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Saudi honey alleviates indomethacin-induced gastric ulcer via improving antioxidant and anti-inflammatory responses in male albino rats

Abstract

Recent years have reported a rise in the occurrence of gastric ulceration especially among young children and adults. This study investigated the mechanism by which two types of Saudi honey: Alnahal Aljawal honey (Wadi) or Bin Ghaithan honey (Talh) exerted their antiulcer potential in indomethacin-induced gastric ulceration. Four cohorts of rats were used: Group 1; Healthy controls, Group 2; Ulcerative animals, Group 3; Ulcerative + Wadi honey treatment, Group 4; Ulcerative + Talh honey treatment. We profiled the levels of different indicators of oxidative stress including the activities of gastric mucosal glutathione superoxide dismutase (SOD), catalase (CAT), peroxidase (GPx), reduced glutathione (GSH), and lipid peroxidation (measured as malondialdehyde; MDA). CRP content, IL-10, and plasma tumor necrosis factor-α were also evaluated. The stomach was visually examined for macroscopic lesions and using light microscope for histopathological changes in the glandular mucosa.

Wadi or Talh honey significantly reduced the ulcer indices, and essentially protected the glandular mucosa from lesions. Wadi or Talh honey also significantly reduced the gastric mucosal concentrations of GPx, SOD and GSH. In addition, the administration of Wadi or Talh honey decreased gastric mucosal plasma TNF-α and MDA, CRP content, and IL-10 levels. In conclusion, Wadi or Talh honey possibly exerted their antiulcer, potential via restoring the homeostasis and stabilizing the enzymatic (SOD and GPx) and non-enzymatic (GSH) antioxidants as well as reducing the levels of inflammatory cytokines (TNF-α, CRP content, IL-10 and, NF-κB activity), and inhibiting the lipid peroxidation in the gastric mucosa. Consequently, Wadi or Talh honey may be of beneficial therapy for patients diagnosed with gastric ulceration. Clinical studies need to be conducted to further support these findings.

Keywords: Saudi honey; Indomethacin; Gastric ulcer; Lipid peroxidation; Antioxidant; Anti-Inflammatory.

Introduction

Gastric ulcer is a common type of peptic ulcer that affects the stomach and nearly around 8% to 10% of the worldwide population are affected (Boligon et al., 2014; Woolf and Rose, 2021). The pathophysiology of this illness is a multifactorial process resulting from the imbalance between aggressive factors like pepsin and acid on one side and mucosal defense factors which include blood flow and prostaglandins on the other side (Sistani Karampour et al., 2019). There are many factors that may increase the risk of gastric ulcers. Such factors include smoking and alcohol consumption (help in causing chronic inflammation of the stomach), stress (which can cause severe damage to the epithelial surfaces and submucosa) (Fazalda et al., 2018), *Helicobacter pylori*, and the use of nonsteroidal anti-inflammatory drugs as well which inhibits prostaglandins (PGs) (Kamada et al., 2021). Prostaglandins, mucus, heat shock proteins and phospholipids are normally generated from the surface of epithelial cells that are firmly connected by tight junctions and named as epithelial barrier. Together with mucus, bicarbonate-phospholipid barrier maintain the mucosal integrity under normal conditions and provides a defense mechanisms (Laine et al., 2008). The cure or even prevention of gastric ulcers is a challenge facing medicine because most of the drugs that are currently sold on market have limited efficacy against the illness and mostly associated with adverse side effects (Kawai et al., 2018; Scally et al., 2018). Accordingly, research is directed more towards identifying potentially active agents from natural resources that may provide safe and effective therapy either when used alone or as complementary therapy to ongoing conventional treatment (Almasaudi et al., 2017).

Honey is a product that is produced naturally and has long been reported for having medicinal properties (Kuropatnicki et al., 2018; Siegmund et al., 2018). Human use of honey dates to 8,000 years ago. It has been used by the ancient Egyptians, Romans, Chinese and Greek in wound healing and diseases of the gut (Al-Waili et al., 2011; Yilmaz and Aygin, 2020). Honey is produced by different species of bees around the globe (Almasaudi et al., 2017; Pita-Calvo and Vázquez, 2018). The

processing of nectar by bees results in this viscus dense solution which is rich in enzymes, water, sugar (sucrose (0.7%–1%), fructose (38.2%), glucose (31.2%) and disaccharides, pigments, minerals and other substances which support healing and provide antioxidant effects as flavonoids (quercetin, galangin, kaempferol, apigenin, chrysin, pinocembrin, and hesperetin) and phenolic acids (p-coumaric, caffeic, ellagic, and ferulic acids), tocopherols, ascorbic acid, reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Akefe et al., 2020; Eteraf-Oskouei and Najafi, 2013; Yilmaz and Aygin, 2020). In addition to the key role, it plays in traditional medicine, in the past few decades honey's antibacterial properties was one of the most important findings to be first recognized by van Ketel in 1892 and since then honey is being subjected to laboratory and clinical investigations (Kuropatnicki et al., 2018). It has been reported that honey used to treat and protect against gastritis, duodenitis and gastric ulceration which are caused by bacteria and rotavirus (Beena et al., 2018; Tsang et al., 2015). The mechanism of action was reported by Alnaady et al (2005) when they found out that honey prevent the adherence of microorganisms to the intestinal epithelium, an initial step by which bacterial infection develops (Eteraf-Oskouei and Najafi, 2013). Wadi and Talh honey, are two important original types of honey available in market with proven effectiveness in the management of wound healing (Roshan et al., 2017).

In an earlier study, we reported the curative efficacy of a New Zealand Manuka honey on chronic gastric ulcer induced by acetic acid (Almasaudi et al., 2017), following, in this study we studied the potential of Wadi and Talh honey in mitigating indomethacin induced gastric ulceration in rats.

Materials and Methods

Experimental design:
Animals:

Eight-week-old male albino rats varying from 222 to 245 g in body weight, were used as experimental subjects. The rats were housed for a week at 24 °C and 5% relative humidity and provided with water and standard rat feed ad libitum.

4 groups (5 rats /group) of rats were used, and each set was replicated three times.

Group 1: Healthy controls, Group 2: Ulcerative animals, Group 3: Ulcerative + Wadi honey treatment, Group 4: Ulcerative + Talh honey treatment. Thirty-six hours preceding to the experiment, all the rats were deprived of food to ensure an empty stomach but were freely allowed to access water. The rats were housed individually in metabolic cages with raised floors and wide mesh during this period to reduce coprophagic vice. Animals were handled with care according to the animal care and use guidelines set by our university (ACUC-21-08-32).

Indomethacin-induced gastric ulceration and treatment

Gastric ulcer was induced in the ulcerative group using single dose of Indomethacin (30 mg/kg body weight) dissolved in 3% tween 80 which was administered via oral intubation. While the control group received the same dose of 3% tween 80 dissolved in distilled water. The administration of treatment to animals began two days following ulcer induction. Group 3 and group 4 received the highest dose (2.5 g/kg) of Wadi or Talh honey respectively by gastric gavage once daily for 10 days consecutively.

Twenty-four hours prior to the last treatment dose, the rats were weighed again prior to sacrifice by cervical dislocation which was done under humane conditions. Afterwards the stomachs were dissected, and assessed for mucosal damage following standard grading: petechiae (light, moderate, and intense with 1, 2, and 3 points, resp.), thickening of the ulcer (1 point/mm2), edema (1 point),

hyperemia (1 point), ulcers (not perforated and perforated, 1 point/mm2 and 2 points/mm^2, resp.), and hemorrhagic lesion (3 points) (Matalka et al., 2013).

$$\text{Ulcer inhibition rate} = \frac{\text{Control (ulcer index)} - \text{Test (ulcer index)}}{\text{Control (ulcer index)}} \times 100\%$$

Assessment of kidney and liver function

Serum concentrations of alanine aminotransferase, aspartate aminotransferase, uric acid, urea, alkaline phosphatase, creatinine testosterone, interleuken-10, C- reactive protein, and cystatin C MBS763996, were determined using ELISA kits of MyBioSource (USA) catalogue No. MBS9719084, MBS9719085, MBS2903804, MBS2600001, MBS7606443, MBS749827, and MBS1600166 respectively, following manufacturer's directions.

Preparation of Sample for Analysis of Oxidative Stress

A segment of the stomach tissue samples was homogenised in 2% Triton X-100 solution containing 0.32M sucrose for evaluation of SOD activity. Another portion of the stomach was homogenized in 50mM potassium phosphate at pH 7.5 and in the presence of 1Mm EDTA for assaying MDA and GSH. Thereafter, the homogenates obtained were sonicated twice at 4^0C for 30s intervals before centrifuging at 4^0C for 10 min, at 1800g.

Evaluation of Reduced Glutathione (GSH) Activity

The activity of GSH was assayed as described by Ellman et al. (Almasaudi et al., 2017). Using a commercial kit (Biodiagnostic, Egypt) the GSH activity of the stomach homogenates was determined and expressed as nmol/g of gastric tissue.

Evaluation of Lipid Peroxidation (MDA)

The MDA was assessed (Mihara and Uchiyama, 1978). Briefly, MDA content of gastric tissues were assayed by using commercial kits (Biodiagnostic, Egypt) to determine the difference between two

optical densities (OD) at 525 and 535 nm. The MDA concentration in gastric tissue was shown in nmol/g.

Evaluation of Superoxide Dismutase (SOD) Activity

Using (Biodiagnostic, Egypt) kits, the SOD activity was assayed in stomach homogenates and displayed in U/g tissue.

Evaluation of Tumor IL-10, Necrosis Factor-*α* (TNF-*α*), and NF-κB levels

ELISA kits (Assaypro, USA; MBS2507393), (Novex, USA; MBS825017), and (MyBioSource; USA; MBS268833), were used to measure the concentrations of TNF-*α* IL-10 and NF-κB in stomach homogenates respectively. While the concentration of cytokines was estimated using a recombinant cytokine purified standard, according to the manufacturer's instructions.

Preparation of Tissue for Histological and Immunohistochemical Analyses

Histological and histochemical studies

The gastric tissues were fixed in 4% paraformaldehyde solution. Then the specimens were processed using standard histological procedure. Portions of Five *μ*m thick gastric tissue were stained with hematoxylin and eosin (H&E) for general histological studies (Carleton, 1980). The condensation of polysaccharide was examined by Periodic Acid Schiff (PAS) staining and viewed under a light microscope (Mc, 1946).

Immunohistochemical studies

Immunohistochemical staining for detection of tumor necrosis factor alpha (TNF-α), was done. The primary monoclonal antibodies used were the mouse anti-TNF-α (Santa Cruz Biotechnology, Santa Cruz, California, USA) (1:300 with PBS).The cellular site of the reaction was the cytoplasm which appeared brown in color (El-azab et al., 2018)**.** The avidin– biotin peroxidase method was used in the immunohistochemical study which was carried out by adding the chromogen, diaminobenzidine (DAB) ,(Dakopatts, Glostrup, Denmark), to slides. The stained slides were washed with distilled water, and

hematoxylin was employed to counterstain the sections. The phosphate buffer saline was used in the case of the negative control instead of the specific primary antibody (Cardiff et al., 2014).

Statistical analysis

At the end of the study, the obtained data were recorded as mean ± standard error of the mean (± SEM) and subjected to analysis of variance (ANOVA), to determine significant difference between the groups. GraphPad prism Software (San Diego, CA, USA) was used to analyse all the data and values of $P < 0.05$ were considered statistically significant.

Results

Effect of Wadi or Talh Honey on Body Weight Gain (BWG)

The results showed that neither Wadi nor Talh honey caused a significant change on the body weight of treated groups as compared to controls (Table 1) and respectively.

Table (1): *Effect of* ***2.5 gm/kg*** *either Wadi or Talh honey on body weight gain (BWG).*

Treatment Regimen	**BWG (%)**	
	Mean	SD
Control	11.33	1.53
Wadi honey	11.67	2.52
Talh honey	12.33	1.53

Data are expressed as mean ± SEM (n = 5 in each group) and analyzed by ANOVA followed by Tukey's test

Non-significant versus (treated) ($P = 0.994$)
Non-significant versus (controls) ($P = 0.879$)

Effect of either Wadi or Talh honey on rat's livers or kidneys functions in indomethacin-induced gastric ulcers

Table (2)*: Effect of 2.5 mg/kg of Saudi Honey (Wadi or Talh) on liver and kidney function test parameters in indomethacin-induced gastric ulcer.*

Treatment regimen	ALT (u\l)	AST (u\l)	ALK.P (IU/L)	Serum Urea (mg/dL)	U.A (mg\dl)	Serum Creatinine (mg/d)
Control	15.00 ±1.78	18.27 ±2.53	42.33 ±3.21	13.77 ±1.12	5.07 ±1.07	0.63 ±0.11
Ulcerative	73.67 ±8.50 [a]	108.83 ±11.36 [a]	137.33 ±16.9 [a]	52.33 ±6.81 [a]	8.97 ±0.55 [a]	1.56 ±0.22 [a]
Wadi honey	19.47 ±7.90 [b]	25.33 ±7.37 [b]	48.67 ±4.16 [b]	14.20 ±1.59 [b]	5.33 ±0.59 [b]	0.71 ±0.22 [b]
Talh honey	27.00 ±5.57 [b]	35.33 ±7.23 [b]	61.00 ±7.94 [b]	19.33 ±1.53 [b]	6.07 ±0.31 [b]	1.03 ±0.06 [b]
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data are expressed as mean ± SEM (n = 3 in each group) and analyzed by ANOVA followed by Tukey's test
a = Significantly different from the value in the control group ($P < 0.05$).
b = Significantly different from the value in the ulcerative group ($P < 0.05$).

The results showed a notable increase in the concentrations of ALT, AST, alkaline phosphatase, urea, uric acid, and creatinine (480%, 600%, 325%, 379%, 175.5% and 247%, resp.) in the gastric mucosa of the ulcerative group compared to the controls (P = <0.001, 0.015, 0.024, 0.018 and 0.001, resp.). Hereby, we demonstrated that treatment with either Wadi or Talh honey led to a significant reduction in the gastric mucosal ALT, AST, alkaline phosphatase, urea, uric acid, and creatinine content in comparison to the ulcerative group. Wadi honey (26.42%, 23.27%, 35.44%, 27.13%, 59.42% and 45.51%, resp.), (P = 0.034, 0.013, 0.022, 0.014, 0.002 and 0.001, resp.). Talh honey (36.64%, 32.46%, 44.41%, 36.93%, 89.96% and 66.02%, resp.) (P = 0.001, 0.027, 0.012, 0.004 and 0.005, resp.).

Assessment of the effects of honey on Oxidative Stress Biomarkers

In the current study, the antioxidant enzymes' activities like GSH, catalase, SOD, and lipid peroxidation product MDA were determined in the gastric tissue homogenate for all the different groups.

Glutathione (GSH)

A significant decrease in the activities of GSH was noted in rats in the ulcerative group (19.9%) when compared with the control group ($P = 0.001$). This substantial decline was reversed upon treatment of rats with 2.5 gm/kg of either Wadi honey or Talh honey leading to a significant rise in the GSH content (~425%) of gastric mucosal compared to the ulcerative group ($P < 0.001$). (Fig1A).

Malondialdehyde (MDA)

A significant elevation of 483% in the gastric mucosal levels of MDA were noted in the ulcerative group when compared to the control group ($P = 0.002$). The treatment of animals with either type of the two honeys Wadi or Talh has resulted in a significant reduction in the MDA levels (~22%) in the gastric mucosal as compared to the ulcerative groups ($P < 0.001$ and $P = 0.003$, resp.). (Fig2, B)

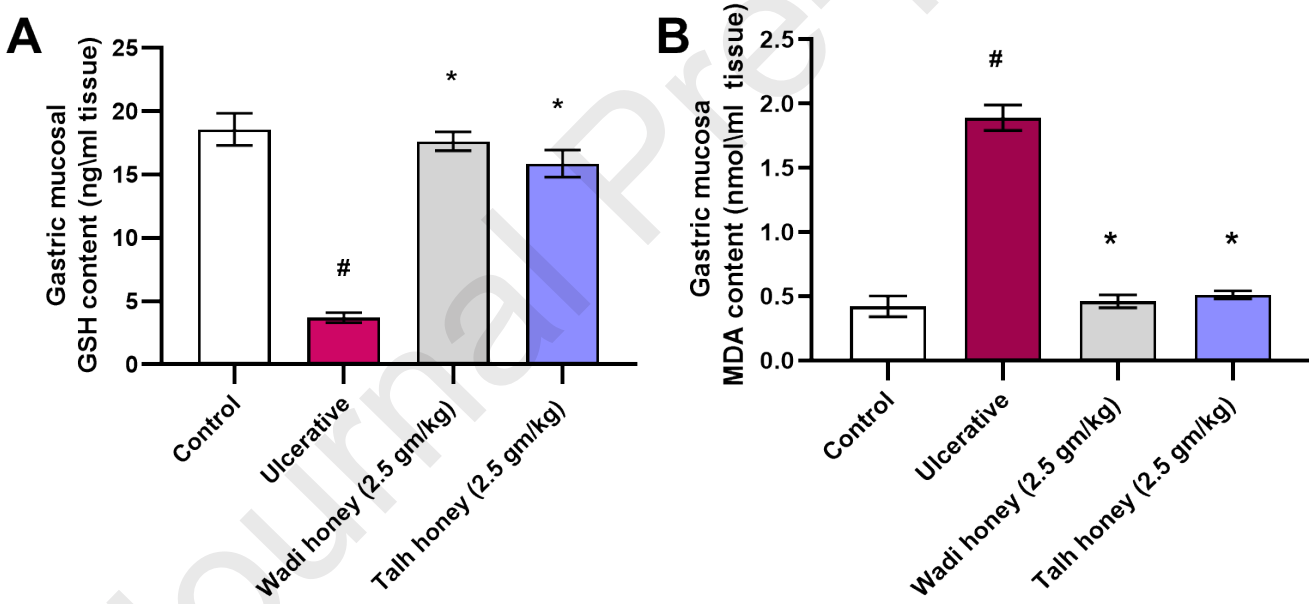


Fig. 1: *Effect of treatment with 2.5 gm/kg* Saudi Honey *(Wadi or Talh) on gastric mucosal* **A.** GSH **B**. MDA levels. Each value is the mean ± SEM ($n = 5$). #Significant versus control ($P \leq 0.05$). ∗Significant versus indomethacin-induced gastric ulcer ($P \leq 0.05$).

Effect of treatment on CAT and SOD Activities

In the ulcerative group, a prominent decline in the levels present in the gastric mucosa of both CAT and SOD, activities (45.7%, and 48%, resp.) when compared to the control value ($P = 0.001$, and

<0.001, resp). The treatment of rats with Wadi honey resulted in an elevation of the levels of gastric mucosa CAT and SOD enzyme (211%, and 215%, resp.) when compared to the ulcerative group (P = 0.001, and 0.002, resp.). The same was noted in the case of treatment of rats with Talh honey resulting in a significant increase in the gastric mucosal enzyme activities of both SOD and CAT as well (210% and 207%, resp.) in comparison to the ulcerative group (P = 0.000 and P = 0.003, resp.; Table 3).

Table (3): *Effect of treatment with 2.5 gm/kg Saudi Honey (Wadi or Talh) on CAT, SOD enzyme activities.*

Treatment regimen	SOD (u\ml tissue)	Catalase (Mu\L tissue)
Control	180.67 ±7.02	121.00 ±2.65
Ulcerative	82.67 ±14.57[a]	58.67 ±3.51[a]
Wadi honey	177.67 ±10.50[b]	123.67 ±6.43[b]
Talh honey	173.33 ±7.77[b]	121.67 ±3.51[b]
P value	<0.001	<0.001

Data are expressed as mean ± SEM (n = 3 in each group) and analyzed by ANOVA followed by Tukey's test
a = Significantly different from the value in the control group (P < 0.05).
b = Significantly different from the value in the ulcerative group (P < 0.05).

Effect of treatment with Wadi or Talh Saudi honey on gastric Mucosa Proinflammatory Cytokines: CRP and TNF-α.

The ulcerative group showed high gastric mucosal levels of TNF-α and CRP (275%, and 411%, resp.) as compared to the control group (P = 0.001, and 0.001, resp.). The treatment of rats with Wadi honey significantly reduced the gastric mucosal content of both TNF-α, and CRP (35%, and 21%, resp.) as compared to the ulcerative group (P = 0.001, and 0.001, resp.). Treatment with Talh honey showed a significant reduction in gastric mucosal TNF-α, and CRP levels as well (35%, and 44%, resp.) (P = 0.001, and 0.005, resp.) **Table (4).**

Table (4): *Effect of treatment with Saudi Honey on gastric mucosal levels of CRP and TNF-α.*

Treatment regimen	TNF-α (pg\ml tissue)	CRP (pg\ml tissue)
Control	14.90 ±1.01	5.67 ±1.14
Ulcerative	41.00 ±3.00[a]	23.33 ±5.03[a]
Wadi honey	14.50 ±0.95[b]	4.97 ±0.45[b]
Talh honey	14.30 ±1.54[b]	10.40 ±1.44[b]
P-value	<0.001	<0.001

Data are expressed as mean ± SEM (n = 3 in each group) and analyzed by ANOVA followed by Tukey's test

a a = Significantly different from the value in the control group (P < 0.05).

b b = Significantly different from the value in the ulcerative group (P < 0.05).

Effect of the tested Saudi Honey on Gastric Mucosal levels of Interleukin-10 (IL-10) Levels

The levels of IL-10 in the gastric mucosa in the honey treated rats were significantly reduced (37%) in relation to the control ($P = 0.004$). The treatment of rats with either Wadi or Talh honey significantly increased the IL-10 levels (293% and 377%, resp.) when compared to the ulcerative group ($P = 0.002$ and 0.001, resp.; **Fig 2A**).

Effect either Wadi or Talh honey on Gastric Mucosal NF-κB Levels

There was a significant rise in the gastric mucosal NF-κB activity (386%) in the ulcerative group when compared to the control group ($P = 0.001$). A significant reduction in the gastric mucosa NF-κB activity was noted in both treatments' groups of either honey Wadi or Talh (26% and 25%, resp.) compared to ulcerative control group ($P = 0.001$ and $P = 0.002$, resp.) **(Fig 2).**

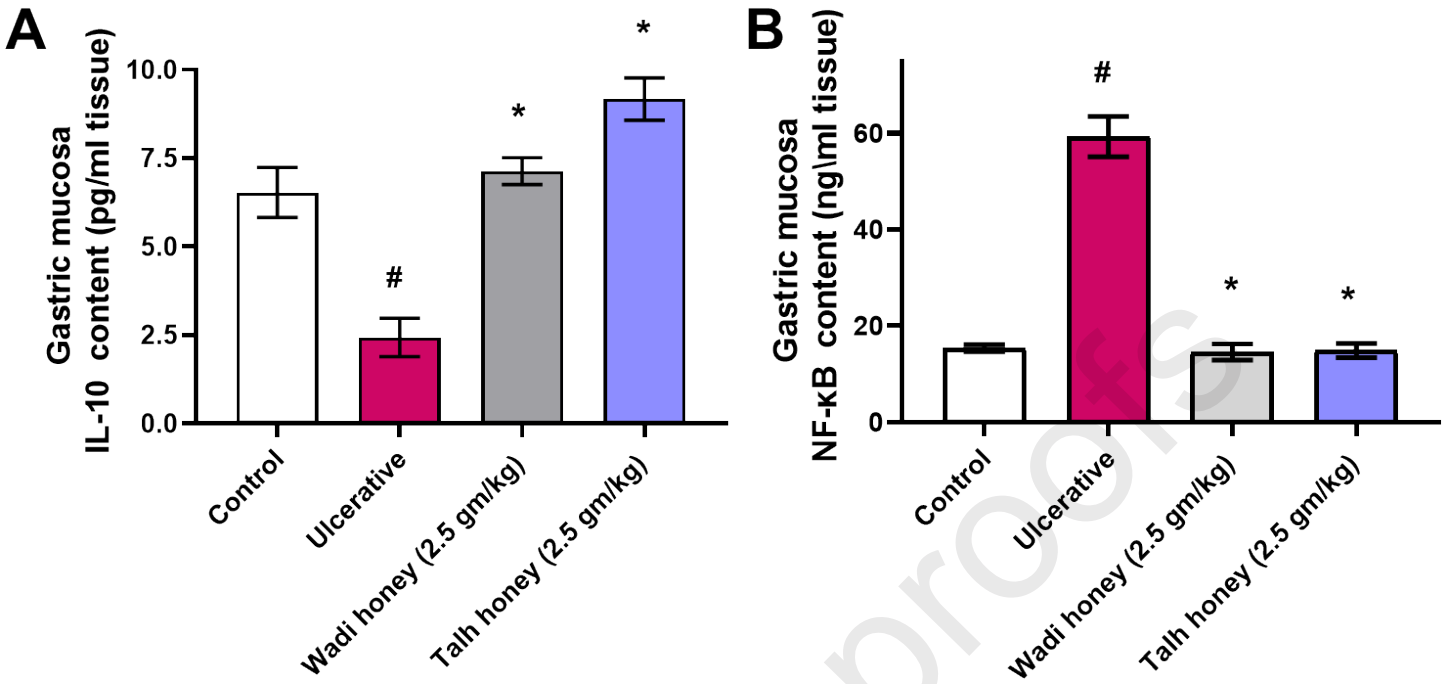


Fig 2: *Effect of treatment with 2.5 gm/kg* Saudi Honey *(Wadi or Talh) on gastric mucosal* A. IL-10 content and **B.** NF-κB level. Each value is the mean ± SEM (n = 6). #Significant versus control ($P \leq 0.05$). ∗Significant versus indomethacin (ulcer) ($P \leq 0.05$).

Histological examination (Hematoxylin and eosin sections).

In the control group, normal glandular structure of the gastric mucosa; the base and the upper parts was observed **(Fig. 3a)**. The basal part revealed many of normal chief cell's structure and few parietal cells **(Fig. 3b)**. While the upper part cells showed many of parietal cell and surface mucous cells opened into the lumen by short narrow gastric pits **(Fig. 3c)**. Loss the architecture of fundus mucosa of stomach in group received indomethacin **(Fig. 3d)** and its basal part showed disorganization, degenerated parietal cells and many dark chief cells with pycknotic nuclei **(Fig. 3e).** Dilated congested blood vessel in submucosa layer was prominent **(Fig. 3d & e).** Meanwhile the upper part exhibited severe gastric ulcer with desquamation of the lining epithelium into the lumen and some cells were shrunken with pyknotic nuclei **(Fig. 1f)**. Treatment with Wadi honey improved the effects induced by Indomethacin and the architecture returned to some extent to the normal form of gastric mucosa **(Fig. 3g)** and most of glandular cells appeared normal structure but still some areas were affected **(Figs. 3 h & i).** Examination of Talh honey group showed the same changed observed in Wadi honey group but the ameliorative effect of Talh honey against Indomethacin harmful effects less than Wadi honey **(Figs. 4a-d)**.

Histochemical examination (PAS reaction sections)

The control group showed strong positive PAS reaction in the mucous cells of the basal part and strong PAS positive of the mucus cells of apical part of the lining surface cells. **(Figs. 5a & b).** High depletion in PAS staining of the stomach mucosa of indomethacin treated group when compared to the control group **(Figs. 5c & d).** In other section, positive reaction in ruptured superficial mucus cells was observed **(Fig. 5e).** The Wadi honey group or Talh honey group exhibited positive reactions in many of the fundus mucous cells of stomach compared to indomethacin treated group**.** But the ameliorative effect of Wadi honey treatment **(Figs. 5 f & g)** was clearer than Talh honey treatment **(Figs. 5h & i).**

Immunohistochemistry examination (TNFα & Ki-67 immunoreaction)

TNF-α immunohistochemical expression

Positive TNF-α immunohistochemical staining was demonstrated as brown cytoplasmic reaction (index for inflammation). The expression of TNF-α in control group showed negative reaction **(Figs. a & b).** High positive immunoreactivity cytoplasmic reaction in basal and upper parts cells of the stomach fundus in indomethacin treated group **(Figs. 6c & d)** when compared to the control group. Wadi honey treated group revealed negative TNF-α immunoreactivity like control **(Figs. 6e & f).** In addition, Talh honey treated group exhibited mild positive TNF-α immunoreaction of basal and upper parts cells **(Figs. 6g-i).**

Ki-67 immunohistochemical reaction.

Investigation of Ki-67 immunoreaction in the control group showed a positive reaction in very few nuclear cells **(Figs. 7a & b)**. In comparison to the control group, the indomethacin treated group represented high positive Ki-67 immunoreactivity in leukocytic infiltration area in submucosa layer and in many nuclear cells of basal **(Fig. 5c)** and upper part layers was observed **(Fig. 5d).** In case of the Wadi honey treated group, few positive nuclear cells were observed in fundus of stomach **(Figs. 5e & f)** as compared to previous treated group. While in the Talh honey treated group, many positive reactions in nuclear cells were observed **(Figs. g & h).**

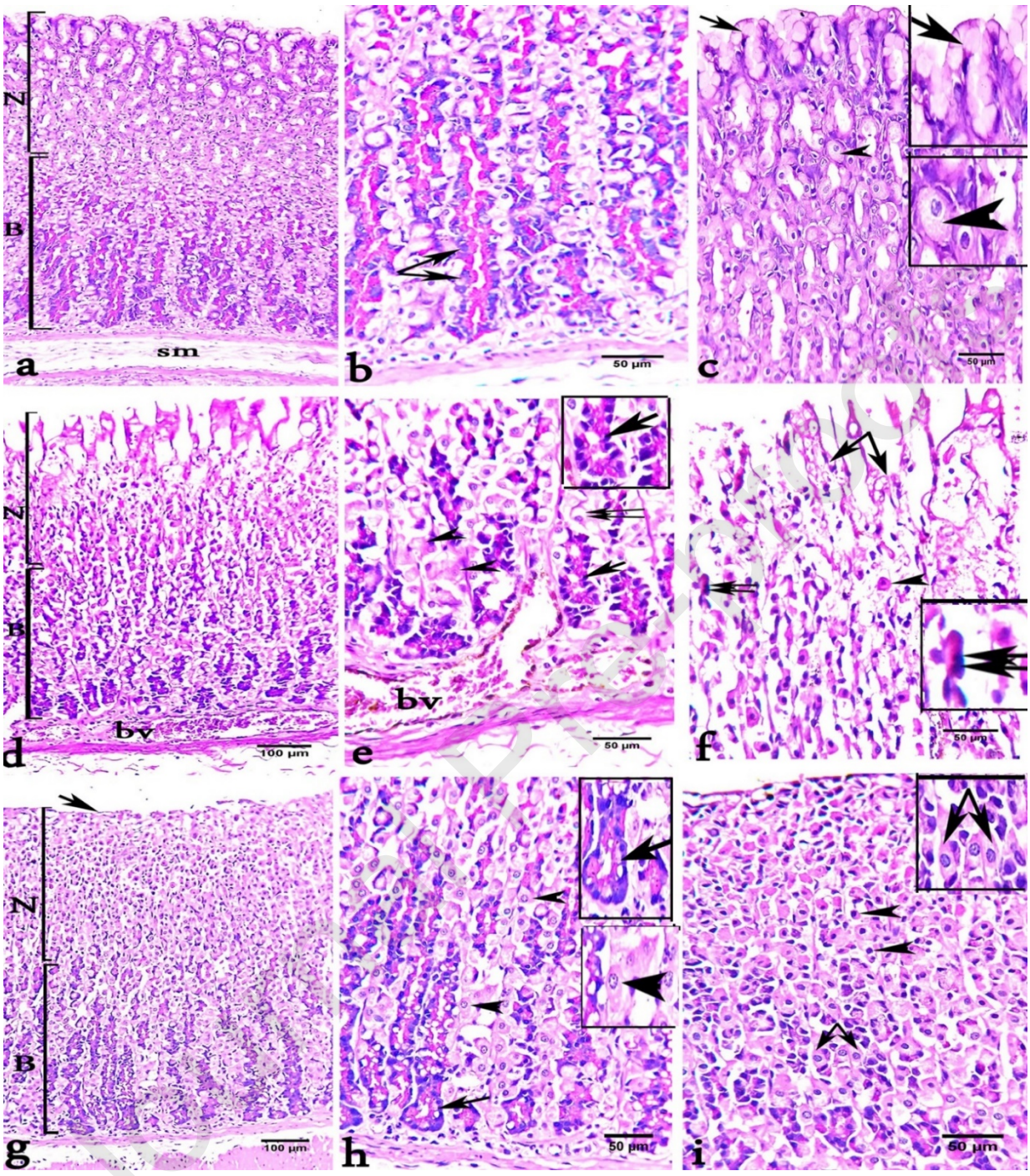


Fig 3. H&E stain a-c) Fundus of the stomach of control showing **a)** normal architecture of stomach mucosa and submucosa (sm) layers, base part (B) and upper part (N). **b)** normal structure of base layer cells showed multiple chief cells (arrows). **c)** normal structure upper part cells revealed surface mucous cells (arrow) opened into the lumen by short narrow gastric pits, and many parietal cells (arrowhead). **d-f)** group received Indomethacin exhibited gastric ulcer showed **d)** disorganization of base part (B), ulceration of upper part (N) and dilated congested blood vessel (bv) in submucosa layer. **e)** base part revealed degenerated parietal cells (arrowhead) and many dark chief cells with pycknotic nuclei (arrow). Many

of vacuolated cells (two arrows) and dilated congested blood vessel (bv) were observed. **f)** distorted the architecture of upper stomach part exhibits sever gastric ulcer (arrows) with desquamation of the lining epithelium into the lumen. Some cells are shrunken with pyknotic nuclei (two arrows) and few degenerated acidophilic cells separated from normal position (arrowhead). **g-i) Wadi honey group** showed **g)** improvement in the architecture structure to some extent to the normal form of fundus stomach, base part (B) and upper part (N). note: no desquamation of lining epithelium into the lumen. **h)** base part revealed normal structure of many cells, chief cells (arrow) and parietal cells (arrowhead). **i)** many parietal cells with normal structure (arrows) while still some cells being degenerated (arrowhead) and partly disarrangement of the upper part.

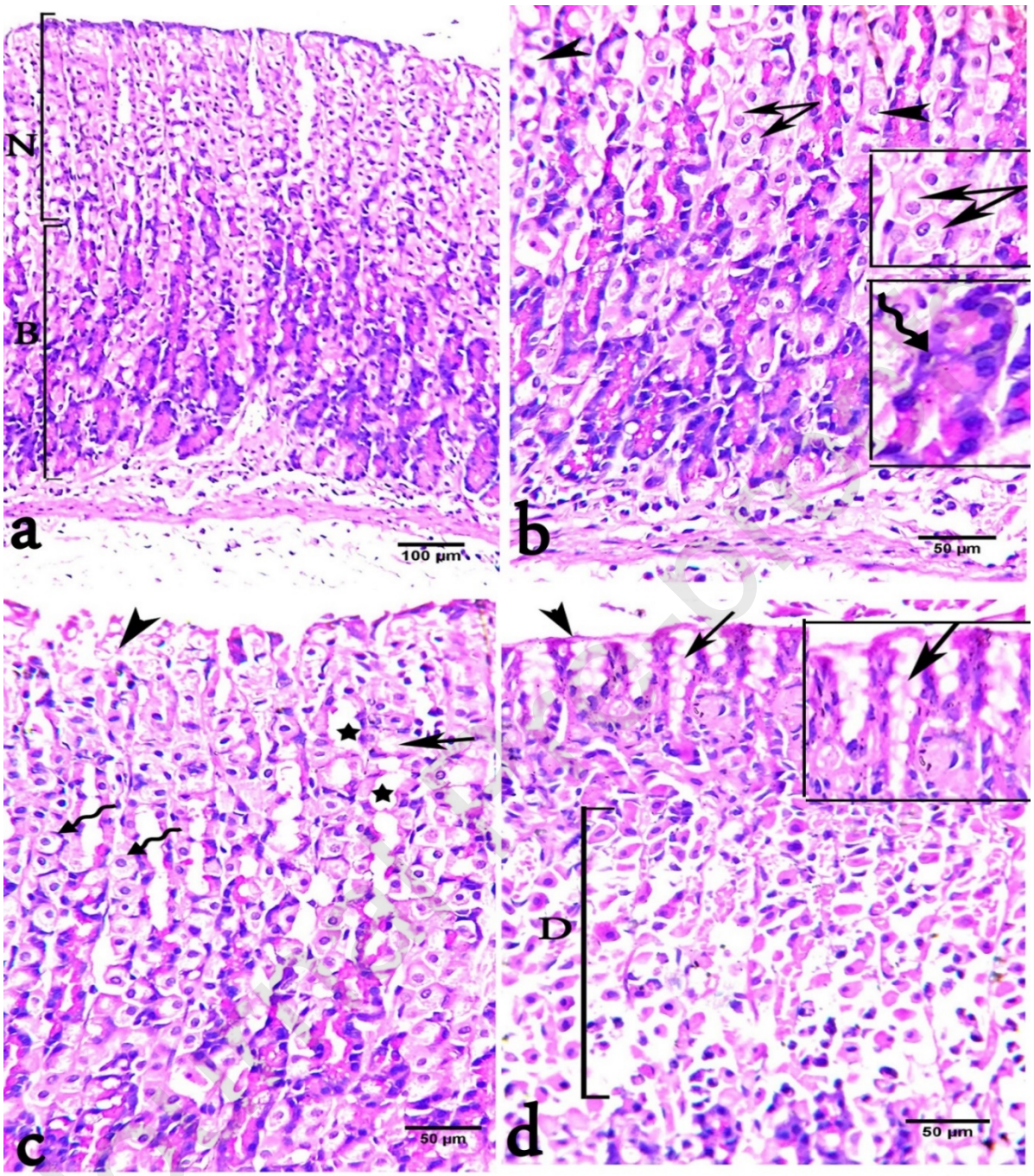
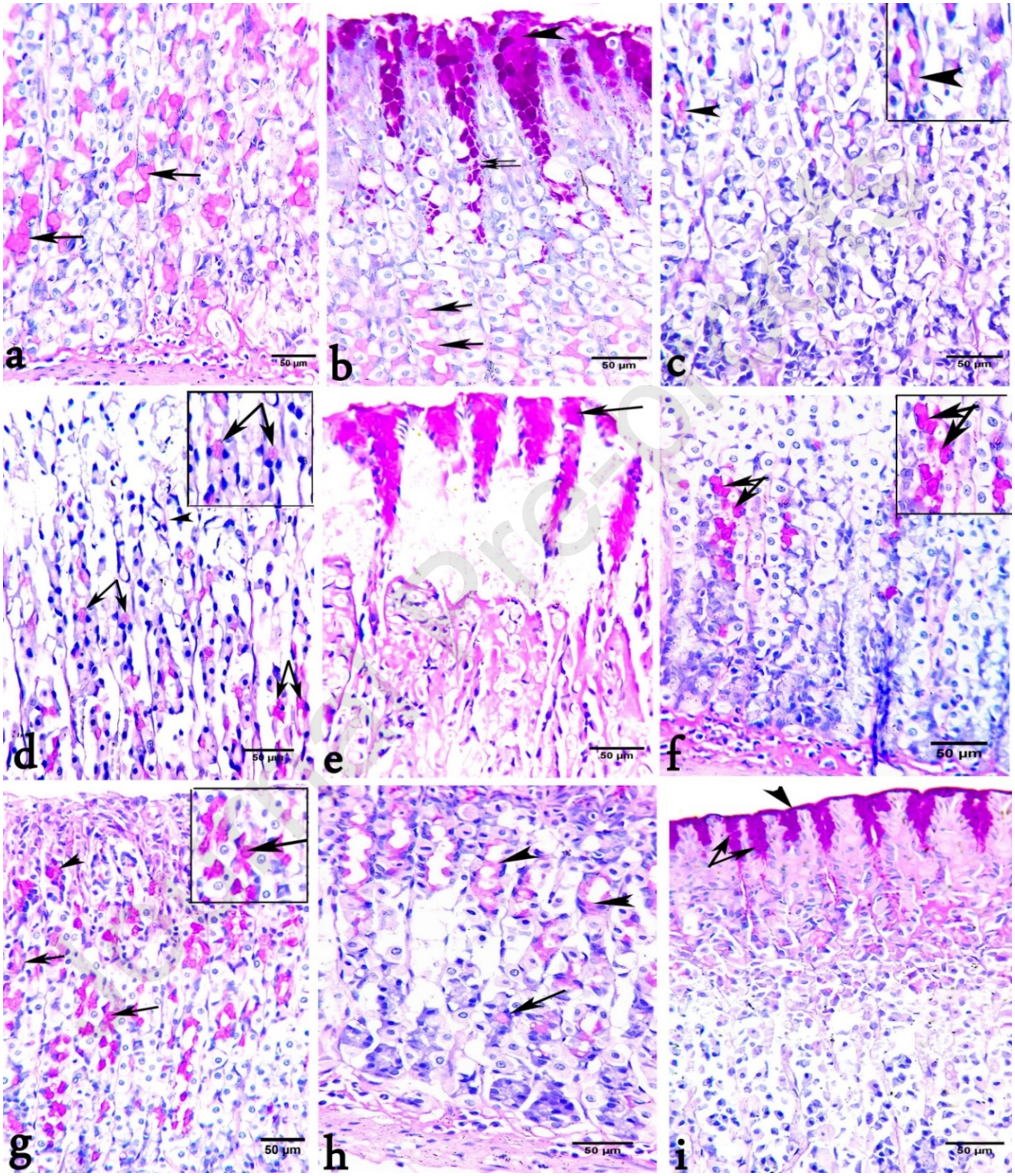


Fig 4. H&E stain a-d) Talh honey group showed improvement of fundus stomach architecture in

base part (B) and reduced the ulceration in the upper part (N). **b)** base part: normal structure of most base layer cells; chief cells (zigzag arrow) and parietal cells (arrows)**.** Some cells still affected (arrowhead). **c)** upper part: exhibits minute gastric ulcer, widening of gastric glands (stars), damage of



some cells (arrow) and many parietal cells appeared normal (zigzag arrow). d). Other sections of upper part layer showed normal mucosal cells (arrow) covered with mucous secretion (arrowhead) of superficial layer of the gastric mucosa, while (D) revealed destruction of most glandular cells.

Fig 5. PAS **stain a&b)** control group showed **a)** strong positive reaction of many base part mucous cells (arrows) and also **b)** positive PAS stain noted as a bright-magenta color to the mucus cells lining the gastric pits (arrowhead), lower part glandular cells (two arrows) and cells of basal part (arrows). **c-e)** depletion of PAS reaction was marked in most areas of basal and upper part cells compared to control. **c)** very few cells with positive PAS reaction (arrowhead). **d)** some cells with positive PAS reaction (arrows) while others with negative PAS reaction (arrowhead) in most affected areas. **e)** other section of stomach fundus upper part showed strong positive reaction in ruptured superficial epithelial layer (arrow). **f & g)** Wadi honey group showed many mucous cells in base and upper part with strong positive PAS reaction (arrows and arrowhead). **h & i)** Talh honey group showed strong positive reaction in many mucous cells in **h)** basal part (arrow and arrowheads) and **i)** upper mucosal cells (arrows) which covered with mucosal secretion (arrowhead).

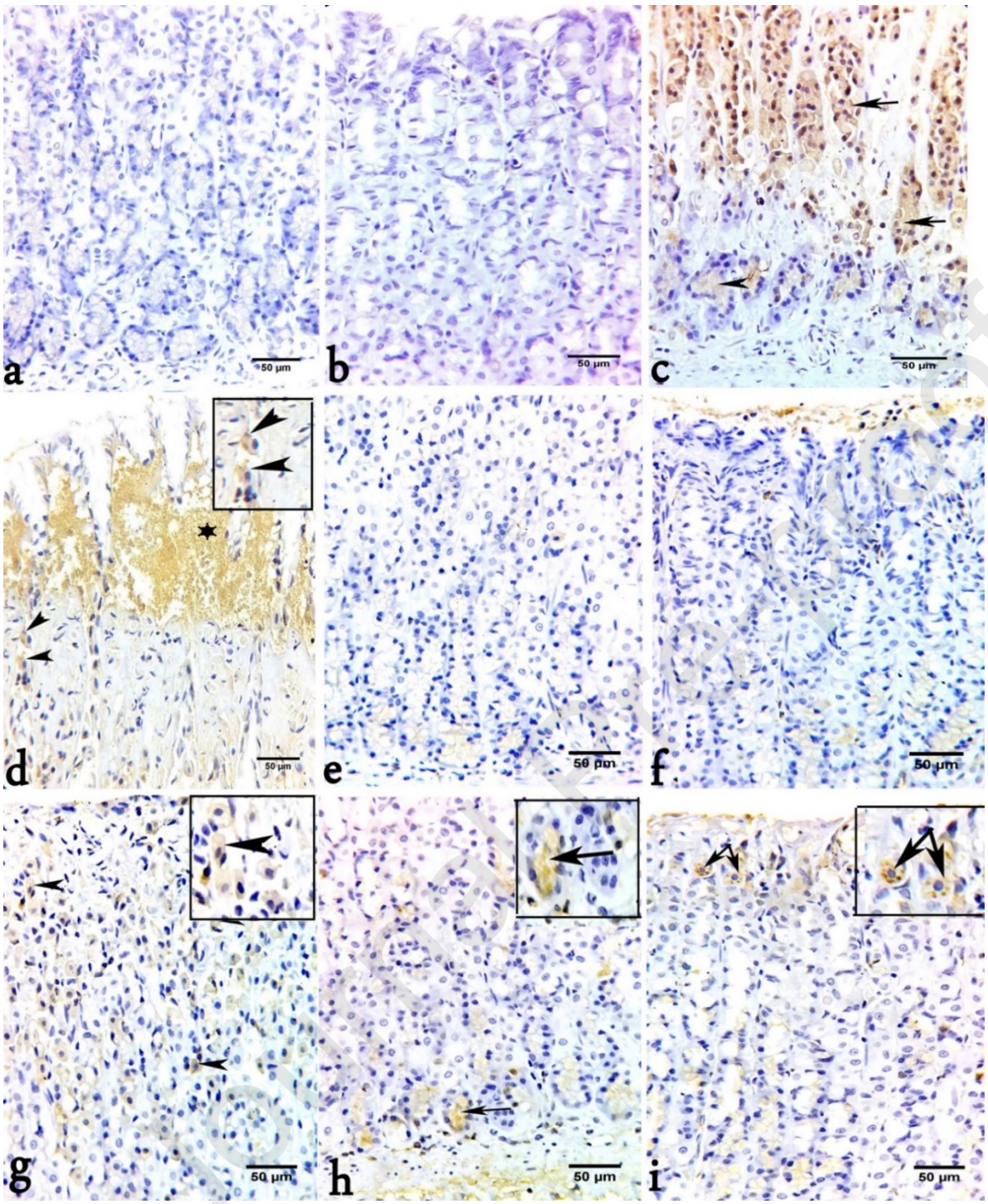


Fig 6. Immunohistochemical staining of TNF-α reaction ((brown cytoplasmic reaction): a & b) control group: negative TNF-α immunoreaction of **a)** the base part and **b)** the upper part of gastric mucosa. **c & d)** group received Indomethacin: **c)** highly positive TNF-α immunoreaction in most chief

cells (arrows) and weak positive in few chief cells (arrowhead) while **d)** many cells revealed positive immunoreaction (arrowheads) and brown color stain in destructed superficial mucosal cells pits were observes (star). **e & f)** Wadi honey group showed negative immunoreaction in gastic mucosa as control group. **g-i)** Talh honey group showed negative immunoreactivity in most areas in upper and base part of stomach fundus but some cells with positive reaction (arrowheads, arrow& arrows).

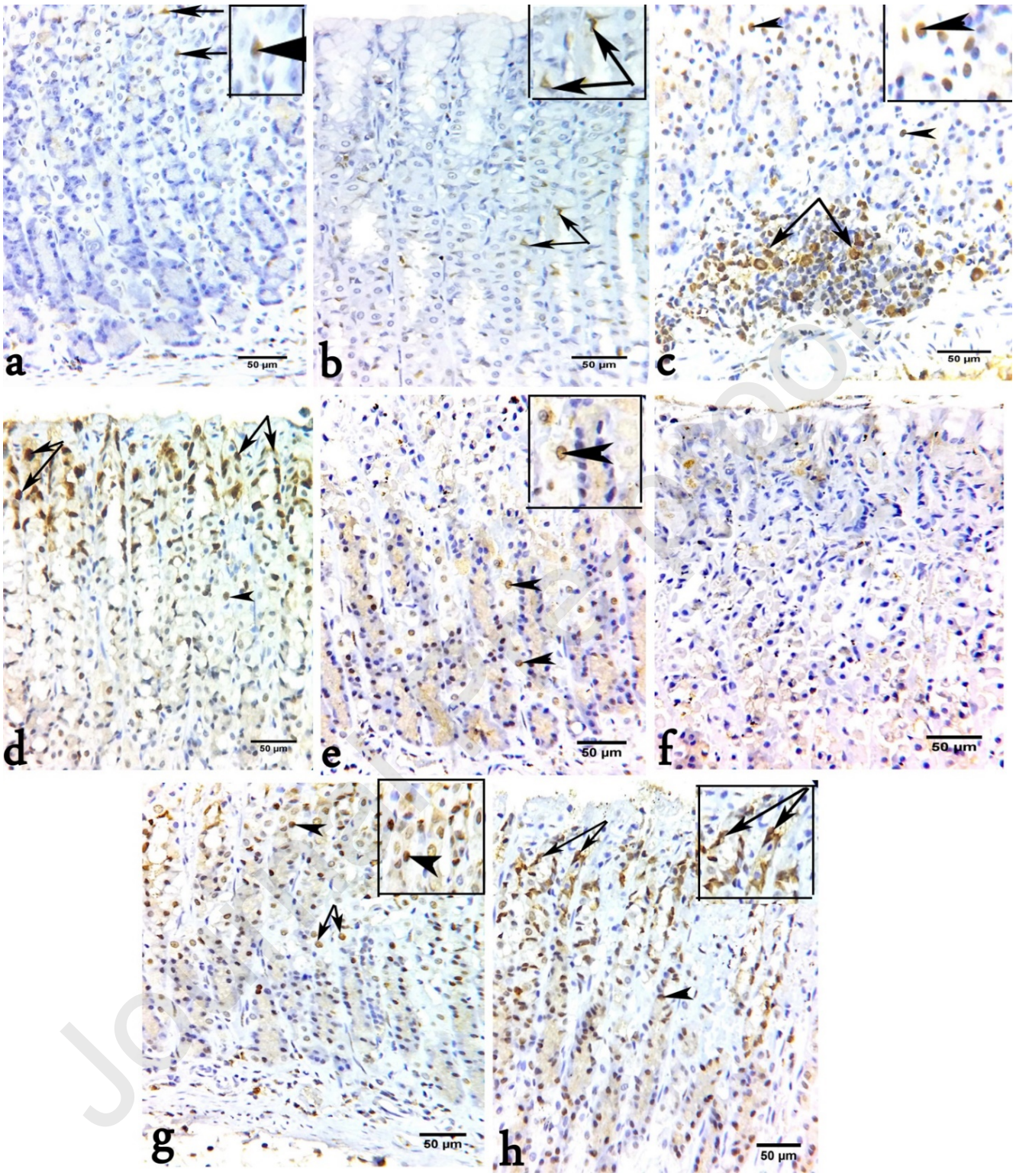


Fig 7. Immunohistochemical staining of Ki67 expression: a&b) control group: immuno-positive reaction in very few nuclear cells (arrows) of **a**) base part layer and **b**) upper part layer. **c & d)** group received Indomethacin showing c) strong immunoreactivity of nuclear leukocytic infiltrated area in sub mucosa layer (arrows), nuclear cells of most basal part (arrowhead) and d) most of nuclear mucosal (arrows) and parietal (arrowhead) cells of the upper part. **e & f**) Wadi honey group showed e) the basal

part revealed few immuno-positive reactions of nuclear cells (arrowheads) while **f**) the upper part revealed negative nuclear cell reaction. **g-h**). Talh honey group showed **g**) some positive immunoreaction of basal part nuclear cells (arrows and arrowhead) and in **h**) the upper layer (arrowhead & arrows).

Table (5): Histological, histochemical and immunohistochemical finding and their score among various study groups.

		Treated groups			
Examination	***Finding**	**Control group**	**Indomethacin group**	**Wadi honey** group	**Talh honey** group
Histology (H&E stain)	Congestion of blood vessels	-	++	-	-
	Distortion of architecture	-	+++	+	+
	cells degeneration	-	+++	+	++
	Pyknotic nuclei	-	+++	+	+
	Gastric ulcer	-	+++	+	+
Histochemistry (PAS stain)	PAS reactivity	+++	+	++	++
Immunohistochemistry	TNFα expression	-	+++	-	+
	Ki-67 reaction	+	+++	+	++

***Findings were scored based on their severity as (-) none, (+) mild, (++) moderate, (+++) severe

Discussion

Indomethacin, which is one of the non-steroidal drugs prescribed as an analgesic and anti-inflammatory medication have been known to cause gastrointestinal lesions as a side effect and it's ulcerogenic potential is higher than other no-steroidal drugs. It is characterized by having an inhibitory action on the synthesis of prostaglandins, together with free radicals are a critical step in the pathogenesis of gastric ulceration (Sabiu et al., 2016). Rats with gastric ulceration induced by indomethacin has been used as a useful model for testing new drugs for treatment of ulcers. Owing to the unfavorable side effects and the high cost of synthetic drugs, taking advantages of nontoxic affordable natural products like honey are believed to be the most appropriate treatment of gastric ulcers (Simões et al., 2019). Our laboratory has previously demonstrated that Manuka honey has a significant protective effect against acute ulceration in ulcerative rats. Consequently, we extended this observation to investigate the potency of two types of Saudi honey (Wadi and Talh) in protecting against gastric ulcer induced by indomethacin.

In this study, a single dose of Indomethacin (30 mg/kg) was used to induce the ulcer in rats and our findings agreed with previous reports which showed that indomethacin induce the ulcer by increasing the gastric acidity. This data revealed the benefits of honey in the treatment of gastric ulcer induced by indomethacin. In corroboration of previous reports, our study confirmed that 7 weeks feeding of honey (2.5 g/kg) yielded improved outcomes in the treatment of gastric ulcer with no significant alteration in the percentage of BWG, when compared to rats fed a sugar-free diet. This, therefore, supports the role of honey in maintaining the physiological weight balance in treated animals (Chepulis and Starkey, 2008). In addition, it is safe and non-toxic to the animals as no mortality was detected during the study at the dose used (Fazalda et al., 2018).

Previous studies have also reported that the levels of free radicals, lipid peroxidation and activities of antioxidant enzymes including: SOD, catalase, and GPx is related to gastric mucosal damage induced

by indomethacin (Eteraf-Oskouei and Najafi, 2013). Nevertheless, reactive oxygen and nitrogen species (ROS and RON) produced after treatment of indomethacin alters the activities of cellular antioxidant enzymes that serve as the first line of defense against oxidative stress. This study revealed that there was a significantly higher level of MDA and a decrease in the activities of GSH, SOD, and catalase in the ulcerative group when compared with the control group. Conversely, the levels of antioxidant enzymes such as GSH, SOD, catalase and lipid peroxidation by-product MDA were significantly lower after administration of honey as compared to the ulcerative group. These findings are in line with previous reports highlighting the beneficial role of honey in ameliorating lipid peroxidation and scavenging free radicals implicated in oxidative stress typified in the pathogenesis of gastric ulcer (Almasaudi et al., 2017).

Proinflammatory cytokines have been shown to play critical roles in the formation of gastric ulcers, as TNF-α essentially modulates gastric mucosal apoptotic cell death (Du et al., 2013). The treatment of animals with either Wadi or Talh honey significantly reduced the levels of gastric mucosal TNF-α, and CRP, which are pro-inflammatory mediators actively contributing to intestinal damage observed in gastric ulcer. Also, the anti-inflammatory cytokine; IL-10 help in reducing the tissue damage induced by inflammation. Interestingly, we uncovered a significant decrease in IL-10 levels of the ulcerative rats, however, this was reversed in the treatment group following treatment with either Wadi or Talh honey. This observation was in line with the report of Almasaudi et al., (Almasaudi et al., 2017) and further confirms the gastroprotective effect of either Wadi or Talh honey which is mediated via the inhibition of TNF-α, CRP content, and IL-10 (Angioi et al., 2021; Du et al., 2013). The observation of this study corroborates preceding reports highlighting the healing properties of honey, and this is the first report on the beneficial effects of the administration of Saudi honey (Wadi and Talh) in protecting against indomethacin-induced gastric ulceration in rats.

Conclusion

In conclusion, the mechanism by which Wadi and Talh honey prevents indomethacin-induced gastric ulcer may be mediated via improving the homeostasis of the antioxidants enzymes (SOD and GPx) and nonenzymatic GSH antioxidant, inhibiting MDA, and decreasing the formation of inflammatory cytokine (TNF-α, CRP content, and IL-10). Consequently, Wadi and Talh honey may be a beneficial therapy for patients diagnosed with gastric ulceration.

Conflict of Interests

The authors confirm that there is no conflicting interest among them.

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Conflict of Interest Statement

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Saudi honey alleviates indomethacin-induced gastric ulcer via improving antioxidant and anti-inflammatory responses in male albino rats

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