups (IV and V), it displayed a prominent brownish color in the cytoplasm of gastric cells when compared with Group II [Figure 5]. It could be concluded that *H. thebaica* extract was able to modulate the levels of these mitochondrial-related proteins of apoptosis.

The ability of *H. thebaica* extract to inhibit the harmful effects of ethanol on gastric mucosa was assessed using the measurement of lipid peroxidation as an oxidative stress marker. The results demonstrated that the ethanol-induced lipid peroxidation was significantly reduced in the treated animals (Group III, IV, and V) compared with the nontreated ones (Group II) as shown in Table 3. SOD activity and total protein were also affected by the potential gastroprotective properties of *H. thebaica*, as shown in Table 3. In addition, the NPSH level, as shown in Table 3, was significantly increased in animals treated with *H. thebaica* extract, whereas the level of NO was significantly reduced compared with Group II [Figure 6].

DISCUSSION

The current study aimed to investigate the *in vivo* antioxidative and protective properties of Doum fruit (*Hyphaene thebaica* Mart.) against experimentally triggered gastric injury in rats. Toxicological evaluation of *H. thebaica* extract was also performed. The findings of this study revealed that *H. thebaica* extract did not show any abnormal effects on rats when administered for 2 weeks at doses of 250 and 500 mg/kg. This high margin of safety was confirmed by the histological findings for various organs. These histological findings were further confirmed using biochemical investigations such as hepatic function tests (ALT and AST). Our findings are in line with previous published

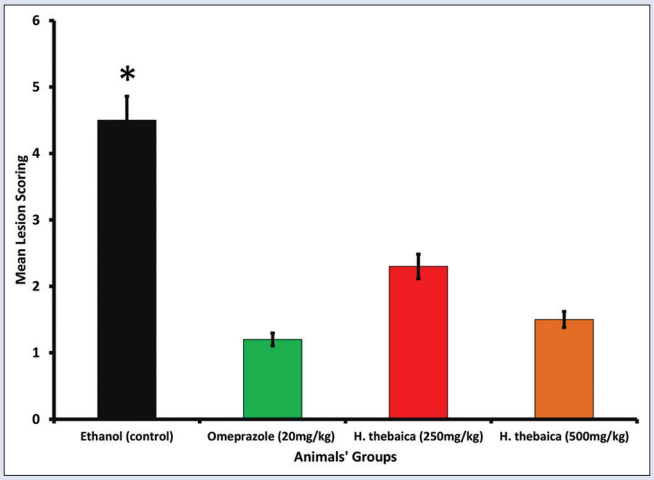

Figure 3: Microscopic scores of ethanol-induced gastric ulcers in control and treated animals. Normal animals presented macroscopic lesions scored as zero. *Represents $P < 0.05$ and the results are shown as mean ± SE

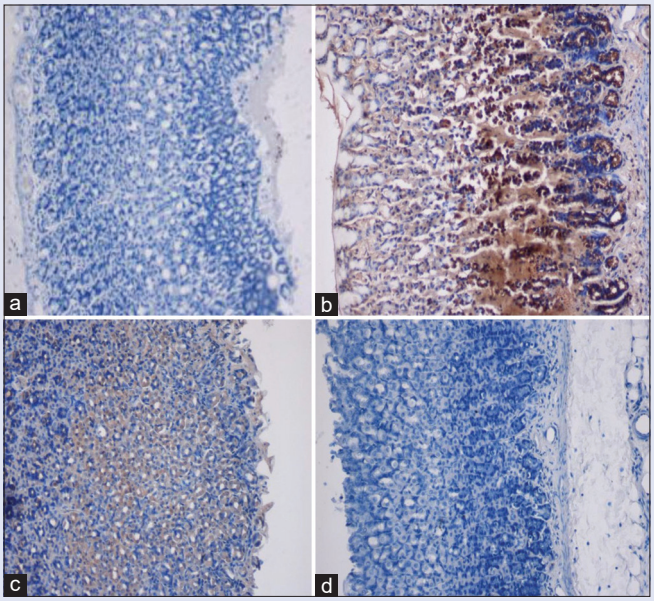

Figure 5: Bax staining in immunohistochemically stained gastric tissues. (a-d) are representative photos from Groups I, II, IV, and V, respectively. Expressed cytoplasmic proteins are stained with brown color

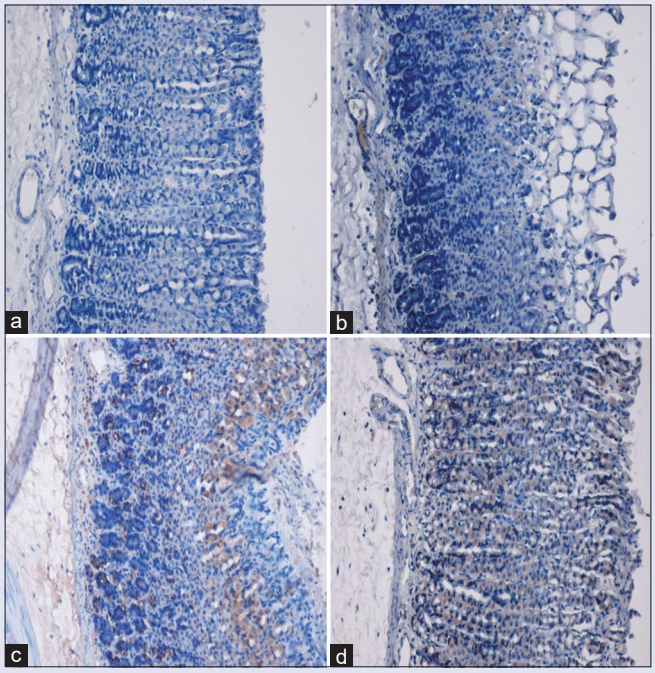

Figure 4: Bcl-2 staining in immunohistochemically stained gastric tissues. (a-d) are representative photos from Groups I, II, IV, and V, respectively. Expressed cytoplasmic proteins are stained with brown color

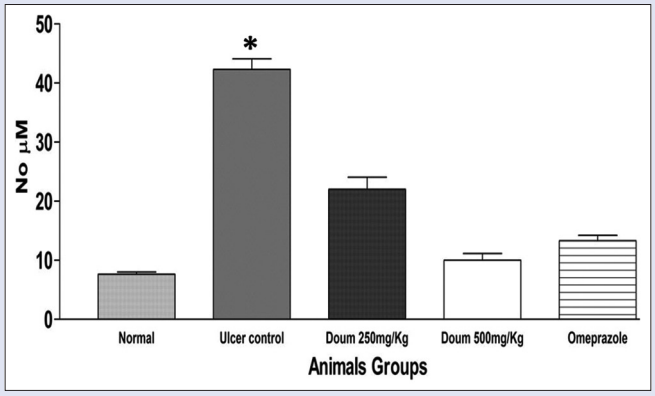

Figure 6: Effect of *H. thebaica* extract on the level of nitric oxide (NO) in the gastric tissues of animals. *Represents $P < 0.05$ and the results are shown as mean ± SE

studies, which demonstrated that *H. thebaica* extract is nontoxic and safe.[5,16]

Our findings demonstrated that *H. thebaica* extract was capable of showing significant protective effects on rat stomach subjected to ethanol-induced gastric injury. Experimental induction of ulcers was performed using ethanol, a well-established animal model of oxidative stress and gastric ulcer.[25] Hydrocarbon solvents are intensively utilized as ulceration agents because of their rapid and easy penetration of gastric mucosa. The permeability of the gastric mucosa and the release of vasoactive products are also modulated by the administration of ethanol.[26,27] The primary mechanistic role of ethanol pathogenesis is the production of free radicals.[26]

From the results of microscopic examination, the ethanol group showed the most severe hemorrhagic lesions on the glandular part of the stomach, whereas there were no lesions formed in the normal group. In addition, our data showed that animals pretreated with *H. thebaica* extract had the fewest hemorrhagic lesions, with almost the same effects compared with the reference antiulcer drug (Omeprazole) treatment. In this context, the exploitation of medicinal herbs for the treatment and prevention of gastric ulcers is continuously expanding all over the world.[28] Preadministration of ethanol-fed rats with *H. thebaica* extract led to the decrease or absence of submucosal edema and leucocyte penetration in a dose-dependent manner. This anti-inflammatory effect of *H. thebaica* extract was previously reported in an *in vitro* model,[29] and was found to be mainly driven through suppression of cyclooxygenase (COX-1),

an enzyme known to be associated with inflammatory pathways.[4] An earlier study reported that the indicators of inflammation were reduced after administration with *H. thebaica*.[29]

The possible role of antioxidant effects by *H. thebaica* extract was examined using the MDA assay, which is a well-established marker for lipid peroxidation. *H. thebaica* extract has the ability to protect gastric tissues from ethanol-induced lipid peroxidation.[30] Lipid peroxidation and free radical involvement in ethanol-triggered gastric injury were reported earlier.[31] Dietary antioxidant polyphenols can diminish toxicant-induced oxidative stress. The role of NO and its bioavailability level has been shown to be altered by the ROS level.[32] Administration of *H. thebaica* to the animals in Group IV and V led to a significant ($P < 0.05$) decrease in NO levels as compared with the animals in Group II. The effect of *H. thebaica* extract on ROS was further confirmed using some enzymatic antioxidant methods. Our data showed that *H. thebaica* extract was able to modulate the levels of SOD and NPSH. Reversal of ethanol NPSH depletion using herbal extract was previously reported.[33]

Activation of apoptosis signaling pathways by ROS is a well-known cellular event. Ethanol is known to induce apoptosis via interference with the mitochondrial pathway of apoptotic Bcl-2 and Bax proteins.[34,35] ROS mediates the downregulation of antiapoptotic Bcl-2 and upregulation of Bax proapoptotic proteins.[36] The present study revealed that the pretreatment with *H. thebaica* extract led to the upregulation of Bcl-2 protein and downregulation of Bax, confirming the involvement of *H. thebaica* extract treatment in the regulation of apoptosis. Numerous studies have shown that apoptosis is associated with the development of gastric ulcers, and that the excessive generation of apoptosis over time will ultimately impair the integrity of the gastric mucosa, resulting in gastric mucosa malfunction and eventual ulcer development. On a mechanistic level, our findings demonstrated that ethanol consumption resulted in a significant downregulation of the antiapoptotic protein Bcl-2, which was mediated by ROS and proinflammatory signals. As a result, the restriction to the apoptotic protein Bax was reduced, which, in turn, resulted in the mitochondrial escape of cytochrome C, which, in turn, resulted in the activation of Caspases 9 and 3. This is consistent with the findings of earlier research. Following treatment with *H. thebaica*, it was clearly apparent that the antiapoptotic potential of the gastric mucosa had been increased, as shown by the recovery of BCL-2 levels and the control of Bax expressions. In fact, earlier investigations have indicated that the compound has antiapoptosis action that is latent. Furthermore, our findings clearly suggested that *H. thebaica*, by its antiapoptotic properties, might contribute to the amelioration of the damage produced by ethanol.

CONCLUSION

To our knowledge, this study is the first of its kind to investigate the *in vivo* antioxidative and protective properties of Doum fruit (*H. thebaica*) against experimentally induced gastric injury. Our findings confirmed the importance of *H. thebaica* extract in protecting rat stomach from the chemical injury induced by ethanol, and it might be a potential nutraceutical candidate not just for preventing gastric ulceration but also for preventing other types of ulceration. The exact mechanism of *H. thebaica* extract involvement in regulating oxidative stress and apoptosis remains to be investigated. Further phytochemical analysis of this extract should be conducted in future work.

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Authors' contributions

MMET, HAH and AAM designed the study and wrote the manuscript and conceptualization. MMET and ESS collected the samples and analysed the data. MMET performed the experiments and all investigations. AMM, AF, HAH and AAM reviewed the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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