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# Role of Some Types of Bee's Honey in Gastric Ulcer Healing by Regulating HSP47 and VEGF Expression

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**Abstract**

Healing of gastric ulcer is a regenerative process including cell migration, proliferation, re-epithelialization, angiogenesis, and matrix deposition. Anti-ulcer potentials of some natural products were investigated as alternative drugs probably due to their safety and effectiveness. This study aimed to investigate the gastroprotective, antiulcerogenic and ulcer healing activities of Clover, Sidr and Samar honey. Physicochemical and antioxidant activity of honey were investigated. Six groups of albino rats were used in this study (control, ethanol ulcerated , recovery, ulcerated rats + clover honey , ulcerated rats + sidr honey and ulcerated rats + samar honey). In vivo studies including histopathological examinations, detection of collagen deposition, histochemical demonstrations with PAS and Perl's Prussian blue stains, immunohistochemical demonstration of VEGF, HSP47 and iNOS, and biochemical investigations of gastric MPO and PEG2 and gastric juice were performed. In vitro Wound scratch and *anti-H. pylori* assays were done. Ethanol-ulcerated rats cotreated with Clover, Sidr or Samar honey achieved gastroprotection, antiulcerogenic, and ulcer healing activities when compared with ethanol and recovery groups. Samar honey achieved the best results by comparing with clover and sidr honey. The gastric injury score decreased, collagen deposition increased. Histochemical demonstration showed improvement of PAS-positive materials, and reduction in hemosiderin deposits. iNOS immunoexpression decreased significantly , while VEGF and HSP47 correlated strongly with the healing status. MPO and PGE2 levels, mucus contents, gastric juice volume, PH and acidity were improved. Honey accelerates cell migration in wound scratch assay and showed anti-*H. pylori* activity.

Keywords: HSP47 ;VEGF ; iNOS ; Samar honey ;Sidr honey ; Clover honey ; Wound Healing

## 1. Introduction

Stomach ulcers are common gastrointestinal disorders characterized by an interruption of the mucosal integrity, degeneration and erosions of the gastric mucosa that extends towards the muscularis mucosa and may penetrates submucosa. Different factors cause ulceration including malnutrition, *Helicobacter (H.) pylori* infection, alcohol consumption, drugs, stress, and burns. Gastric ulcers can result in major complications such as bleeding, perforation and finally death [1,2]. The aetiology of stomach ulcers has been linked to a number of factors such as inhibition of cell proliferation, oxidative stress, neutrophil infiltration, inflammatory agent overexpression, and the production of pro-inflammatory cytokines [3]. Furthermore, gastric ulcer pathogenicity is associated mainly with the imbalance of the homeostasis between the defensive factors (cellular regeneration, cell shedding, growth factors and the production of mucin, bicarbonate, prostaglandin and nitric oxide) and the offensive aggressive factors (infection with *H. pylori* and increased secretion of pepsin and gastric acids). Gastric ulcer may be also related to the disturbances in the microcirculation leading to ischemia, loss or reduction in blood and oxygen supply into the gastric mucosa [3,4]. Healing of gastric ulcer is a complex tissue regenerative process of gastric tissue including cell migration, proliferation, re-epithelialization with reconstitution of underlying connective tissue, including vessels and muscle layers, gland reconstruction, angiogenesis, vasculogenesis and matrix deposition, all finally resulting in scar formation [5]. Different growth factors have been involved in the ulcer healing process, such as vascular endothelial growth factor (VEGF), the

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platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) [6,7]. HSP47 is a specific glycoprotein that specifically binds collagen and localizes to the endoplasmic reticulum (ER) of collagen-producing cells and it is essential for the modification of procollagen and maturation of collagen. HSP47 concentrates the premature procollagens within the ER and prevents their secretion from the ER into the Golgi apparatus [8]. However, the current therapies of gastric ulcer have adverse effects and high recurrence rate of ulcer. Anti-ulcer potentials of some botanicals like plants, herbs, fruits, dietary antioxidants of natural products and vegetables are investigated as alternative drugs probably due to their safety and effectiveness as a result of notable inherent active phytochemicals (such as flavonoids, terpenoids, and saponins) as well as their easy availability and low cost [4,5]. Honey is a natural sweet material produced by Apis mellifera bees from the plants nectar. There are different types of honey (e.g., Manuka, Pasture, Jelly bush, Sidr [Ziziphus spina-christi], Sumra, and Jungle) which depends on the different proportions of possible nectar sources [9,10]. Honey is utilised not only as a food, but also as a traditional medicine, and its phytochemical, anti-inflammatory, antibacterial, and antioxidant qualities may have therapeutic value. Honey is used as a medication to treat hepatic and gastric disorders, pulmonary infections, digestion problems, constipation, infections following burns, wounds and surgery [10,11]. Honey has long been used to treat wounds and/or skin illnesses (such as eczema, dermatitis, burns, ulcers, and Fournier gangrene) due to its antibacterial, immunostimulatory, and inflammatory properties [12]. In this context, this study aimed to investigate the gastroprotective, antiulcerogenic and ulcer healing activities of three types of honey namely Clover, Sidr and Samar honey, in an attempt to clarify a novel mechanism of gastric ulcer healing.

## 2.Materials and Methods

### 2.1. Materials

**a. Honeys:**

Three types of pure, unprocessed, non-boiled commercial honey namely (Clover, Sidr and Samar) were used in the current study. Clover honey was collected in brown bottles after ripening in May 2019 from Faculty of Agriculture, Minya University, Egypt. Sidr and Samar honey were offered from Bin Tairan Bees Market, Al-Baha, Kingdom of Saudi Arabia. Samples were labelled and stored in a dark room at (25 ± 5 °C) until further use.

**b. Ethanol:** Absolute ethanol was obtained from Sigma (St. Louis, MO, USA).

### 2.2. Physicochemical Analysis and Antioxidant Activity of Honey:

Honey samples were investigated to determine each of PH, moisture content, reducing sugar, hydroxymethyl furfural content (HMF), total soluble solids (TSS) and sucrose content [13 & 14]. Glucose and fructose analysis were determined by HPLC according to Aljohar et al. [15]. The total phenolic content was measured according to Velioglu et al. [16], while total flavonoid content was measured according to Chang et al. [17]. Antioxidants DPPH assay was quantified as described by Chizzola et al., [18]. Data of all honey samples were evaluated and compared within the scope of the international honey standards of GSO [19] and the Egyptian standard [20]. Analysis of each sample was carried out in triplicate for each test.

**Preparation of Honey solution:** For *in vivo* studies, Fresh 10% w/v solution was prepared with distilled water and used as concentration (150 mg/kg/day) of honey solution for two weeks according to Mahdy et al. [21].

### 2.3. Animals

A total of 42 healthy adult male albino rats weighing 162 ±7 g were used. Rats were purchased from the National Research Centre (Giza, Egypt) and were kept under ideal sanitary conditions. Rats were fed *ad libitum* and allowed a free supply of water. All rats were left for one week before the experimentation for optimization and to exclude any infection.

**Induction of gastric ulcer**

All rats, except those of the control group, were fasted for 18 hours and were allowed only to free access of water prior to the administration of ethanol. On the second day of starvation, gastric ulcer was induced by administering intra-gastric 100% ethanol (1 ml/200 g) as a single oral dose [22].

**2.4. Experimental Design:** Animals were classified into six groups, seven rats of each as follows:

1. **Control Group:** Rats of this group were left without any treatments for two weeks.
2. **Ethanol-ulcerated group:** Rats received 100% ethanol (1 ml/200 g) orally by gastric

tube. Rats of this group were sacrificed one hour after administration of ethanol.

3. **Recovery group:** Ethanol-treated rats were left without treatment for two weeks to investigate the normal recovery, and this group was used a standard control group to compared with honey treated groups.
4. **Clover honey group:** Ethanol-ulcerated rats were treated with Clover honey solution (150 mg/kg/daily for two weeks) [21].
5. **Sidr honey group:** Ethanol-ulcerated rats were treated with Sidr honey solution (150 mg/kg/daily for two weeks).
6. **Samar honey group:** Ethanol-ulcerated rats were treated with Samar honey solution (150 mg/kg/daily for two weeks).

**2.5. Methods:**

**2.5.1. Biochemical Investigations:**

1. **Measurement of gastric juice volume PH and total acidity:**

Stomach's contents of gastric juice were collected and centrifuged at 4000 rpm, 25°C for 10 min. The total acidity of the extracted gastric acid was assessed via titration with 0.1 N NaOH solutions, then, measured with a digital pH meter and expressed as meq/l. [23]. The supernatant volume of gastric juice was measured with a graduated cylinder and expressed in ml.

## 2. Measurement of gastric mucus contents:

The mucosa of stomach was scraped gently using histological section slides, then the mucus was collected and weighed using electronic balance **[24].**

**3. Measurement of gastric PEG2 and MPO levels:**

Gastric tissues were homogenized, and the clear supernatant was separated to investigate prostaglandin-E2 (PGE2) using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Myeloperoxidase (MPO) was assessed spectrophotometrically using commercial kits (BioDiagnostic, Egypt).

**2.5.2. Histological studies:**

Small specimens of the stomachs of rats from different experimental groups, were fixed in Bouin solution for about 24 h, then washed, dehydrated, cleared in xylene, and impregnated in parablast paraffin wax for blocking. Five µm thick sections were prepared using rotary microtome, and then section stained by:

1. Hematoxylin and Eosin (H&E) stain for microscopic examination of gastric injury and for any regenerative process [25].
2. Masson trichrome stain to evaluate collagen fibers accumulation [25].

**2.5.3. Histochemical studies:** For histochemical demonstrations, sections were stained by:

**1. Periodic acid Schiff's (PAS) stain:** To demonstrate mucosal glycoprotein production **[25].**

**2. Perl's Prussian Blue (PPB) stain:** To detect "non-hem" iron in tissues such as hemosiderin **[26].**

**2.5.4. Immunohistochemical Studies**

Avidin-biotin peroxidase complex method was used in immunohistochemical examinations by following the manufacturer's instructions. Briefly, paraffin sections of 4-µm thickness were deparaffinized on charged slides, cleared with xylene, rehydrated in a gradient ethanol series, and washed by distilled water. Then, sections were heated in a 10% citrate buffer solution, and allowed to cool at room temperature for 20 mn. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and proteins Block Serum-Free (Dako, Japan) was applied to prevent nonspecific protein binding. Microwave-assisted antigen retrieval was performed for 20 min. Sections were incubated overnight at 4ºC with the primary antibody of:

1. **Inducible Nitric Oxide Synthase (iNOS):** Sections were incubated with rabbit anti-iNOS polyclonal antibody (Invitrogen, PA1-036, USA).
2. **Vascular endothelial growth factor (VEGF) (index for angiogenesis):** The primary monoclonal antibody used was the rabbit anti VEGF (Santa Cruz Company, California, USA). The cellular site of the reaction was cytoplasmic brown in color.
3. **Heat Shock Protein-47 (HSP47):** Sections were incubated with rabbit anti-HSP47 (Biotechnologies Corp, Canada).

Then , sections were washed in PBS for five min, incubated with ready to use. Biotinylated Goat-anti-rabbit immunoglobulin secondary antibody (#BP-9100; undiluted; Vector Laboratories, Burlingame, CA, USA) for 20 min at 30˚C, and finally washed in PBS for 5 min and incubated with the peroxidase detection system Ready to Use conjugated antibody (#RE7110-K; Novocastra; Leica Microsystems, Inc.) for 20 min. Sections then were

rinsed PBS for 5 min, incubated with chromogenic 3,3'-diaminobenzidine (DAB) (Leica Microsystems, Inc.) for 5 min, washed with tap water and counterstained with hematoxylin; all steps were completed at room temperature **[27].**

**2.5.5. Morphometric Assessments and Image Analyses:**

1. The detection of microscopic gastric injury was evaluated according to Ortaç et al. [28]. Five non-overlapping randomly selected fields from each slide (x10) were investigated to evaluate the gastric histomorphometric parameters then the average was recorded. Gastric structural derangement was determined on a 0–4 scale, and each tissue section was examined for haemorrhagic damage (0–4), mucosal oedema (0–4), presence of inflammatory cells infiltration (0–3), and epithelial cell loss (0–3).
2. Morphometric analysis of images obtained from different slides was done using image analyser software (Image- Pro Plus program ;version 6.0; Media Cybernetics Inc., Bethesda, Maryland, USA). Five fields (10x) were measured from each sections of all groups and their mean was taken to evaluate each of the following:

1. Area percentage (%) for collagen fibres deposition in gastric tissues.
2. Area percentage (%) for PAS +ve reaction.
3. Area percentage (%) for Pearl +ve reaction.
4. Area percentage (%) for iNOS positive reaction.
5. Area percentage (%) for VEGF positive reaction.
6. Area percentage (%) for HSP47 positive reaction.

**2.6. *In Vitro* studies**

**2.6.1. Wound Scratch Assay:**

To determine the effect of honey samples on wound healing, a scratch-wounded BJ.1 dermal fibroblast monolayer model was employed as migration rates of cells were assessed by the scratch assay method by Sato and Rifkin [29]. Data were recorded and analyzed statistically using the following formula:

*Wound closure (%) = [(Measurement at 0 h −Measurement at 24 h)/ Measurement at 0 h] X 100.*

**2.6.2 .Anti-*H. pylori Assay*:**

Antibacterial activity of tested honey samples against *H. pylori* was determined by a micro-well dilution method [30]. The minimum concentration of honey samples (inhibitors) required for inhibition of 90% of *H. pylori* growth (The minimal inhibitory concentration (MIC 90) was determined from corresponding dose-response curves (fig 9). The MIC90 values were measured in triplicate. The inhibition percentage was calculated using the formula:

***[% inhibition = (Abs Control – Abs Sample /Abs Control) × 100]***

**2.7.Statistical Analysis**

Results were presented as mean ± standard division (SD), and all statistical comparisons were analyzed by means of one-way ANOVA test followed by Post hoc analysis. A ($P < 0.01$) value was considered as significant difference.

## 3. Results and Discussion

**3.1. Physiochemical and Antioxidant Activity of Different Honey Samples:**

Results shown in table (1) revealed that all types of honey samples were of the best quality and met the international quality parameters. HMF is an excellent indicator for honey purity and freshness as it is present in trace amounts in fresh honey, increased content of HMF in honey indicates poor storage conditions and/or overheating[31]. HMF is affected by different factors such as sucrose content, temperature, pH, floral source, type of sugars, and fructose/glucose ratio [31,32]. The average of HMF content of clover honey was significantly ($P < 0.01$) lower than both Samar and Sidr honey and it was within the limits prescribed by Egyptian standard (2005) and GSO (2014).

During honey storage, moisture content affects significantly the shelf life of honey and can results in unfavorable honey fermentation [33]. Our results showed that the moisture values of Samar and Sidr honey ,were significantly ($P < 0.01$) lower than those of clover honey, and it was within the limit recommended by Egyptian standard and GSO [19,20].These differences could be attributed to a variety of factors, including degree of maturity in hive, harvesting season, environmental condition and manipulation by beekeepers during harvest period [3].As shown in table (1), the TSS content percentage in Clover, Samar and Sidr honeys did not differ significantly ($P < 0.01$). Noticeably, there was an inverse relationship between moisture content and TSS in all samples. Our results agreed with Roby et al. [35] who found that TSS in Egyptian clover honey was 80 % , and with Abdulaziz et al. [36] who found that the TSS values of Egyptian honey was about 77.5%. The pH of honey is related to its

storage stability and microorganism's growth [37]. The current findings revealed acidic characters of all honey samples with values ranging from 3.30 to 5.22, as clover honey was significantly ($P < 0.01$) less than those found in Samar honey and Sidr honey. The high acidity of honey could be attributed to the fermentation of sugar into organic acids, in addition to the presence of inorganic ions such as phosphate and chloride ions which are responsible for the stability of honey's against microbial spoilage, while variation in among the honey samples could be returned to the plant floral types [32]. Our findings revealed that no significant difference ($P < 0.01$) were observed In TSS% and total reducing sugar and glucose contents between Samar , Sidr and clover honeys. Clover honey was significantly ($P < 0.01$) higher than Samar and Sidr in sucrose content. All samples had sucrose levels below the maximum allowable limit of 5% presented by Egyptian standard (2005) and GSO (2014). Data indicated that glucose content in the investigated honeys showed no significant difference ($P < 0.01$) between them. On the other hand , Samar honey was significantly ($P < 0.01$) higher than clover and Sidr ($P < 0.05$) in fructose content .

The results of antioxidant activity of clover , Sidr and Samar honeys are portrayed in table (2). It might be deduced that the total flavonoids, total phenolic substances and DPPH% were significantly ($P <0.01$) higher in Samar honey when compared with Sidr and Clover honeys. The obtained results elucidate positive correlation between the honey color and its antioxidant activity as the darker honey contains more Flavonoids, phenolic compounds and DPPH% activity than the lighter ones. Our results agreed with those presented by Smetanska et al.,[32] and Ferreira et al., [38]. El-Borai et al., [39] suggested that high radical scavenging activity of honey is due to its high phenolic and flavonoid contents, thus indicating high antioxidant potential.

### 3.2. Biochemical and Physiological Results:

Comparing data obtained from ethanol treated group with those of control group (table 3) revealed a significant increase of gastric MPO, and significant decrease of gastric PEG2 and mucus contents. Our finding is in agreement with previous studies reported that ethanol causes marked increase of MPO while it reducing PEG2 levels and mucus contents [3,40,41]. PGE2 is an important mucosal protective factor and plays an essential role in preserving the mucosal integrity, which is reflected in the increase in blood supply, and in balancing between protective and destructive factors in the gastric tissue [40]. Concerning gastric juice investigations, ethanol induced a significant increase in gastric juice volume, decreased PH value with significant increase in total acidity. These results were in compatible and confirming the findings of some investigators who reported that ethanol-induced ulcer evoked an increase in gastric juice volume, free acidity and total acidity, while it reduced its PH [42-44]. Recovery group showed significant improvement in all gastric physiological parameters when compared with ethanol treated group. Treatment of ulcerated rats with clover, Sidr or Samar honey caused significant improvement of physiological parameters when compared with ethanol treated group and recovery group. However, by comparison with recovery group, ulcerated rats treated with clover honey showed significant decrease in MPO and total acidity values, while there was insignificant difference in all other parameters. Samar honey showed the best results in almost all physiological parameters, as there was insignificant difference when compared with control group. Honey has been reported to induce reduction in MPO, and upregulation of PGE2 [12,45,46]. Normal gastric secretion and acidity are required for appropriate digestion and to prevent bacterial overgrowth and enteric infections, whereas increased secretion and acidity results in gastric ulcerogenesis. Compounds that limit stomach secretion and lower acidity, have potent gastroprotective and antiulcerogenic properties [3]. The efficiency of honey in increasing gastric pH could be attributed to the presence of flavonoids and phenolic compounds. According to Liu et al [40] and Mousa, et al., [43], flavonoids have a main role in the mechanism of gastroprotection by rising pH of gastric juice. Flavonoids have a great ability to decrease stomach acid production and neutralize stomach acidic environment. Taha et al., [47] reported that, Sidr honey induced protective properties against stress induced gastric ulcer in rats juice volume, total acidity and PH.

### 3.3. Histological Results

#### 3.3.1. H and E staining results:

Microscopic examination of H&E stained slides obtained from the control group revealed the normal histological structure of gastric layers (mucosa, submucosa and musculosa) (Figs. 1a & 1b). Gastric

mucosa occupied with the long tubular gastric (oxyntic) glands extend as far down as the muscularis mucosa, and lined by the tall mucus secreting columnar epithelium. The gastric gland appeared narrow, numerous, straight, perpendicular to the surface epithelium and opened onto the surface through narrow gastric pits, and they were separated from each other by lamina propria.

Gastric glands were divided into three regions, inner isthmus lined with surface mucous cells formed of simple columnar epithelium with basal oval nuclei and foamy cytoplasm, middle neck region, or the gland body, lined with surface mucous neck cells which were cubical with basal flattened nuclei and large polyhydral eosinophilic parietal cells that were the more distinctive cells. The outer basal regions lined with parietal cells and small chief cells with basal nuclei and deeply basophilic cytoplasm. Second layer is submucosa composed of loose connective tissue (CT) containing, smooth muscle fibres and blood vessels. Third layer is Muscularis composed of longitudinal and circular muscle bundles. The last layer is serosa composed of C.T and blood vessels. Contrary to the control group, ethanol administration produced gastric damage as manifested by intense cellular degenerative changes, erosion of the superficial epithelial cells, which seem to be acute ulcer that appears in some areas in the gastric mucosae, which was confirmed by morphometric assessments that revealed a significant increase in macroscopic evaluation of ulcer index in comparison with control group.

**Table (1): Physical and chemical properties of honey samples compared with some international standards (mean±SD ; n=3 ).**

| Honey samples / Parameters | Clover | Samar | Sidr | Egyptian standard 2005 | GSO 2014 |
|---|---|---|---|---|---|
| **HMF (mg/kg)** | 15.36±1.11* | 22.00±2.03 | 26.98±1.44 | ≤80 | ≤ 40 |
| **Moisture** | 18.05±0.68** | 16.11±0.32 | 14.31±0.50 | ≤ 20 | ≤ 20 ; Acacia ≤ 17 |
| **pH** | 3.30±0.07* | 4.30±0.10 | 5.22±0.03 | - | - |
| **Reducing Sugars%** | 69.04±0.64 | 76.76±2.14 | 71.96±2.96 | ≥ 65.00% | ≥ 60.00% |
| **TSS (%)** | 76.96±1.40 | 80.02±0.94 | 81.93±0.66 | - | - |
| **Glucose (%)** | 34.94±1.42 | 34.22±1.27 | 33.52±1.88 | - | - |
| **Sucrose (%)** | 4.74± 0.45** | 2.10± 0.07 | 2.88± 0.10 | ≤ 5% | ≤ 5% |
| **Fructose (%)** | 34.15±1.05 | 42.05±1.40 ** | 38.44±1.21 | - | - |

** Significant increase when compared with other types of honey ; * Significant decrease when compared with other types of honey **,( (P <0.01).**

**Table (2): Antioxidant Activity of Different Honey Samples (mean±SD ; n=3 ):**

| Honey / Parameters | Clover | Samar | Sidr |
|---|---|---|---|
| **Total Flavonoids (mg Catechin /100 g)** | 5.04±0.18 | 13.07±1.00** | 8.59±0.62 |
| **Total phenolic (mg GAE/100 g)** | 25.70±4.82 | 69.63±2.75 ** | 51.23±1.82 |
| **DPPH%** | 38.48±0.65 | 64.57±1.19 ** | 56.29±1.03 |

** Significant increase when compared with other types of honey ; * Significant decrease when compared with other types of honey , **( (P <0.01).**

**Table 3: Alterations in Biochemical Parameters among Different Experimental Groups (Mean±SD , n=7 ; p<0.01)**

| Groups / Parameters | Control | Ethanol | Recovery | Clover | Sidr | Samar |
|---|---|---|---|---|---|---|
| **MPO (units/mg tissue)** | 5.96±0.58 | 64.52±4.04$^{a}$ | 38.81±2.56$^{b}$ | 25.07±1.77$^{b,c}$ | 16.40±0.76$^{b,c}$ | 8.01±0.68$^{b,c,d}$ |
| **PEG2 (pg / g tissue)** | 39.20±3.15 | 12.69±1.20$^{a}$ | 20.11±1.30$^{b}$ | 23.71±3.98$^{b}$ | 32.22±2.40$^{b,c}$ | 35.94±1.36$^{b,c,d}$ |
| **G. juice volume (ml)** | 3.04±0.39 | 4.65±0.34$^{a}$ | 4.05±0.27 | 3.60±0.24$^{b}$ | 3.42±0.32$^{b,c}$ | 3.25±0.45$^{b,c,d}$ |
| **G. juice pH** | 3.98±0.27 | 1.99±0.15$^{a}$ | 2.71±0.28$^{b}$ | 2.96±0.23$^{b}$ | 3.38±0.32$^{b,c}$ | 3.65±0.27$^{b,c,d}$ |
| **Total acidity (Meq/l)** | 31.23±1.40 | 89.35±3.54$^{a}$ | 51.27±3.66$^{b}$ | 39.08±2.33$^{b,c}$ | 35.41±2.98$^{b,c,d}$ | 31.81±4.02$^{b,c,d}$ |
| **Mucus weight (mg)** | 4.04±0.34 | 0.83±0.08$^{a}$ | 1.77±0.48$^{b}$ | 2.16±0.14$^{b}$ | 3.00±0.16$^{b,c}$ | 3.40±0.16$^{b,c}$ |

***a:** Significant difference when compared with control group ; **b:** Significant difference when compared with ethanol treated group ; **c:** Significant difference when compared with Recovery treated group ; **d:** Insignificant difference when compared with control treated group*

Moreover, several ulcerative changes were prominent in almost all parts of the gastric wall of rats treated with ethanol such as, necrosis, haemorrhages, submucosal edema, blood vessel congestion, sloughing of gastric pits, in addition to infiltration by neutrophils and lymphocytes in the submucosa and in lamina propria. Morphometric results showed significant increase in histological injury score (fig 1c & table 4). These results were compatible and confirming the findings of previous authors who reported that the ethanol model is widely used to induce gastric ulcer, since ethanol administration to fasting rats penetrates easily and rapidly into the gastric tissues, causing mucosal damage, decreases blood flow within gastric mucosa, as well as, enhances release of histamine and free radicals production [3,43]. Moreover, ethanol causes damage of the gastroprotective mucus barrier and the associated phospholipids layers causing acid back diffusion and gastric mucosal injury, which in turn increases mucosal permeability in addition to the release of vasoactive factors, which finally result in gastric ulcer formation [3,43]. Ethanol induced gastric lesions associated with submucosal edema, haemorrhage, desquamation of epithelial cells and infiltration of inflammatory cells [43], which are typical patterns of ethanol injury in our results. Increased neutrophils infiltration into the gastric mucosa due to ethanol treatment in the presented histological results, was confirmed with our biochemical results represented by the significant increase of the gastric MPO activity, which is considered as neutrophil infiltration index marker. The MPO activity was known to increase under ulcerated conditions [3,48]. Recovery group showed marked improvement of gastric tissue when compared with ethanol treated group as a significant reduction in the ulcer index and histological scoring of gastric tissue injury were recorded. However, desquamated surface epithelial cells, and small-unhealed gastric ulcer were still observed in all animals of this group (Figs. 1d; and table 4). Clover treated group showed marked improvement of gastric tissue when compared with ethanol-ulcerated group and with recovery group as a significant reduction in the ulcer index and gastric injury histological score were recorded. However, degenerative changes, and inflammatory infiltration were still observed in all animals of this group (figs. 1e & table 4). Sidr honey treated animals showed significant improvement in gastric histoarchitecture with some degenerative changes, neutrophils infiltration and pyknosis in some mucosal cells (Fig 1f & table 4). Ulcerated rats treated with Samar honey as compared to ethanol treated group exhibited the classical histological structure of normal gastric layers (mucosa, submucosa and musculosa) with no histopathological changes except slight submucosal edema in some examined sections (Fig 1h & table 4). Previous studies reported that honey showed protective role on the gastric mucosal injury. It was reported that treatment with honey for two weeks induced recovery from gastric ulcers in about 66% of the animals, while the rate of recovery increased to 83.4% after six weeks of treatment [49,50]. Manuka honey reduced significantly the gastric ulcer index and protected the gastric mucosa completely from lesions in ethanol-ulcerated rats and the related histopathological changes as mucosal degeneration, necrosis, haemorrhage and submucosal edema [51]. The gastro-protective and anti-ulcerogenic effects of Sidr honey on ethanol-induced ulcers were investigated and it was observed that Sidr honey achieved gastroprotective effects in a dose-dependent manner [47, 52]. It has been reported that honey affected positively in cutaneous wound healing in rabbits and decreased related edema, infiltration of mononuclear and polymorphonuclear cell, and necrosis, as well as , honey improved wound contraction and epithelialization [53].

**3.3.2 Masson Trichrome staining results.**

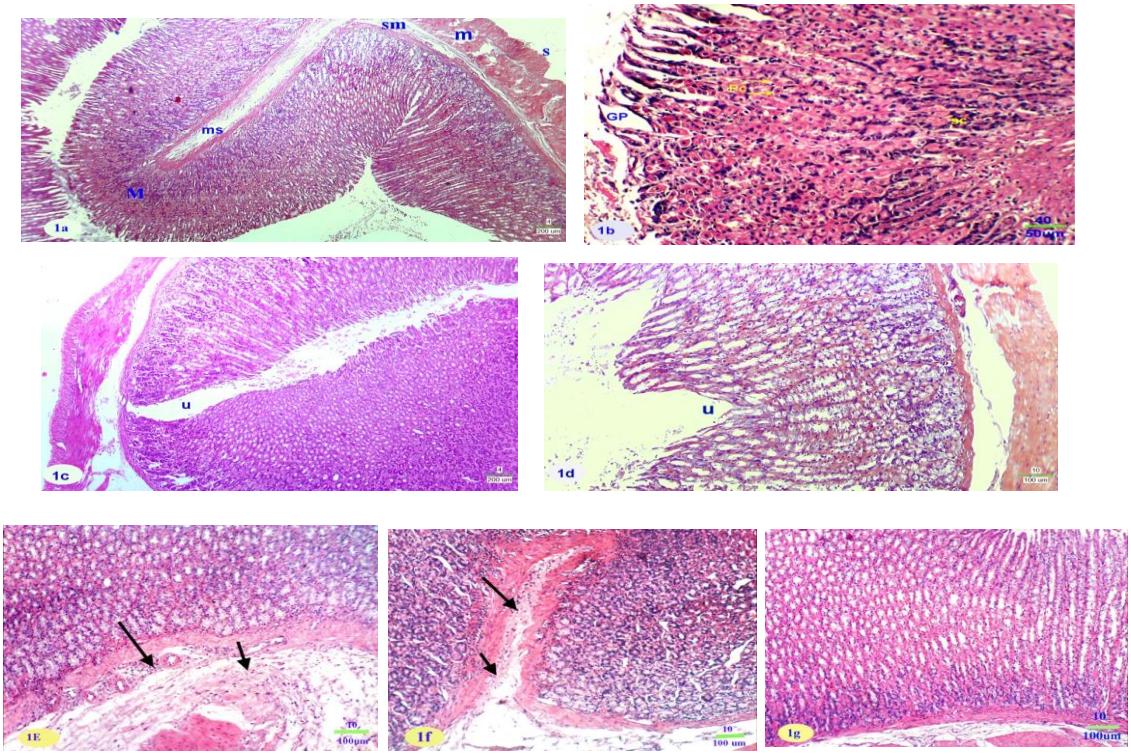
Microscopic examination of Masson trichrome stained section obtained from control animals showed abundant dense collagen fibers irregularly arranged between the fundic glands in the lamina propria as well as in the submucosa (Fig. 2a). Ethanol-ulcerated rats revealed significant reduction in % collagen fibers area in gastric tissues as few collagen fibers were detected in the thin lamina propria and in the submucosa (Fig. 2b & table 4). Our findings are in accordance with those of Sharma et al. [54], who reported that the collagen content is significantly reduced in different gastric ulcer models. Sharma et al. [54] reported that the collagen content was reduced approximately 1.5-fold by comparing to that in normal tissues in indomethacin-induced gastric ulcer. The importance of assessment of collagen expression is often used to evaluate the healing rate of wounds [55]. Because collagen is the fundamental component of the extracellular matrix (ECM), ulceration appears to be caused by a disruption in ECM turnover, which is restored by a balance of collagen deposition and degradation in stomach tissues [54]. During wound healing, collagen is mainly secreted by fibroblasts and represents an essential step for granulation tissue formation which is important for a high quality of ulcer healing [55,56]. In our results; recovery group showed limited restoration of collagen fibres deposition in submucosa when compared with ethanol treated group (Fig. 2c). Ulcerated rats treated with clover honey, Sidr honey and Samar honey showed significant increase of percentage collagen deposition area when compared with ethanol treated rats and recovery group ,as abundant thick collagenous fibers

bundles extended in the gastric glands and within submucosa (Fig. 2d:2f & table 4). Animals treated with Samar honey exhibited nearly normal quantity and distribution of dense collagen fibres in each of gastric mucosa, lamina propria and submucosa ,moreover, morphometric evaluations revealed insignificant difference when compared with control group ($P < 0.01$) (fig 2f & table 4). Honey was reported to stimulate synthesis of collagen and tissue growth [53]. Acacia honey with different doses induced an important impact on skin wound healing in rats, as the epithelization area, wound contraction, skin-breaking strength, and tissue granulation , significantly increased. The hydroxyproline levels also elevated indicating an increase in collagen formation [57]. Honey improves wound healing by enhancing activity of glycolytic enzymes, and via supplying sufficient energy for cellular repair. Collagenation, wound contraction, inflammation, macrophasia, fibroplasias and epithelization are crucial phases of wound healing and are interlinked. Therefore, any intervention in one or more of these phases can induce either promotion or depression of collagen deposition during ulcer healing [57]. Honey contains almost 80% sugars, which is the main source of energy required for fibroblasts to synthesize collagen through the glycolytic pathway. Furthermore, lactate, the end product of glycolysis, was found to accelerate the rate of hydroxylation of proline and collagenation after fibroblasts were exposed to oxygen [57]. Honey stimulates the production of inflammatory cytokines (TNF-α , IL-6, IL-1, and NO) by monocytes during the inflammation process, which stimulates and plays a significant role in collagen synthesis by fibroblasts [58].

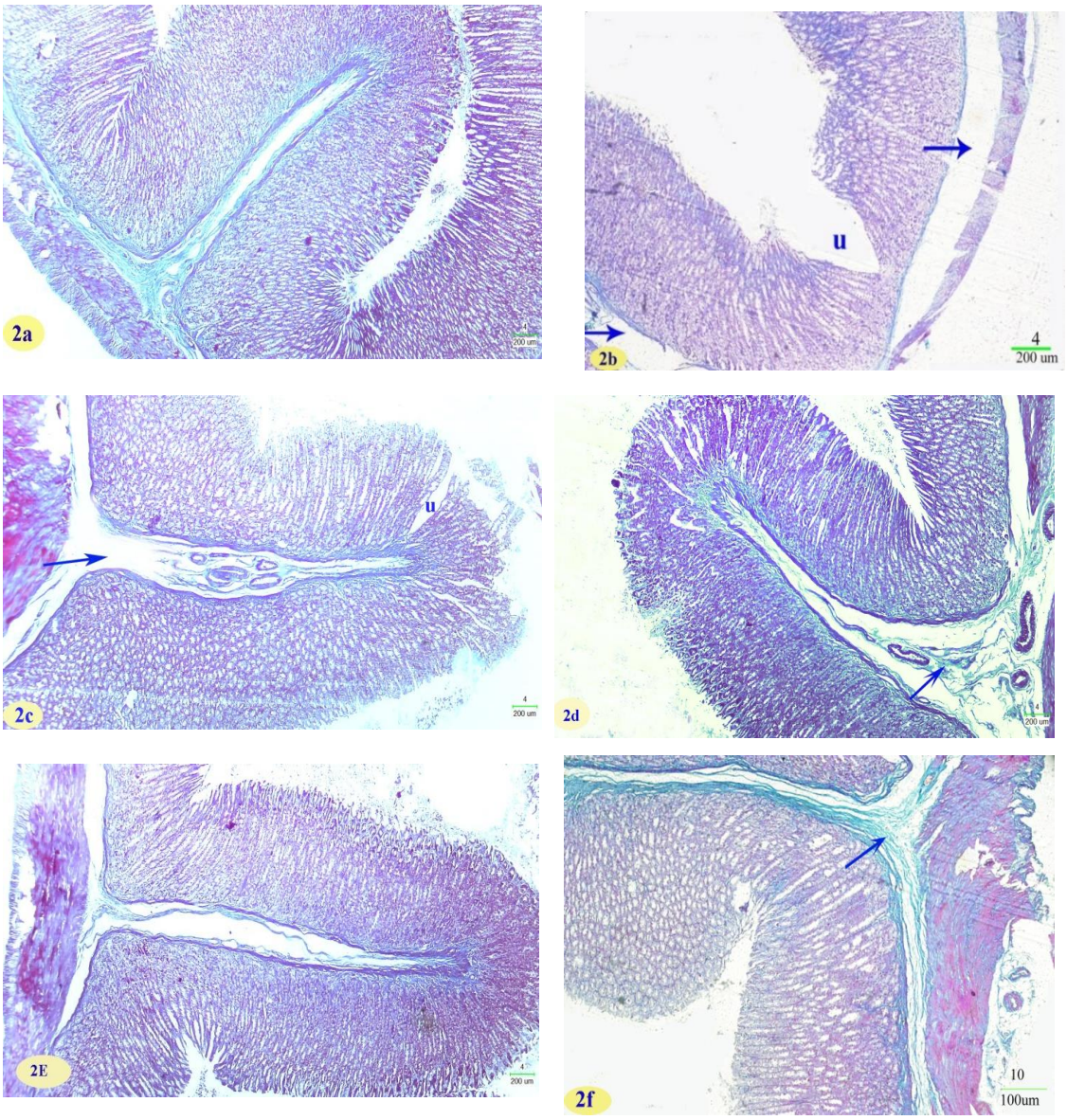
### 3.4. Histochemical Results:

#### 3.4.1. Periodic acid Schiff Reaction (PAS)

The gastric mucosae of control animals revealed intense positive PAS reaction on mucosal surface which was covered by a thin film of PAS positive mucous coat spread down to fill the gastric pits, isthmus and neck regions (Fig. 3a). Ethanol ulcerated rats showed mucosal surface epithelium that lacked the PAS positive mucous coat, while weak reaction was observed in pits and isthmus areas but the reaction was negative in the neck region, with significant decrease in % PAS positive area ($P < 0.01$) when compared to control rats (Fig. 3b & table 4).




**Figure(1) :** Microscopic examination of H&E stained sections showing histopathological alterations in gastric tissue among different groups. **(1a)** Section from a control rat showing normal histological structure of stomach and its layers mucosa(M), submucosa (sm), muscularis mucosa (mm) , musclosa (m) , and serosa (s) (scale bar 200 µm). **(1b)** Section from a control rat showing normal gastric glands with normal gastric pits (Gp) and different cells (parietal cells (PC) and chief cells (sc)) (scale bar 50 µm). **(1c)** Section from a rat from ethanol ulcerated group, showing deep gastric ulcer (u) eroding through the mucosa (scale bar 200 µm). **(1d)** Section from a rat from recovery group showing gastric ulcer (u) (scale bar 100 µm) . **(1e)** Section from an ulcerated rat treated with clover honey showing submucosal oedema and inflammatory cells infiltration (arrows) (scale bar 100 µm). **(1f)** Section from an ulcerated rat treated with Sidr honey showing slight submucosal oedema and few inflammatory cells infiltration (arrows) (scale bar 100 µm). (1g) Section from an ulcerated rat treated with Samar honey showing normal gastric tissue with normal gastric layers (scale bar 100 µm).




Figure(2) : Microscopic examination of Masson trichrome stained sections showing collagen fibres deposition in gastric tissue among different groups. (2a) Section from a control rat showing normal distribution of thick collagen fibres in gastric submucosa. (2b) Section from a rat from ethanol ulcerated group showing deep gastric ulcer (u), with absence of collagen fibers (arrow). (2c) Section from a rat from recovery group showing gastric ulcer (u) with marked depletion of collagen fibres in gastric submucosa. (2d) Section from an ulcerated rat treated with clover honey showing moderate deposition of collagen fibres in gastric submucosa (arrow). (2e) Section from an ulcerated rat treated with Sidr honey showing moderate deposition of collagen fibres in gastric submucosa. (2f) Section from an ulcerated rat treated with Samar honey showing normal gastric tissue with normal thick collagen fibres in gastric submucosa (arrow) (2a :2e ;scale bar 200 µm) ; (2f ; scale bar 100 µm).

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Those observations were confirmed by biochemical analysis of gastric tissue that showed significant reduction of PGE2 levels and gastric mucus weight. However, our results are in agreement with several studies that proved the diminishing effect of ethanol on gastric PAS-positive materials, mucus contents and PGE2, as ethanol was found to cause necrotic lesions by reducing the production of gastric mucus that preserves neutral pH, forms defense mechanism against gastro ulcerative agents and supports the healing process [3, 4, 40 ,41, 59].

Recovery group showed gastric mucosa with moderate restoration of positive PAS reaction on gastric mucosa while the PAS negative reaction still pronounced in some regions. However, statically significant increase in % PAS positive area was recorded in each of recovery group, clover honey, Sidr honey and Samar honey treated rats by comparison with ethanol ulcerated group (Fig 3c: 3f & table 4). Examination of gastric tissues obtained from clover honey treated animals showed insignificant difference when compared with

recovery group. Sidr honey and Samar honey showed intense positive PAS reactivity on the mucosal surface extending down into glandular region; with significant increase in % PAS stained area when compared with recovery group. Samar honey achieved the nearest normal appearance of gastric mucosa with PAS-positive reaction in mucosal surface and neck mucus cells. However there was no significant difference by comparing with control group ($P < 0.01$) (Fig 3c : 3f & table 4). Our results are in accordance with those of Taha et al., [47], who found that various doses of Sidr honey resulted in significant increase ($P < 0.05$) in the mucus production of gastric mucosa, and increase the expansion of PAS-positive substantial continuous mucous layer lining the gastric mucosal. Almasaudi, et al., [51] reported that pretreatment with Manuka honey significantly preserved gastric mucosal glycoprotein in ethanol-injected rats. Mahdy, et al. [21], invistigated the antiulcer impact of honey in rats and concluded that honey restored mucus production through increased mucosal glycoproteins synthesis. The protective effect of honey against mucosal damage was owed to their phenols contents which stimulate PGE2 synthesis [21].

### 3.4.2. Perls' Prussian Blue stain results:

Examination of gastric sections of control group showed normal mucosa with negative ferric iron (hemosiderin) reaction. Gastric mucosae of ethanol-ulcerated rats showed significant accumulation of dens amounts of hemosiderin in degenerated mucosal epithelium extending down into gastric gland as expressed by dark blue color when compared with control group. By comparing recovery group with ethanol treated group, hemosiderin deposits were significantly diminished in gastric mucosa. Clover honey treated rats showed significant decrease of iron staining when compared with ethanol group, while no difference was detected when compared with recovery group. Sidr honey and Samar honey treated rats showed significant reduction in hemosiderin deposits when compared with recovery group, as if normal gastric mucosa appeared with purple color with fine hemosiderin deposits. However, Samar honey treated rats showed insignificant difference when compared with control group (Figs 4a, 4f & table 4). Excess iron in the body accumulates as hemosiderin in the liver, then in other organs such as the pancreas, heart, and joints, resulting in cell damage and organ diseases. Because the stomach has no recognized role in iron metabolism, the identification of hemosiderin in gastric tissue raises a slew of clinical and pathophysiologic issues. [60]. Excess iron accumulation in gastric mucosa is known as gastric siderosis (GS), which has previously been described in alcoholics [60-62]. Some studies attributed the demonstration of iron pigments in gastric mucosa as a result of gastric inflammation, gastric ulcer, haemorrhagic necrosis, or before mucosal haemorrhages [60,62,63,64]. The reducing manner of honey on hemosiderin may be attributed to its anti-inflammatory and anti-ulcerogenic properties as reported in different studies [46, 47, 51, 52]. The antioxidant activity of honey can reduce the damage caused by free radicals, and therefore synergistically prevent tissue necrosis [46]. Gelam honey had an anti-inflammatory effect in a rat paw ulcer model, reducing paw edoema and limiting the synthesis of proinflammatory mediators such NO, PGE2, TNF-α, and IL-6 in plasma, as well as suppressing the expression of iNOS, COX-2, TNF-, and IL-6 in paw tissue [45].

### 3.5. Immunohistochemical Results

#### 3.5.1. iNOS

Examination of gastric tissue sections obtained from ethanol ulcerated rats showed that iNOS immunoreactivity was significantly increased ($P<0.01$) in the degenerated gastric mucosa when compared to those of control animals, which exhibited scanty immunostaining of iNOS in the gastric glands (table 4 & Fig. 5). This increase was significantly diminished in recovery group, and then, in clover honey, Sidr honey and Samar honey treated groups, respectively, as they revealed a significant decrease in iNOS immunostaining when compared with recovery group. However, Samar honey treated group showed the weakest localization of iNOS immunostaining, as there was insignificant difference when compared with control group. Our results are similar to those of Yoo et al. [65], who reported that iNOS immunoexpression was increased within gastric tissues in ethanol induced ulcer, while it was not observed in the normal control group. Massive amounts NO produced by iNOS acts an essential impact in ulcer progression via the induction of peroxynitrite radicals (ONOO-) cell toxicity, protein tyrosine nitration, hydroxyl radical production, and tissue damage [66].

The anti-inflammatory activity of honey was attributed to its phenolic compounds and flavonoids [46]. These compounds were demonstrated in several studies to suppress the activity inducible nitric oxide synthase (iNOS), hence resulting in an anti-

inflammatory response [67]. Gelam honey represented an inhibitory and suppresses the expression of iNOS in paw tissue [45]. Honey inhibits TNF-α and the pro-inflammatory cytokines expression via suppressing nuclear factor kappa B (NF-κB), a key factor in the activation of iNOS. NO is a free radical that functions as a mediator in both acute and chronic inflammation and promotes tissues healing [46, 68].

**3.5.2. VEGF**

Strong positive VEGF immunohistochemical staining was demonstrated as brown cytoplasmic reaction (index for angiogenesis) in gastric mucosa of control rats, while gastric tissues obtained from the ethanol-ulcerated rats revealed significant depletion of VEGF (Fig 6) and showed statistically significant decrease when compared with control group as shown in table (4). Significant restoration of VEGF immunoexpression in gastric mucosae was observed in recovery rats when compared with ethanol treated ones (fig 6b). Furthermore, it was observed that clover honey, Sidr honey and Samar honey treated animals showed more degree of VEGF immunoreactivity when compared with recovery group. However, Samar honey still represents the best results, as VEGF immunostaining returns to normal levels, as there was insignificant statistical difference when compared with control group as shown in fig 6 and table (4). Our findings corroborate with some studies, which reported that mucosal VEGF expression was significantly decreased in different gastric ulcers models [7, 69]). Ibrahim et al. [69] attributed this observation to the inhibitory effect of indomethacin on gastric mucosal PGE2 level, as PGE2 was found to stimulate VEGF immunoexpression and angiogenesis in the ulcerated mucosa by the activation of EP4 receptors [70]. VEGF is dimeric glycoproteins, which is angiogenic mediator and essential component of wound healing, and in mucosal protection and repair [6].

Honey stimulates tissue growth, and formation of new blood vessels in the wounds bed [53]. Honey improved angiogenesis in an *in vitro* study by Chaudhary et al. [71], and they observed that hydrogen peroxide (released from glucose by the action of the enzyme glucose oxidase found in raw honey) causes the migration of leukocytes to wounds via a concentration gradient mechanism [71]. Scepankova et al., [46] and Oryan et al., [58], attributed the wound-healing activity of honey to its anti-inflammatory and antioxidant properties, as well as its ability to promote re-epithelialization and angiogenesis. Thus, our findings can suggest that honey stimulate VEGF synthesis via upregulation of PGE2. Prostaglandins have a role in inflammatory response by causing vasodilation, improving blood vessels permeability and facilitate the passage of leukocytes, acting as an antiplatelet agent, and activating the nerve ends. Inflammation, edoema, and discomfort may all be reduced by lowering prostaglandin levels in the blood [46].

The expression of VEGF increases during healing in different acute gastric damage models, and it was found that pretreatment with a single dose of oral VEGF might exert a gastroprotective effect against acute ethanol damage in the gastric mucosa in rats. Furthermore, it has been discovered that daily treatment of VEGF enhances the healing of cysteamine-induced duodenal ulcers in rats by improving angiogenesis and granulation tissue development. The addition of exogenous VEGF induced gastric wounds healing via TGF-b signaling [54]. Earlier studies have shown that obestatin protects the stomach mucosa against stress-induced gastric lesion by upregulating VEGF mRNA, resulting in pro-angiogenic effects. Furthermore, obestatin was reported to improve skeletal muscle healing by boosting the production of VEGF and VEGFR2 [69]. The ulcer margin of human peptic ulcer disease has been shown to express VEGF and its receptors during healing [6]. VEGF enhances the formation of granulation tissue, angiogenesis, and connective tissue remodeling during ulcer repair by acting particularly on vascular endothelial cells and improving vascular permeability, migration, and cell proliferation around the ulcer's edge [69]. Furthermore , interstitial collagenase (matrix metalloproteinase [MMP]-1), tissue inhibitor of metalloproteinases, and gelatinase A (MMP-2) production by endothelial cells is improved by VEGF Bao et al., [72] related the VEGF's wound healing activity to its anti-apoptotic properties in human endothelial cells.

**3.5.3. HSP47**

Immunohistochemical examination of gastric mucosae obtained from control rats showed quite low HSP47 expression in cytoplasm of mucosal epithelium. Ethanol-ulcerated tissues showed rare HSP47 expression with insignificant difference in HSP47 % area when compared with control group.

The increase of HSP47 expression in either recovery group or honey treated groups was correlated with the ulcer healing rate, thus the HSP47 expression decreased with the progress of ulcer healing. In recovery group, where the minimal ulcer healing score was recorded, HSP47 showed the maximal immunoexpression when compared with other cotreated groups. HSP47 strong immunoexpression was still observed in clover honey group, and then decreased in Sidr honey. Uninterestingly, in Samar honey group, where the maximal ulcer healing score was recorded, HSP47 showed fine immunoexpression and there was insignificant difference when compared with control group (Figs 7a :7f & tble 4) . Our findings are consistent with Tsukimi and Okabe (2001), who reported that HSP47 was found to be strongly expressed in the ulcer base during ulcer development, and its expression reduced with ulcer healing progression. During wound healing and/or normal development, HSP47 is required for the synthesis and processing of different types of collagen **[56]**. HSP27 and HSP47 were detected during healing of oral ulcer and periosteal defects [73]. During skin wound healing in a pig model, HSP47 mRNA levels were highly related with mRNA for type I and III collagen[74]. A positive correlation between heat shock proteins (HSPs) synthesis and cytoprotection of gastrointestinal mucosa at the intracellular level was reported [75].

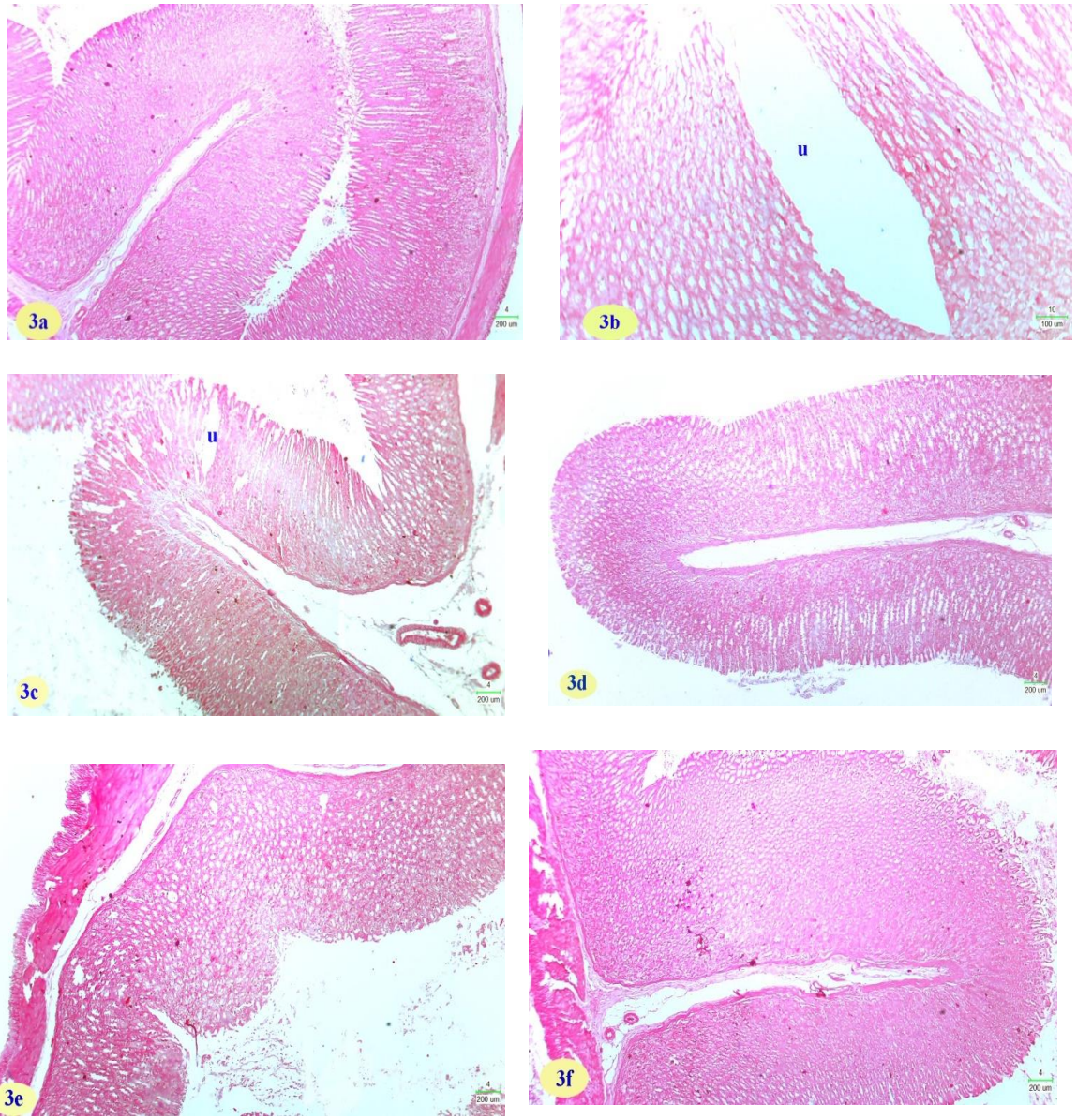
The increased expression of HSP47 in retrobulbar adipose tissue in TAO patients was related with some inflammatory factors [76]. Cytokines such as TGF-$\beta$, IL-4, and IL-1 was found to promote collagen synthesis and deposition in each of liver, conjunctiva and lung tissues through regulation of the expression of HSP47 [76]. Based on these findings, we can attribute the modulatory effects of honey on HSP47 immunoexpression to its anti-inflammatory properties [45 ,46 ,50]. However, further studies are required to verify the relationship between HSP47 and inflammatory factors.

An additional advantage of the anti-inflammatory activity of honey is it reduces edema, which relieves pressure on the microvasculature of wounded tissue, allowing for the availability of oxygen and nutrients needed for tissue growth and wound repair. This impact maintains a proper moisture balance for wound exudate, which is still a persistent challenge in wound healing[46]. Furthermore, honey's acidic pH inhibits proteases enzymes from inactivating tissue growth factors and destroying plasma fibronectin and collagen matrix, both of which are required for fibroblast activity and tissue re-epithelialization. Moreover, the lower pH in the wound bed makes more oxygen available from the blood's haemoglobin [68,77]. The acidification of the wound environment also encourages macrophage activity, inhibits bacterial development, and neutralises ammonia produced by bacterial metabolisms, which can damage tissues[58]. As observed from all above in vivo results, Samar honey achieved a significant preference when compared with both of clover honey and Sidr honey. By considering the phytochemical and antioxidant screening of Samar honey, thus we can attribute the increased effect of Samar honey to the highest contents of flavonoids, phenolic contents and DPPH% when compared with clover and Sidr honey. Honey's high flavonoid content is thought to prevent the gastric ulcers by acting as an antioxidant and anti-secretory mechanisms [50]. Antioxidant, anti-ulcerogenic, and gastroprotective properties of phenolic natural products and flavonoids have been demonstrated [47,78,79]**.** Some studies demonstrated the protective effect of flavonoids especially quercetin, on alcohol-induced gastric lesions through increasing gastric mucus production which accompanied by a parallel reduction of gastric lesions [21]. Other derivatives of flavonoids, such as kaempferol, hesperetin, and naringin, possess antiulcer efficacy for the treatment of the gastric and duodenal ulcers. Their antiulcer activity manifests as the reducing of lipid peroxidation, the neutralization of cell proliferation, and an increased susceptibility to apoptosis [80]. Treatment with *Acacia* honey attenuated oxidative stress and enhanced antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase, as well as suppressing lipid peroxidation as evident by a decrease in malondialdehyde levels [78].

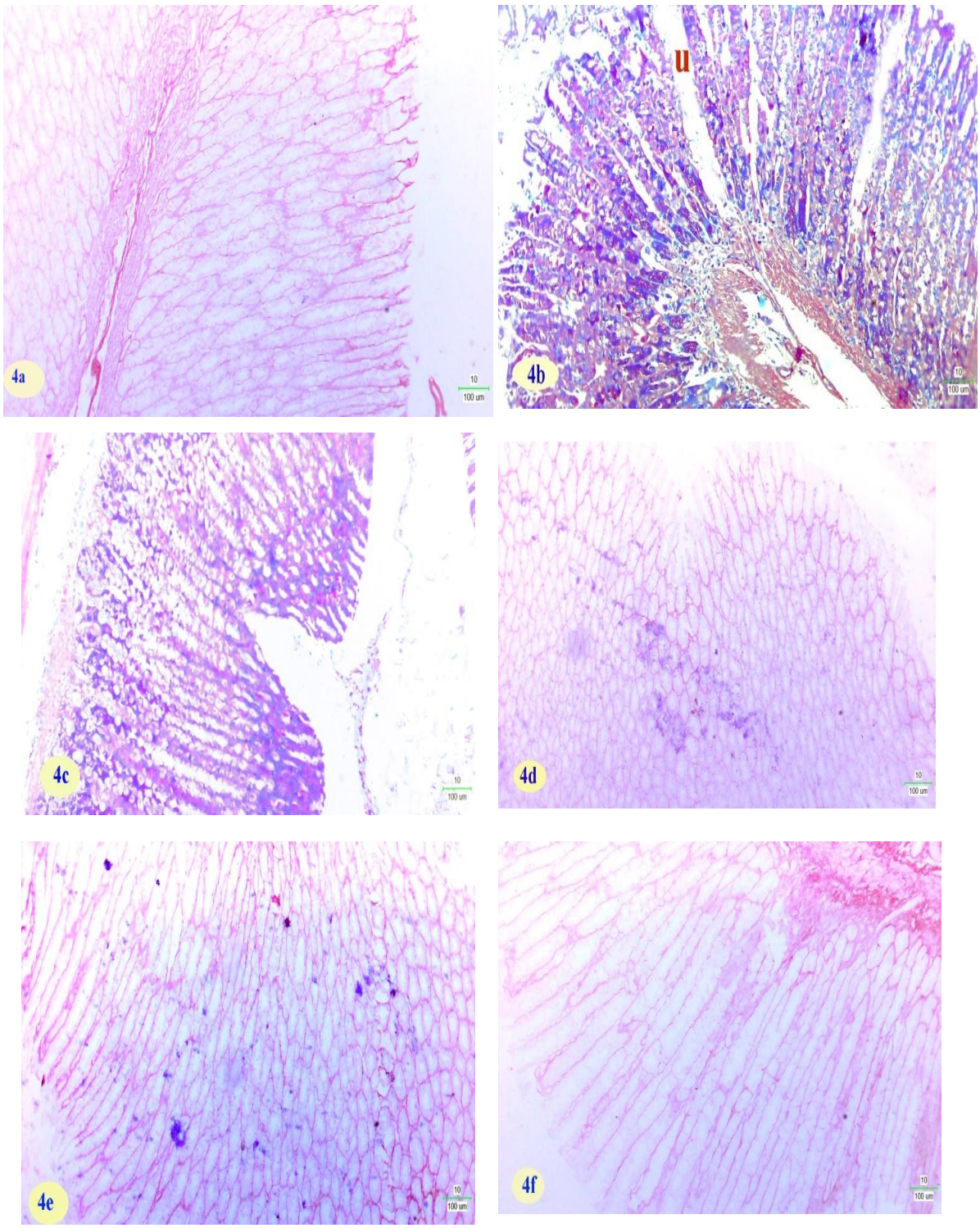
**Table (4): Histomorphometric Evaluations and Alterations Among Different Experimental Groups (Mean±SD , n=7 ; p<0.01).**

| Groups / Parameters | Control | Ethanol | Recovery | Clover | Sidr | Samar |
|---|---|---|---|---|---|---|
| **Ulcer index** | 0.00±0.00 | 7.42±1.51$^{a}$ | 3.57±2.29$^{b}$ | 2.14±0.89$^{b}$ | 1.00±0.57$^{b,c,d}$ | 0.28±0.48$^{b,c,d}$ |
| **G. injury score** | 0.00±0.00 | 9.57±1.51$^{a}$ | 5.85±1.06$^{b}$ | 3.71±0.75$^{b,c}$ | 1.57±0.97$^{b,c}$ | 0.85±0.69$^{b,c,d}$ |
| **Masson%** | 34.32±0.69 | 6.45±0.90$^{a}$ | 18.38±1.28$^{b}$ | 22.15±1.92$^{b,c}$ | 24.60±0.74$^{b,c}$ | 32.71±0.54$^{b,c,d}$ |
| **PAS %** | 34.53±1.14 | 10.99±1.75$^{a}$ | 22.49±1.30$^{b}$ | 24.25±0.61$^{b}$ | 28.48±0.88$^{b,c}$ | 32.90±0.67$^{b,c,d}$ |
| **Pearl %** | 5.66±0.54 | 60.88±1.48$^{a}$ | 26.01±1.97$^{b}$ | 17.56±0.74$^{b,c}$ | 14.55±4.32$^{b,c}$ | 8.54±0.79$^{b,c,d}$ |
| **iNOS %** | 5.38±0.48 | 67.71±4.64$^{a}$ | 31.52±2.99$^{b}$ | 20.80±0.99$^{b,c}$ | 11.49±1.06$^{b,c}$ | 7.20±0.76$^{b,c,d}$ |
| **VEGF %** | 32.56±0.80 | 5.72±0.41$^{a}$ | 18.76±0.93$^{b}$ | 22.73±1.50$^{b,c}$ | 27.42±0.87$^{b,c}$ | 31.20±1.24$^{b,c,d}$ |
| **HSP47 %** | 4.49±0.66 | 5.23±0.53$^{d}$ | 49.09±4.77$^{a,b}$ | 21.08±1.60$^{a,b,c}$ | 12.07±1.65$^{a,b,c}$ | 5.37±0.75$^{b,c,d}$ |

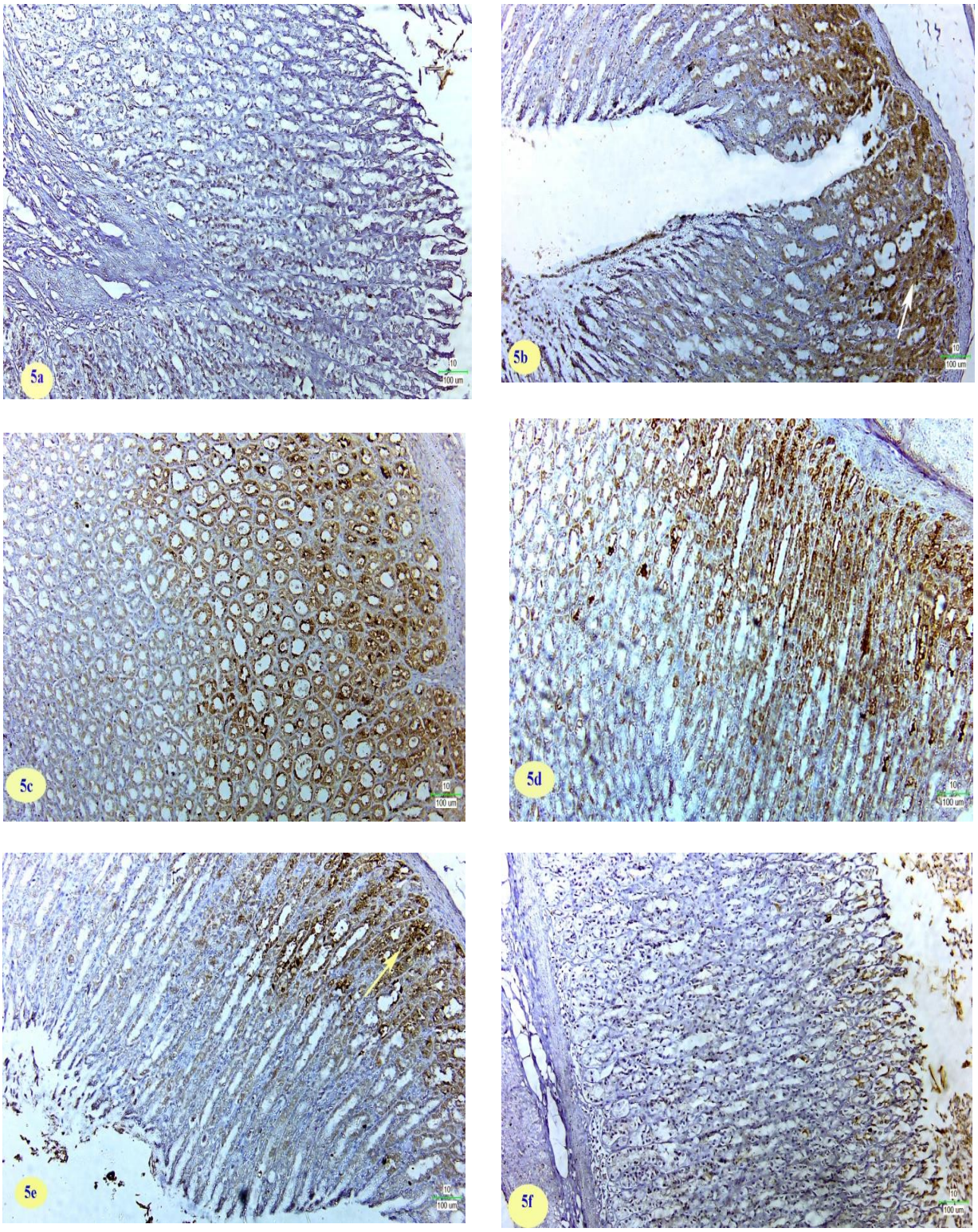
a: Significant difference when compared with control group, b: significant difference when compared with ethanol treated group, c: significant difference when compared with Recovery group, d: insignificant difference when compared with control treated group



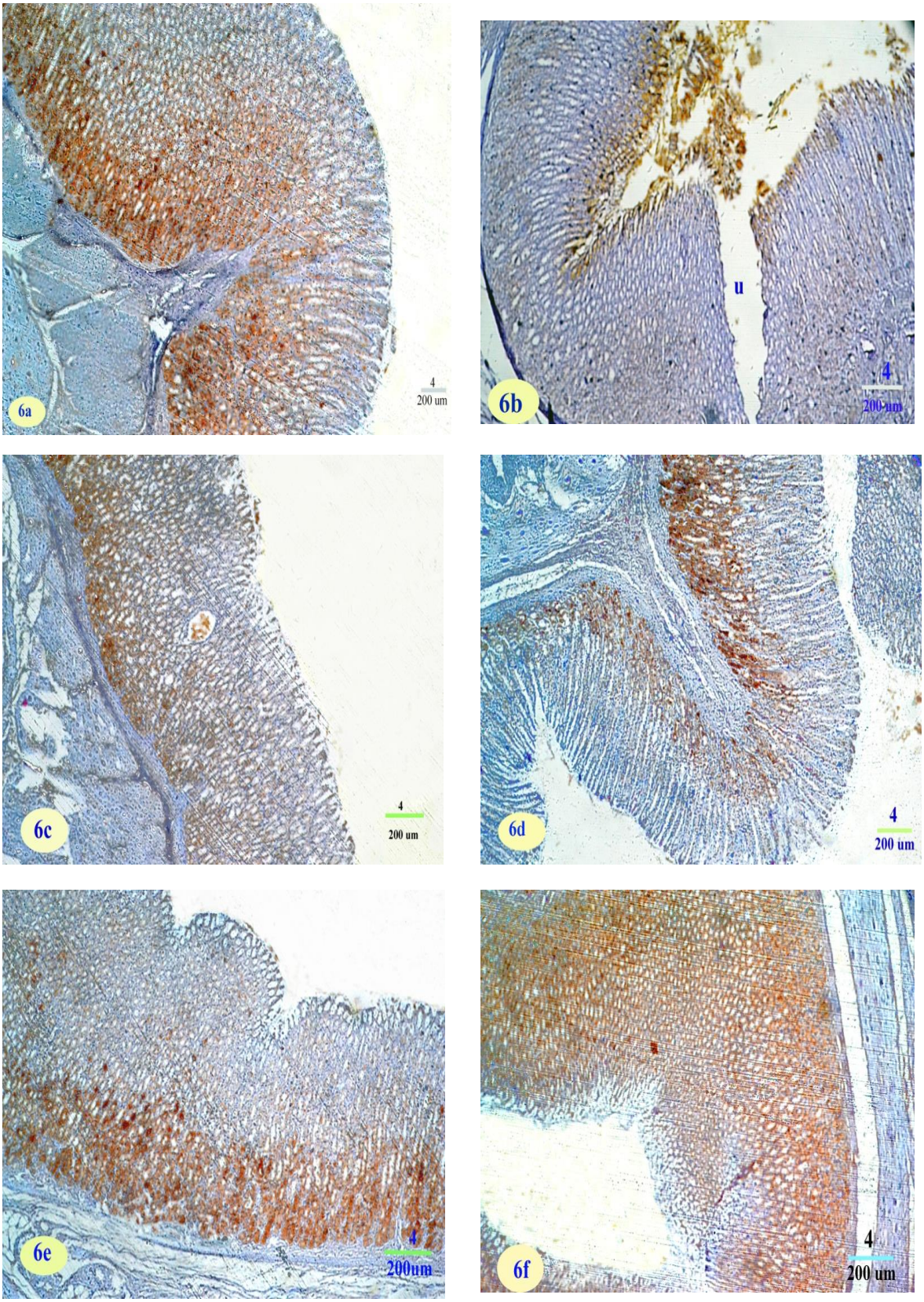

Figure(3) : Histochemical demonstration of PAS-stained sections showing distribution of PAS positive materials in gastric tissue among different groups. (3a) Section from a control rat showing normal distribution of PAS positive materials in gastric mucosa. (3b) Section in a rat from ethanol ulcerated group, showing gastric ulcer (u) with marked depletion of PAS positive materials in gastric mucosa. (3c) Section from a rat from recovery group showing, gastric ulcer (u) with marked depletion PAS positive materials in gastric mucosa. (3d) Section from an ulcerated rat treated with clover honey showing moderate restoration of PAS positive materials in gastric mucosa. (3e) Section from an ulcerated rat treated with Sidr honey showing marked restoration of PAS positive materials in gastric mucosa. (3f) Section from an ulcerated rat treated with Samar honey showing normal gastric mucosa with normal distribution of PAS positive materials in gastric mucosa (3a , 3c:3f : scale bar 100 µm ; 3b :scale bar 200 µm).



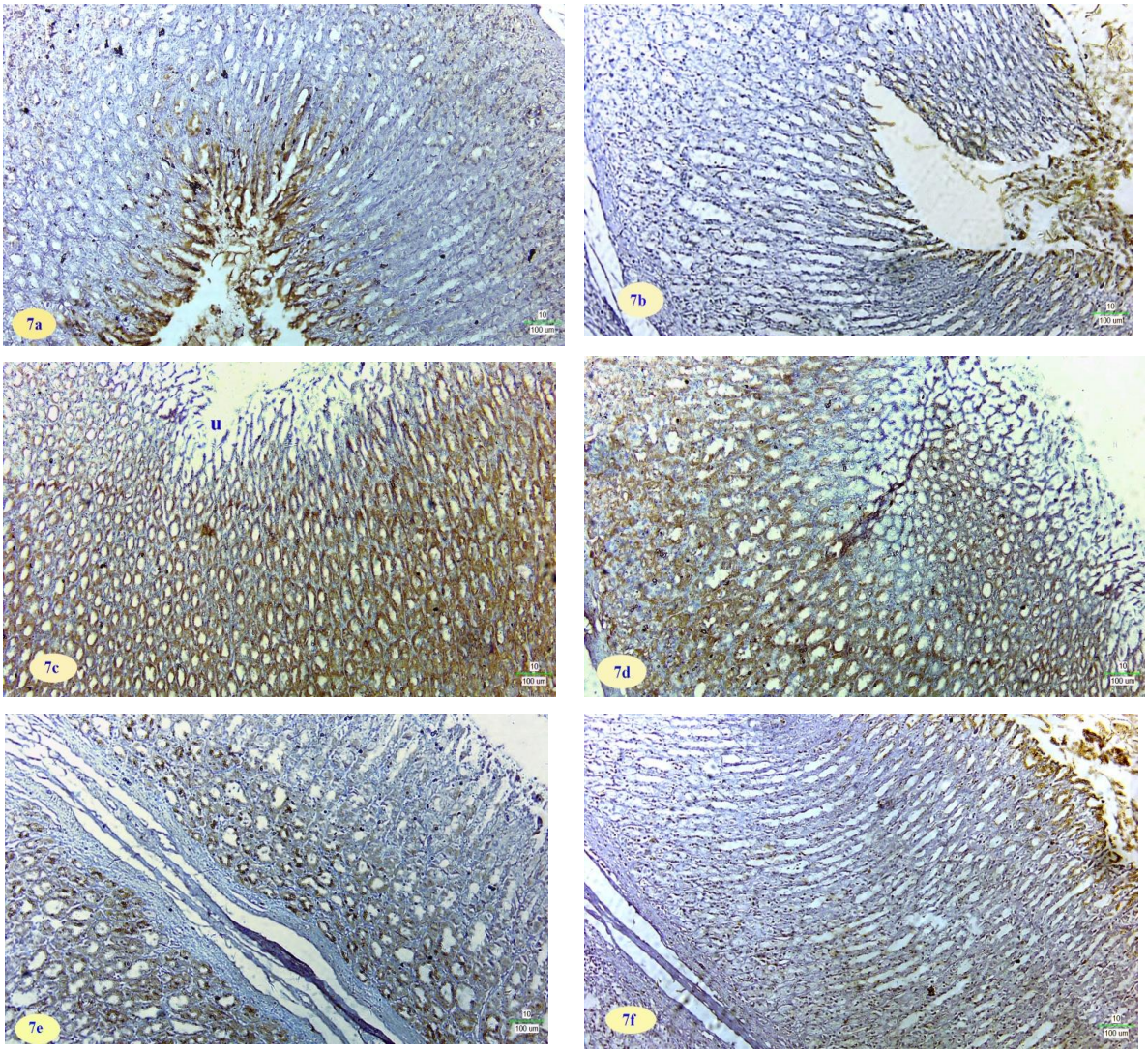

Figure(4): Histochemical demonstration of hemosidrin depodits in Perl stained sections in gastric tissue among different groups. (4a) Section from a control rat showing normal hemosidrin depodits in gastric mucosa. (4b) Section in a rat from ethanol ulcerated group, showing deep gastric ulcer (u), with massive deposition of hemosidrin in gastric mucosa (blue color). (4c) Section in a rat from recovery group showing, gastric ulcer with marked deposition of hemosidrin in gastric mucosa (blue color). (4d) Section from an ulcerated rat treated with clover honey showing fine deposition of hemosidrin in gastric mucosa. (4e) Section from an ulcerated rat treated with Sidr honey showing rare hemosidrin deposits in gastric mucosa. (4f) Section from an ulcerated rat treated with Samar honey showing normal gastric tissue with absence of hemosidrin deposits in gastric mucosa (arrow) (scale bar 100 µm).




Figure(5): Immunohistochemical demonstration of **iNOS** immunoreaction in gastric tissue among different groups. (5a) Section from a control rat showing negative iNOS immunoexpression in gastric mucosa. (5b) Section in a rat from ethanol ulcerated group, showing deep gastric ulcer, with strong iNOS immunoexpression in gastric mucosa (brown color). (5c) Section in a rat from recovery group showing marked iNOS immunoreaction in gastric mucosa. (5d) Section from an ulcerated rat treated with clover honey showing moderate iNOS expression in gastric mucosa. (5e) Section from an ulcerated rat treated with Sidr honey showing rare iNOS immunoexpression in gastric mucosa. (5f) Section from an ulcerated rat treated with Samar honey showing normal gastric tissue with weak immunoreaction of iNOS in gastric mucosa (scale bar 100 µm).




Figure(6): Immunohistochemical demonstration of **VEGF** immunoreaction in gastric tissue among different groups. (6a) Section from a control rat showing strong VEGF immunoexpression in gastric mucosa. (6b) Section in a rat from ethanol ulcerated group, showing deep gastric ulcer (u), with negative immunoexpression in gastric mucosa. (6c) Section in a rat from recovery group showing mild VEGF immunoreaction in gastric mucosa. (6d) Section from an ulcerated rat treated with clover honey showing moderate VEGF expression in gastric mucosa. (6e) Section from an ulcerated rat treated with Sidr honey showing moderate VEGF immunoexpression in gastric mucosa. (6f) Section from an ulcerated rat treated with Samar honey showing normal gastric tissue with strong immunoreaction of VEGF in gastric mucosa (scale bar 200 µm).




Figure(7): Immunohistochemical demonstration of HSP47 immunoreaction in gastric tissue among different groups. (7a) Section from a control rat showing negative HSP47 immunoexpression in gastric mucosa. (7b) Section in a rat from ethanol ulcerated group, showing deep gastric ulcer, with negative HSP47 immunoexpression in gastric mucosa. (7c) Section in a rat from recovery group showing strong immunoreaction of HSP47 in gastric mucosa. (7d) Section from an ulcerated rat treated with clover honey showing moderate HSP47 expression in gastric mucosa. (7e) Section from an ulcerated rat treated with Sidr honey showing rare HSP47 immunoexpression in gastric mucosa. (7f) Section from an ulcerated rat treated with Samar honey showing normal gastric tissue with weak immunoreaction of HSP47 in gastric mucosa (scale bar 100 µm).

### *3.6. In Vitro* Scratch-Wound Healing Assay:

Clover honey, Sidr honey and Samar honey were examined for changing the cellular proliferation migration rate of BJ.1 dermal fibroblast cell line using scratch assay technique, the in vitro wound closure rat of the samples in 0h and 24h were calculated. As shown in fig (8) and table (5), the migration analysis showed that each of Clover honey, Sidr honey and Samar honey achieved a significant ($P < 0.01$) acceleration of wound closure rates with respect to control sample. However, it was far from being complete. The migration analysis showed that clover honey exposed cells significantly ($P < 0.01$) had the highest wound closure rate at 24 h in BJ.1 fibroblast cells and an increased number of fibroblasts in the denuded area by comparing with either control cells, Samar honey and Sidr honey exposed cells. Consequently, the length between the scratch mark edges within Sidr honey exposed cells represented that there was non-significant increase in the percent of wound closure rates of BJ.1 fibroblast cells when compared with Samar honey treated cells. According to Sell et al.[80], Manuka honey accelerates the wound closure rate in a monolayer of human fibroblasts. Furthermore, Ranzato et al.[81] showed that Acacia, Manuka and buckwheat honey promotes wound-closing process in dermal fibroblasts. Acacia and buckwheat honeys, which

were most effective active in encouraging scratch wound closure, also caused considerable increases of primary interleukins found in fibroblast culture, such as, IL-4, IL-6, and IL-8 [81]. Abd Ghafar et al. [83], reported that Acacia honey promotes faster migration and wound closure in *in vitro* corneal ulcer wound healing model, as the corneal fibroblasts in Acacia honey -enriched media promote faster wound closure compared to controls. Furthermore, honey's high concentration of nutritive components (sugars, amino acids, vitamins, and other trace elements) promote cell growth, endothelial cell proliferation, and the acceleration of tissues repair [68,77,83]. Afonso et al. [84] related the biological potential of honey in wound-healing activity, with their antioxidant and anti-inflammatory properties.

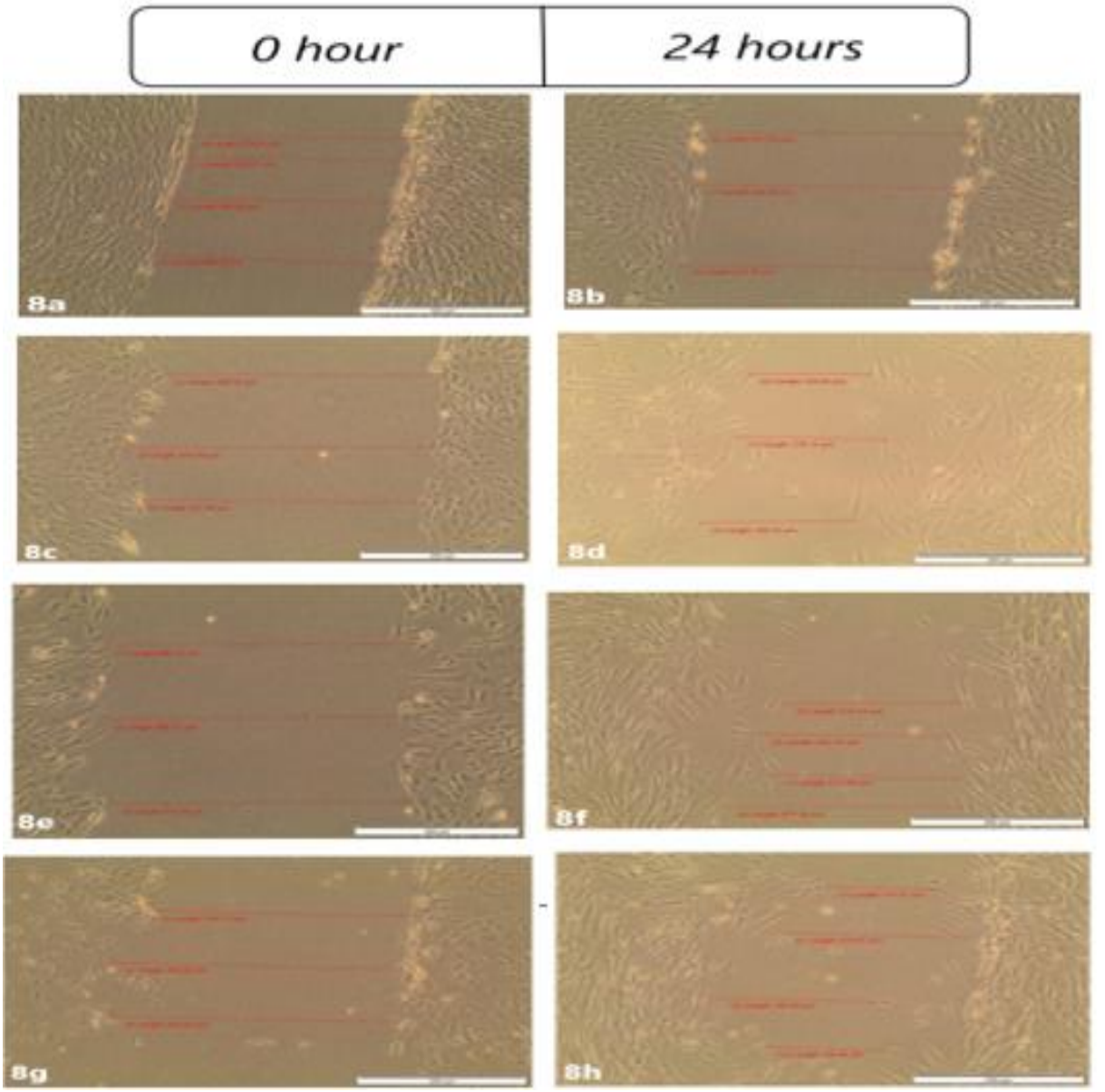



Fig (8) : Alteration in Wound Closure Rate in different cell Samples (8a , 8b : control cells) , (8c,8d :clover honey) (8e , 8f :Sidr honey) (8g ,8h :samar honey) ( scale bar 200µm).

**Table (5): Alteration in Wound Closure Rate in different Samples (n=3, mean±SD ; p<0.01).**

| groups | -ve control | Samar | sidr | clover |
|---|---|---|---|---|
| **Wound Closure (%)** | 25.65± 1.34 | 54.66± 1.47 | 51.91± 0.85 | 47.55± 0.75 |

**3.7. Anti- *Helicobacter pylori* activity:**

A different types of honeys showed different anti-*Helicobacter* activity each with MIC values ranging from 15.63 to >125 µg/mL. The best activity was recorded with Samar honey with MIC values 15.63 µg/ml , followed by sider honey with MIC values 32. µg/mL while, the lowest MIC value (128 µg/mL) was recorded with clover honey (fig 9).Our results were agreed with many studies that reported the efficiency of raw honey in inhibition the growth of *H. pylori* [85-90]. The efficiency of several honey types as an alternative therapy on *H. pylori*-induced gastric ulcer has been reported [90]. The anti *H. pylori* activities of honey in the presented result may be attributed to their high contents of Flavonoid, Phenolics and DPPH% [91,92]. On the other hand, Kim et al. [88] ; Jantakee and Tragoolpua, [91] ; and Petric [92] indicated that honey's high acidity, high osmotic effect, high concentration of hydrogen peroxide, and various photochemicals all help honey to inhibit or kill different bacteria. Moreover, the high viscosity of honey helped to provide the host cellular barrier and protect bacterial infection. Manyi-Loh et al [87], reported that the high concentration of HCl and low pH value, certainly affects the activity of enzymes in consumed food, including glucose oxidase, which generates hydrogen peroxide ($H_2O_2$) which is very important for possibilities of its effective application for prophylaxis and therapy of in *vivo H. pylori* infection. Petric [92] and Szweda [93] related the anti *H. pylori* activity to bee defensin-1, which is a peptide secreted by the honeybee hypopharyngeal glands and exhibits activity against Gram-positive bacteria. *H. pylori* inhabit on gastric cell induces mucosal inflammation via a variety of pathways including the activation of transcription factors such as nuclear factor-kB and activator protein-1 activity , which controls various cellular processes during inflammation[90]. Abdel-Latif and Abouzied [89], demonstrated that honey suppresses nuclear factor-kB and activator protein-1 in a study.

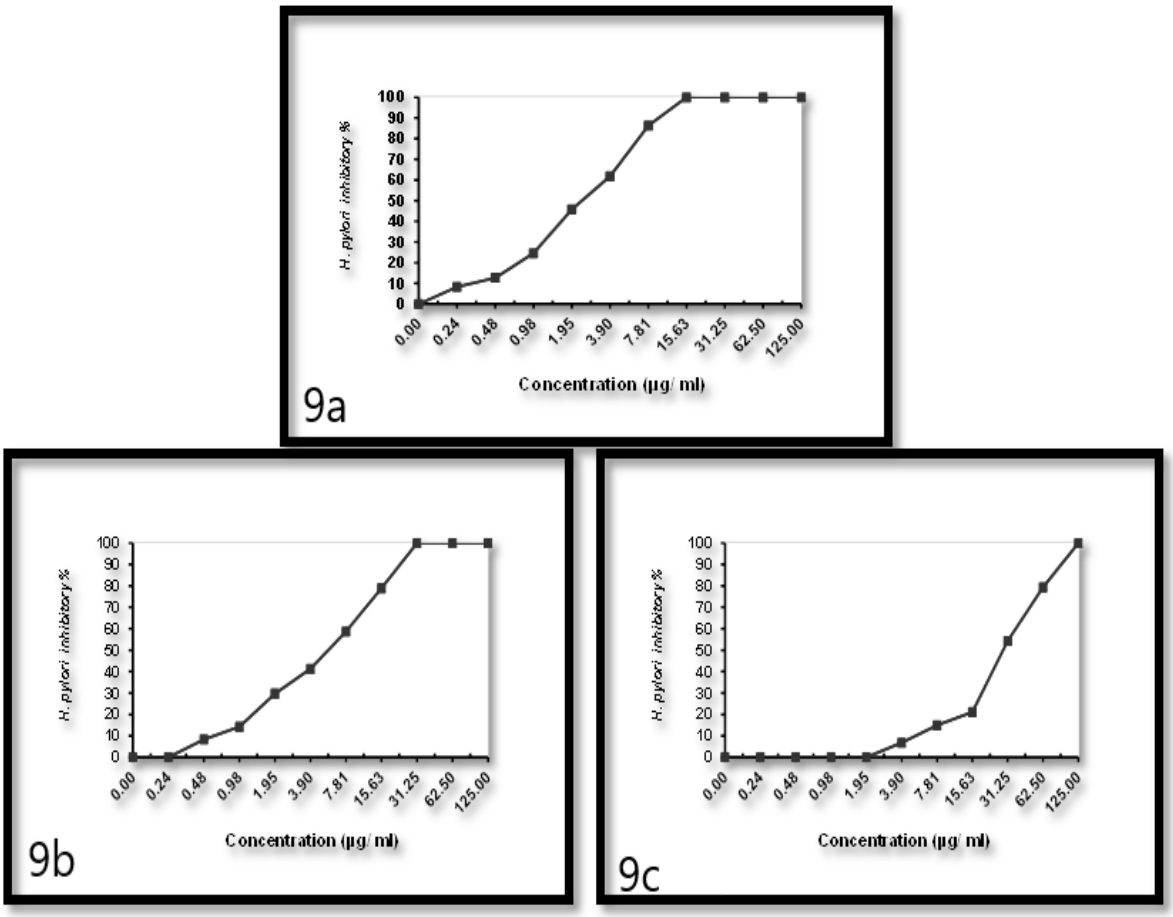



**Fig (9) :** Different types of honeys showed different anti-*Helicobacter* activity each with MIC values **( 9a samar honey , 9b sidr honey , 9c clover honey).**

## 4. Conclusion

The current work concluded that each of clover, Sidr and Samar honey accelerate gastric ulcer healing , in addition to anti-inflammatory, antioxidant, gastroprotective , and anti- *H. pylori* properties. Samar honey achieved the best results when compared with clover and Sidr honey, in all histological, histochemical, immunohistochemical, biochemical results, which may attributed to its high content of phenolic compounds and flavonoids. Our observations recorded that antiulcerogenic properties of honey may attributed to its modulatory effects on HSP47 and VEGF expression. The current work presents HSP47 as immunohistochemical marker for gastric ulcer healing progress, moreover, we can recommend Perls Prussian blue as histochemical marker for gastric inflammation and ulcer.

## 5. Conflicts of interest

*"There are no conflicts to declare".*

## 6.Funding Sources

*"No funding sources"*

## 7. References

[1] Alazzouni AS, Fathalla AS, Gabri MS, Dkhil MA, Hassan BN. Role of bone marrow derived-mesenchymal stem cells against gastric ulceration: Histological, immunohistochemical and ultrastructural study. Saudi J Biol Sci. 2020;27(12):3456-3464. doi:10.1016/j.sjbs.2020.09.044.

[2] Nabil M, El Raey MA, Abdo W, et al. Gastro-Protective Effects of Albizia anthelmintica Leaf Extract on Indomethacin-Induced Gastric Ulcer in Wistar Rats: In Silico and In Vivo Studies. Antioxidants (Basel). 2021;10(2):176. Published 2021 Jan 26. doi:10.3390/antiox10020176.

[3] Al Asmari A, Al Shahrani H, Al Masri N, Al Faraidi A, Elfaki I, Arshaduddin M. Vanillin abrogates ethanol induced gastric injury in rats via modulation of gastric secretion, oxidative stress and inflammation. Toxicol Rep. 2015;3:105-113. Published 2015 Dec 12. doi:10.1016/j.toxrep.2015.11.001.

[4] El-Azab NE, Mansy AE, El-Mahalawy AM, and Sabry D. Comparative study of the therapeutic effect of pomegranate alone or in combination with bone marrow mesenchymal stem cells on experimentally induced gastric ulcer in adult male rats: a histological and immunohistochemical study. Egypt. J. Histol. 2018;41 (2), 150–166. DOI: 10.21608/EJH.2018.13838

[5] Ajayi AF, Folawiyo AM, Salami TA. Ulcer healing mechanism of ethanolic extract of Talinum triangulare in male wistar rats. Unravelled Knowledge J. Pharm. Biosci. 2016;4:41–50. DOI:10.20510/UKJPB/4/I4/110647

[6] Dai XP, Li JB, Liu ZQ, Ding X, Huang CH, Zhou B. Effect of Jianweiyuyang granule on gastric ulcer recurrence and expression of VEGF mRNA in the healing process of gastric ulcer in rats. World J Gastroenterol. 2005;11:5480–5484. DOI: 10.3748/wjg.v11.i35.5480

[7] Luo JC, Peng YL, Chen TS, et al. Clopidogrel inhibits angiogenesis of gastric ulcer healing via downregulation of vascular endothelial growth factor receptor 2. J Formos Med Assoc. 2016;115(9):764-772. doi:10.1016/j.jfma.2015.07.022.

[8] Ruigrok MJR, El Amasi KEM, Leeming DJ, et al. Silencing Heat Shock Protein 47 (HSP47) in Fibrogenic Precision-Cut Lung Slices: A Surprising Lack of Effects on Fibrogenesis?. Front Med (Lausanne).2021;8:607962. doi:10.3389/fmed.2021.607962.

[9] Ghramh HA, Ibrahim EH, & KilanynM. Study of anticancer, antimicrobial, immunomodulatory, and silver nanoparticles production by Sidr honey from three different sources. Food science & nutrition. 2019; 8(1), 445–455.

[10] Qadir J , Wani J, Yatoo A, Zehra A, Rasool S, Ali S, and Majid S. Heath Benefits of Phenolic Compounds in Honey: An Essay. In: Rehman M.U., Majid S. (eds) Therapeutic Applications of Honey and its Phytochemicals. Springer, Singapore. 2020 ; pp 361-388.

[11] Samarghandian S, Farkhondeh T, Samini F. Honey and Health: A Review of Recent Clinical Research. Pharmacognosy Res. 2017;9(2):121-127. doi:10.4103/0974-8490.204647.

[12] Miguel MG, Antunes MD, Faleiro ML. Honey as a Complementary Medicine. Integr Med Insights. 2017;12:1178633717702869.

[13] Bogdanov S. Harmonized Methods of the International Honey Commission. International Honey Commission. International Honey Commission. 2009 ;pp 1-61.

[14] AOAC . Official Method of Analysis Association of Analytical Chemists. 19th Edition, Washington DC, 2012; 121-130.

[15] Aljohar HI, Maher HM, Albaqami J, et al. Physical and chemical screening of honey samples available in the Saudi market: An important aspect in the authentication process and quality assessment. Saudi Pharm J. 2018;26(7):932-942. doi:10.1016/j.jsps.2018.04.013.

[16] Velioglu Y.S., Mazza G., Gao L., Oomah B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agr. Food Chem. 46:4113–4117.

[17] Chang CC., Yang MH., Wen HM , and Chern JC. "Estimation of total flavonoid content in propolis by two complementary colometric methods," Journal of Food and Drug Analysis: (2002) Vol. 10 : Iss. 3 , Article 3. doi :10.38212/2224-6614.2748.

[18] Chizzola R, Michitsch H, Franz C. Antioxidative properties of Thymus vulgaris leaves: comparison of different extracts and essential oil chemotypes. J Agric Food Chem. 2008;56(16):6897-6904. doi:10.1021/jf800617g.

[19] GCC Standardization Organization (GSO), 2014. GSO 5/FDS Final Draft for Honey. pp. 1–12..

[20] Egyptian standard. Honey and method of analysis :Part one: Honey. Egyptian stander (2005/1-355). Egyptian Organization for Standerdization and Quality.A.R.E.P.1-12.

[21] Mahdy A, Shehab NG, Bayoumi FA. Protective Effects of Honey Solution and Fagonia indica Alcoholic Extract Against Ethanol-Induced Gastric Ulcer in Rats. Int J Clin Pharmacol Pharmacother. 2018 ;3: 133. doi:10.15344/2456-3501/2018/133.

[22] Al-Qaraghuli AMS, Abdel Wahab EMN, Al-Ani IM, Faisal GG. Effects of Xiang Sha Yang Wei Wan on Ethanol-Induced Gastric Ulcer in Sprague Dawley Rats: a Histological Study. The International Medical Journal Malaysia. 2013 ;12 (2): 3-10.

[23] Nordin N, Salama SM, Golbabapour S, Hajrezaie M, Hassandarvish P, Kamalidehghan B, Majid NA, Hashim NM, Omar H, Fadaienasab M, Karimian H, Taha H, Ali HM, Abdulla MA. Anti-ulcerogenic effect of methanolic extracts from Enicosanthellum pulchrum (king) Heusden against ethanol-induced acute gastric lesion in animal models. PLoS One. 2014;9(11):e111925. doi: 10.1371/journal.pone.0111925.

[24] Rouhollahi E, Moghadamtousi SZ, Hamdi OA, et al. Evaluation of acute toxicity and gastroprotective activity of curcuma purpurascens BI. rhizome against ethanol-induced gastric mucosal injury in rats. BMC Complement Altern Med. 2014;14:378. Published 2014 Oct 6. doi:10.1186/1472-6882-14-378.

[25] Bancroft JD. And Gamble M. Theory and practice of histological techniques, 5 edition, Churchill Livingstone, New York, London; (2008): p. 153.

[26] Ellis R. Perls Prussian blue Stain Protocol, Pathology Division. Queen Elizabeth Hospita; South Australia: 2007.

[27] Ramos-Vara JA, Kiupel M, Baszler T, et al. Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. J Vet Diagn Invest. 2008;20(4):393-413. doi:10.1177/104063870802000401.

[28] Ortaç D, Cemek M, Karaca T, et al. In vivo anti-ulcerogenic effect of okra (Abelmoschus esculentus) on ethanol-induced acute gastric mucosal lesions. Pharm Biol. 2018;56(1):165-175.

[29] SatoY, Rifkin DB. Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J. Cell Biol. 1988 ; 107(3), 1199–1205.

[30] Bonacorsi C, Raddi MS, Carlos IZ, Sannomiya M, Vilegas W. Anti-Helicobacter pylori activity and immunostimulatory effect of extracts from Byrsonima crassa Nied. (Malpighiaceae). BMC Complement Altern Med. 2009;9:2. Published 2009 Jan 16. doi:10.1186/1472-6882-9-2.

[31] Can Z, Yildiz O, Sahin H, Akyuz Turumtay E, Silici S, Kolayli S. An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chem. 2015;180:133-141.

[32] Smetanska I, Alharthi S, and Selim K. Physicochemical, antioxidant capacity and color analysis of six honeys from different origin. Journal of King Saud University – Science ; 2021 ; 33 101447.

[33] Abselami A , Tahani A, Sindic M , Fauconnier ML, Bruneau E, Elbachiri A. Physicochemical Properties of some Honeys Produced from Different Flora of Eastern Morocco. J. Mater. Environ. Sci., 2018, Volume 9, Issue 3, Page 879-886.

[34] Alvarez-Suarez JM, González-Paramás AM, Santos-Buelga C, Battino M. Antioxidant characterization of native monofloral Cuban honeys. J Agric Food Chem. 2010;58(17):9817-9824. doi:10.1021/jf1018164.

[35] Roby MHH, Abdelaliem YF, Esmail AM, Mohdaly AAA, Ramadan MF. Evaluation of Egyptian honeys and their floral origins: phenolic compounds, antioxidant activities, and antimicrobial characteristics. Environ Sci Pollut Res Int. 2020;27(17):20748-20756. doi:10.1007/s11356-020-08586-7.

[36] Abdulaziz SA , Ayman AO, and Awad AM. Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia. Arab J Chem 2016 ; 9:114–120.

[37] Pontis JA , Costa DA , Silva SJ , Flach A. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. Food Sci. Technol, 2014; vol.34, n.1, pp.69-73.

[38] Ferreira ICFR , Aires E., Barreira JCM , and Estevinho LM. Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. Food Chem. 2009; 114, 1438–1443.

[39] El-Borai AM, Youssef GA, Ghareeb DA , and Abdel-Tawab MM, Antibacterial and Antioxidant Activities of Different Varieties of Locally Produced Egyptian Honey. Egypt. J. Bot., 2018; Vol. 58, No, 1. pp. 97 – 107.

[40] Liu Y, Sui D, Fu W, Sun L, Li Y, Yu P , Yu X, Zhou Y, and Xu H. Protective effects of polysaccharides from Panax ginseng on acute gastric ulcers induced by ethanol in rats. Food & function. 2021; 12, 2741-2749.

[41] Raish M, Shahid M, Bin Jardan YA, et al. Gastroprotective Effect of Sinapic Acid on Ethanol-Induced Gastric Ulcers in Rats: Involvement of Nrf2/HO-1 and NF-κB Signaling and Antiapoptotic Role. Front Pharmacol. 2021;12:622815. doi:10.3389/fphar.2021.622815.

[42] Katary MA, Salahuddin A. Gastroprotective Effect of Punicalagin against Ethanol-Induced Gastric Ulcer: the Possible Underlying Mechanisms. Biomark J. 2017, 3:3. doi: 10.21767/2472-1646.100025.

[43] Mousa AM, El-Sammad NM, Hassan SK, et al. Antiulcerogenic effect of Cuphea ignea extract against ethanol-induced gastric ulcer in rats. BMC Complement Altern Med. 2019;19(1):345. doi:10.1186/s12906-019-2760-9.

[44] Sattar A, Abdo A, Mushtaq MN, Anjum I, Anjum A. Evaluation of Gastro-protective Activity of Myristica fragrans on Ethanol-induced Ulcer in Albino Rats. An Acad Bras Cienc. 2019;91 (2):e20181044.. doi:10.1590/0001-3765201920181044

[45] Ranneh Y, Akim AM, Hamid HA, et al. Honey and its nutritional and anti-inflammatory value. BMC Complement Med Ther. 2021;21(1):30. doi:10.1186/s12906-020-03170-5.

[46] Scepankova H, Combarros-Fuertes P, Fresno JM, et al. Role of Honey in Advanced Wound Care. Molecules. 2021;26(16):4784. doi:10.3390/molecules26164784.

[47] Taha MM, Abdelwahab SI, Elsanousi R, Sheikh BY, Abdulla MA, Babiker SE, et al. Effectiveness of Sidr Honey on the prevention of ethanol-induced gatroulcerogenesis: role of antioxidant and antiapoptotic mechanism. Pharmacognosy Journal. 2015;7(3):157-164. DOI:10.5530/pj.2015.3.3.

[48] Chatterjee A, Chatterjee S, Das S, Saha A, Chattopadhyay S, and Bandyopadhyay, SK. Ellagic acid facilitates indomethacin-induced gastric ulcer healing via COX-2 up-regulation. Acta biochimica et biophysica Sinica, 2012 ; 44(7), 565–576.

[49] Fazalda A, Quraisiah A, Azlina MFN . Antiulcer effect of honey in nonsteroidal anti-inflammatory drugs induced gastric ulcer model in rats: a systematic review. Evid Based Complement Alternat Med 2018:7515692.

[50] Mushtaq S, Imtiyaz Z, Wali AF , Khan A , Rashid SM , Amin I, Ali A, Rehman MU, Arafah A. Honey: A Powerful Natural Antioxidant and Its Possible Mechanism of Action. In Therapeutic Applications of Honey and Its Phytochemicals; Rehman, M.U., Majid, S., Eds.; Springer: Singapore, 2020.

[51] Almasaudi SB, El-Shitany NA, Abbas AT, et al. Antioxidant, Anti-inflammatory, and Antiulcer Potential of Manuka Honey against Gastric Ulcer in Rats. Oxid Med Cell Longev. 2016;2016:3643824. doi:10.1155/2016/3643824.

[52] Al-Ghamdi AA and Ansari MJ. Biological and therapeutic roles of Saudi Arabian honey: A comparative review. Journal of King Saud University - Science, 2021; 33,( 2) : 101329. doi:10.1016/j.jksus.2020.101329.

[53] Al-Waili N, Salom K, Al-Ghamdi AA. Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. ScientificWorldJournal. 2011;11:766-787. Published 2011 Apr 5. doi:10.1100/tsw.2011.78.

[54] Sharma AV, Ganguly K, Paul S, Maulik N, Swarnakar S. Curcumin heals indomethacin-induced gastric ulceration by stimulation of angiogenesis and restitution of collagen fibers via VEGF and MMP-2 mediated signaling. Antioxid Redox Signal. 2012;16(4):351-362. doi:10.1089/ars.2011.4232.

[55] Zhang W, Liu K, Li L, et al. Therapeutic effect of low molecular weight chitosan containing sepia ink on ethanol-induced gastric ulcer in rats. Acta Cir Bras. 2016;31(12):813-820. doi:10.1590/S0102-865020160120000006.

[56] Fornai M, Antonioli L, Colucci R, Tuccori M, and Blandizzi C. Pathophysiology of Gastric Ulcer Development and Healing: Molecular Mechanisms and Novel Therapeutic Options. Peptic Ulcer Disease, 2011. p. 113-142.

[57] Iftikhar F, Arshad M, Rasheed F, Amraiz D, Anwar P, Gulfraz M. Effects of acacia honey on wound healing in various rat models. Phytother Res. 2010;24(4):583-586. doi:10.1002/ptr.2990.

[58] Oryan, A.; Alemzadeh, E.; Moshiri, A. Biological properties and therapeutic activities of honey in wound healing: A narrative review and meta-analysis. J. Tissue Viability 2016, 25, 98–118.

[59] Akhtar M., Malik A., Arshad H., Batool S., Raza A., Tabassum T. , Murtaza M., Riaz M., Noreen M. & Rasool G. Protective effect of newly synthesized indole imines against ethanol induced gastric ulcer in rats, Biotechnology & Biotechnological Equipment, 2021; 35:1, 231-237. DOI: 10.1080/13102818.2020.1868330.

[60] Marginean EC, Bennick M, Cyczk J, Robert ME, Jain D. Gastric siderosis: patterns and significance. Am J Surg Pathol. 2006;30(4):514-520. doi:10.1097/00000478-200604000-00013.

[61] Hattori H. High prevalence of haemosiderin accumulation in the cytoplasm of gastric glands in patients with liver cirrhosis. J Clin Pathol. 2004;57(6):621-624. doi:10.1136/jcp.2003.010405.

[62] Kothadia JP, Arju R, Kaminski M, Mahmud A, Chow J, Giashuddin S. Gastric siderosis: An under-recognized and rare clinical entity. SAGE Open Med. 2016;4:2050312116632109. doi:10.1177/2050312116632109.

[63] Ji H, Yardley JH. Iron medication-associated gastric mucosal injury. Arch Pathol Lab Med. 2004;128(7):821-822. doi:10.5858/2004-128-821-IMGMI.

[64] De Petris G, Gatius Caldero S, Chen L, et al. Histopathological changes in the gastrointestinal tract due to drugs: an update for the surgical pathologist (part I of II). Int J Surg Pathol. 2014;22(2):120-128. doi:10.1177/1066896913502229.

[65]Yoo CY, Son HU, Fanar A, Choi HJ, Alam MB, Lee SH. Opuntia humifusa aqueous extract alleviates ethanol-induced gastric ulcer in a mouse model. Asian Pac J Trop Biomed 2020;10:403-10.

[66] Zaghlool SS, Abo-Seif AA, Rabeh MA, Abdelmohsen UR, Messiha BAS. Gastro-Protective and Anti-Oxidant Potential of Althaea officinalis and Solanum nigrum on Pyloric Ligation/Indomethacin-Induced Ulceration in Rats. Antioxidants (Basel). 2019;8(11):512. doi:10.3390/antiox8110512.

[67] Masad RJ, Haneefa SM, Mohamed YA, et al. The Immunomodulatory Effects of Honey and Associated Flavonoids in Cancer. Nutrients. 2021;13(4):1269. doi:10.3390/nu13041269.

[68] Angioi R, Morrin A, White B. The Rediscovery of Honey for Skin Repair: Recent Advances in Mechanisms for Honey-Mediated Wound Healing and Scaffolded Application Techniques. Applied Sciences. 2021; 11(11):5192. doi: 10.3390/app11115192.

[69] Ibrahim RM, Allam MM, El-Gohary OA, El-Talees AE, El-Hamady MS. Protective effect of obestatin on indomethacin-induced acute gastric ulcer in rats: role of VEGF and TNF-α. Benha Med J. 2018 ;35:369-77.

[70] Takeuchi K, Amagase K. Roles of Cyclooxygenase, Prostaglandin E2 and EP Receptors in Mucosal Protection and Ulcer Healing in the Gastrointestinal Tract. Curr Pharm Des. 2018;24(18):2002-2011. doi:10.2174/1381612824666180629111227.

[71] Chaudhary A, Bag S, Banerjee P, Chatterjee J. Wound Healing Efficacy of Jamun Honey in Diabetic Mice Model through Re-epithelialization, Collagen Deposition and Angiogenesis. Journal of traditional and complementary medicine, 2020; 10, 529–543.

[72] Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. J Surg Res. 2009;153(2):347-358. doi:10.1016/j.jss.2008.04.023.

[73]Vasques MT, Alves MA, de Cerqueira Luz JG, Corrêa L. Immunolocalization of heat shock proteins 27 and 47 during repair of induced oral ulcers. J Oral Sci. 2010;52(4):623-631. doi:10.2334/josnusd.52.623.

[74] Wang JF, Olson ME, Winkfein RJ, Kulyk WM, Wright JB, Hart DA. Molecular and cell biology of porcine HSP47 during wound healing: complete cDNA sequence and regulation of gene expression. Wound Repair Regen. 2002;10(4):230-240. doi:10.1046/j.1524-475x.2002.10406.x.

[75] Tsukimi Y, Okabe S. Recent advances in gastrointestinal pathophysiology: role of heat shock proteins in mucosal defense and ulcer healing. Biol Pharm Bull. 2001;24(1):1-9. doi:10.1248/bpb.24.1.

[76] Luo H, Liu T, Yang H, Ye H and Luo X, "Expression of Collagen (Types I, III, and V), HSP47, MMP-2, and TIMP-1 in Retrobulbar Adipose Tissue of Patients with Thyroid-Associated Orbitopathy", Journal of Ophthalmology, vol. 2020, Article ID 4929634, 5 pages, 2020.

[77] Azmi MF, Abd Ghafar N, Che Hamzah J, Chua KH, Ng SL. The role of Gelam honey in accelerating reepithelialization of ex vivo corneal abrasion model. J Food Biochem. 2021;45(4):e13645. doi:10.1111/jfbc.13645.

[78] Aliyu M, Ibrahim S, Inuwa HM, et al. Ameliorative Effects of Acacia Honey against Sodium Arsenite-Induced Oxidative Stress in Some Viscera of Male Wistar Albino Rats. Biochem Res Int. 2013;2013:502438. doi:10.1155/2013/502438.

[79] Rao PV, Krishnan KT, Salleh N, Gan SH. Biological and therapeutic effects of honey produced by honey bees and stingless bees: A

comparative review. Rev. Bras. Farmacogn. 2016 ; 26, 657–664.
[80] Jibril, F.I., Hilmi, A.B.M. & Manivannan, L. Isolation and characterization of polyphenols in natural honey for the treatment of human diseases. Bull Natl Res Cent 43, 4 (2019). doi.:10.1186/s42269-019-0044-7.
[81] Sell SA, Wolfe PS, Spence AJ, et al. A preliminary study on the potential of manuka honey and platelet-rich plasma in wound healing. Int J Biomater. 2012;2012:313781. doi:10.1155/2012/313781.
[82] Ranzato E, Martinotti S, Burlando B. Honey exposure stimulates wound repair of human dermal fibroblasts. Burns Trauma. 2013;1(1):32-38. Published 2013 Jun 18. doi:10.4103/2321-3868.113333.
[83] Abd Ghafar N, Ker-Woon C, Hui CK, Mohd Yusof YA, Wan Ngah WZ. Acacia honey accelerates in vitro corneal ulcer wound healing model. BMC Complement Altern Med. 2016;16:259. Published 2016 Jul 29. doi:10.1186/s12906-016-1248-0.
[84] Afonso A, Gonçalves J, Luís Â, Gallardo E, Duarte AP. Evaluation of the In Vitro Wound-Healing Activity and Phytochemical Characterization of Propolis and Honey. Applied Sciences. 2020; 10(5):1845 . doi: 10.3390/app10051845.
[85] Nzeako BC, Al-Namaani F. The antibacterial activity of honey on *Helicobacter pylori*. Sultan Qaboos Univ Med J. 2006;6(2):71-76.
[86] Ndip, R.N., Takang, A.E.M., Echakachi, C.M., Malongue, A., Akoachere, J.F.T.K., Ndip, L.M. & Luma, H.N. In vitro antimicrobial activity of selected honeys on clinical isolates of *Helicobacter pylori.* African Health Sciences. 2007 ; 7(74): 228-231.
[87] Manyi-Loh CE, Clarke AM, Munzhelele T, Green E, Mkwetshana NF, Ndip RN. Selected South African honeys and their extracts possess in vitro anti-Helicobacter pylori activity. Arch Med Res. 2010;41(5):324-331. doi:10.1016/j.arcmed.2010.08.002.
[88] Kim SG, Han MS, Jang RH , Hong PI, Woo OS and Kim HS. Composition and Anti-Helicobacter pylori Activity of the Different Floral Honeys Form Korea. Journal of Apiculture. 2015; 30(3) : 217-223.
[89] Abdel-Latif MM, Abouzied MM. Molecular Mechanisms of Natural Honey Against H. pylori Infection Via Suppression of NF-κB and AP-1 Activation in Gastric Epithelial Cells. Arch Med Res. 2016;47(5):340-348. doi:10.1016/j.arcmed.2016.09.002.
[90] Quraisiah A, Fazalda A, Alfizah H, and Nur Azlina MF. In vitro Study of Anti-Helicobacter pylori Activity of Honey: A Systematic Review. Sains Malaysiana , 2020; 49(2): 411-420.
[91] Jantakee K, Tragoolpua Y. Activities of different types of Thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals. Biol.Res. 2015 ; vol.48. ISSN 0716-9760.
[92] Petric D. (2020): Natural Antibiotics. [ in Press].
[93] Szweda P. Antimicrobial Activity of Honey, Honey Analysis, Vagner de Alencar Arnaut de Toledo, IntechOpen, 2017. DOI: 10.5772/67117.