



## The antioxidant impact of Saudi sidr honey against acetyl salicylic acid- induced gastric ulcer

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### General Note



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### ABSTRACT

It was mostly known that nonsteroidal anti-inflammatory drugs (NSAIDs) frequently be a main cause of gastric ulcers (GUs). Honey market is reaching a notable advance due to the strong cultural, social and religious relation between people and using honey. This study investigated the protective effects of Saudi sidr honey on aspirin induced gastric ulcer in rats. Rats were divided into three groups, G1, Healthy negative control. G2: Gastric ulcer rats, ulcer was induced by 4 doses of Aspirin taken orally (200 mg/kg b.w) G3: Saudi Sidr Honey group after induction of gastric ulcer, Sidr honey was administered in the dose. Results showed that Aspirin induced oxidative stress in gastric tissues that manifested by decreased levels of superoxide dismutase (SOD), reduced glutathione

(GSH), together with increased levels of malondialdehyde (MDA) and xanthine oxidase (XO). Our results showed using Saudi Sidr honey modified the results and reduced oxidative stress. For this reason it posses an antioxidant protective role against gastric ulcer.

**Keywords:** Gastric ulcer, Aspirin, Saudi sidr honey, Oxidative stress.

## 1. INTRODUCTION

Gastric ulcer (GU) is a common disease that disturbs the digestive system and causes death for about 5–10 % during human life time, being a major public health burden in the present century. Numerous studies were conducted to observe factors that leads to gastric ulcer, although the etiology and pathogenesis of GU remains controversial, numerous studies have revealed that it may due to the serious imbalance between mucosal invasive factors (such as long period consumption of nonsteroidal anti-inflammatory drugs) and the protective factors of gastric mucosa such as levels of prostaglandins and antioxidant enzymes, causes a disturbance of gastric mucosal defensive barrier and leads to gastric ulcer (Li et al., 2019). There are destructive factors that cause GU such are *Helicobacter pylori*, nonsteroidal anti-inflammatory drugs, ethanol and genetic factors. Ethanol has a devastating effect on gastric mucosa as a result it has been used to induce gastric ulcer in animal models (Ibrahim et al., 2016). Alcohol has been shown to compromise the integrity of gastric mucosa by aiding acid reflux into the subluminal mucosa and submucosa layer. It may also act through a general mechanism affecting the release of hormones and the regulation of nerve function involved in acid secretion (Ortac et al., 2018). Aspirin drug is widely distributed in the whole body and has a strong effect, because it is easily absorbed after oral administration. Aspirin is extensively used to treat fever, headache and neuralgia. Itraconazole, a triazole antifungal agent, is a broad-spectrum triazole (Liu et al., 2019).

Aspirin is widely prescribed for the treatment of inflammatory diseases such as rheumatoid arthritis, and is commonly used for the prevention of cardiovascular thrombosis However; the prolonged use of NSAIDs is usually associated with gastric mucosal erosions, ulceration, bleeding, and perforation. NSAIDs cause gastric injury by inhibiting the synthesis of endogenous prostaglandins (PGs) (Mahmoud and El-Ghaffar, 2019). Treatment of gastric ulcer using usual known therapy aspects a chief delay because, utmost of the drugs that accessible in the market represents incomplete efficiency against gastric diseases and mainly causes side effects. However, several medicinal plants are used in folk medicine to treat gastrointestinal disorders and they have been shown to produce promising results in the treatment of these pathologies (Sathish et al., 2011).

Honey is a valuable functional food with a plenty of nutrients and it has been utilized as traditional and complementary medicine since ancient times. It has numerous beneficial biological activities, like antibacterial, antioxidant, anti-browning, angiotensin converting enzyme (ACE) inhibitor, antiinflammatory, antiparasitic, and immunosuppressive (Ghramh et al., 2019). Recently, its therapeutic role in the treatment of burns, healing of infected and chronic wounds, skin ulcers, eye ailments, asthma, gastrointestinal disorders, and its medicinal effects like anticancer, antimutagenic, antiproliferative, hepatoprotective and hypoglycaemic properties have been ascribed (Moniruzzaman et al., 2013).

Sidr honey is a premium original honey in many countries; it was used for the remediation of different health troubles in humans and used experimentally in disorders of rat models. Moreover, honey contains phytochemical compounds that accounts for its notable antipyretic, anti-inflammatory, analgesic effects (Hegazi et al., 2017). This comparative study investigated the antioxidant effect of Saudi Sidr honey against gastric ulcer

## 2. MATERIALS AND METHODS

### Materials

Saudi Sidr honey was obtained from AL-Shifa Company, KSA.

### Chemicals

Aspirin, (Acetyl Salicylic acid 200 mg), was purchased from Bayer Company, USA.

### Animals

Twenty one adult male Albino rats weighing 150-170 g were operated in this study. Rats were obtained from the Animal House Colony of the King Fahd Medical Research Center (KFMRC), Jeddah, Saudi Arabia. Animals were acclimatized according to laboratory circumstances one week before beginning of experiments. The animals were grouped and housed in cages environmentally controlled ( $24\pm 1$  °C,  $45\pm 5\%$  humidity and 12 h light/dark cycle) at King Fahd Medical Research Center Animal Facility Breeding

Colony. A commercial balanced diet was given and tap water applied *ad libitum*. The experiment was approved by the Ethical Committee of King Fahd Medical Research Center. Jeddah, KSA. Approval number (172-19).

### Experimental design

The rats were divided into three groups of seven as follows:

Group (1): Healthy rats. These rats received only saline. (Negative Control).

Group (2): Gastric ulcer induced rats, ulcer was induced by 4 doses of Aspirin taken orally (200 mg/kg b.w) according to (Wang et al., 2007) (Positive control).

Group (3): Saudi Sidr Honey (Therapeutic group): after induction of gastric ulcer (by Aspirin taken orally (200 mg/kg b.w), Saudi Sidr honey was administered in the dose (0.5g/kg b.w/daily) according to (Al-Yahya et al., 2013) for 30 days.

### Blood sample collection

At the end of experimental period (3 weeks), the rats were fasted overnight, prior to blood collection then scarified under ether anesthesia then blood was collected through retro-orbital puncture. Blood was allowed to set for 30 min. to separate serum at temperature of 25°C then centrifuged at 3000 rpm for 20 minutes, then divided into several aliquots and stored at -20 C until analysis was performed.

### Macroscopic examinations

The stomachs were opened along the greater curvature and washed with 0.9% NaCl and examined for macroscopic lesions in the glandular part.

### Measurement of ulcer index UI

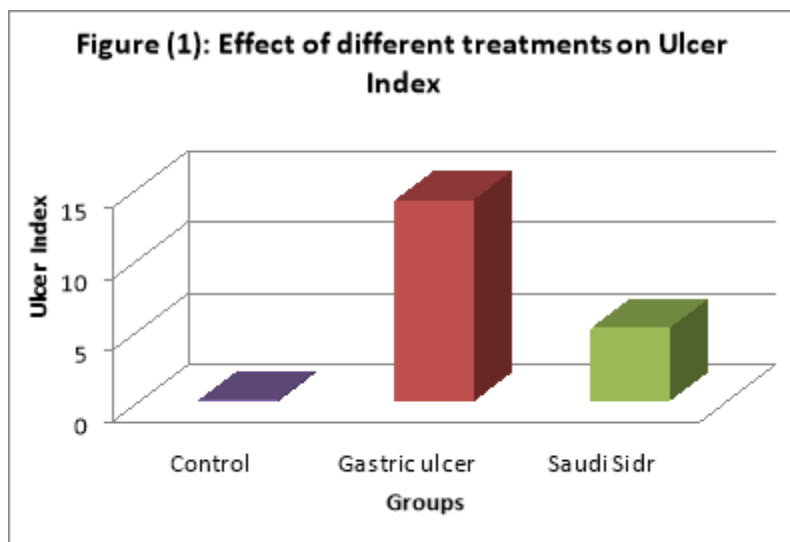
The gastric mucosal lesions were expressed in terms of ulcer index (UI) which depends on the calculation of the severity of each lesion by using 0-3 scoring system. The severity factor was defined according to the length of the lesions, where severity factor 0 = no lesions; severity factor 1 = lesions less than 1 mm length, severity factor 2 = lesions 2-4 mm in length and severity factor 3= lesions greater than 4 mm in length. The lesion score for each rat was calculated as the number of lesions in the rat multiplied by their respective severity factor (Singh et al., 2008).

### Biochemical Analysis

Serum oxidative biomarkers, including superoxide dismutase (SOD), the lipid peroxidation marker, malondialdehyde (MDA), xanthine oxidase (XO), and reduced glutathione (GSH), were evaluated spectrophotometrically using Biovision Kit, CA. USA.

### Statistical analysis

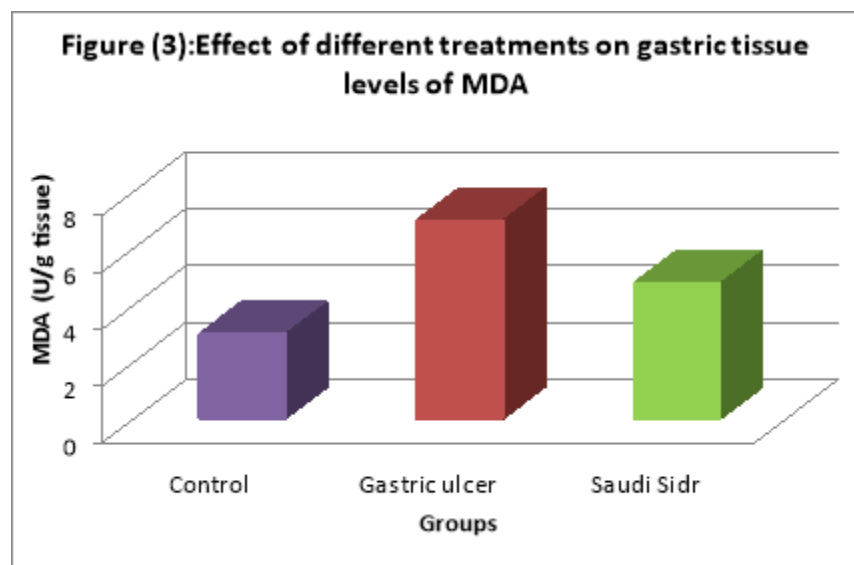
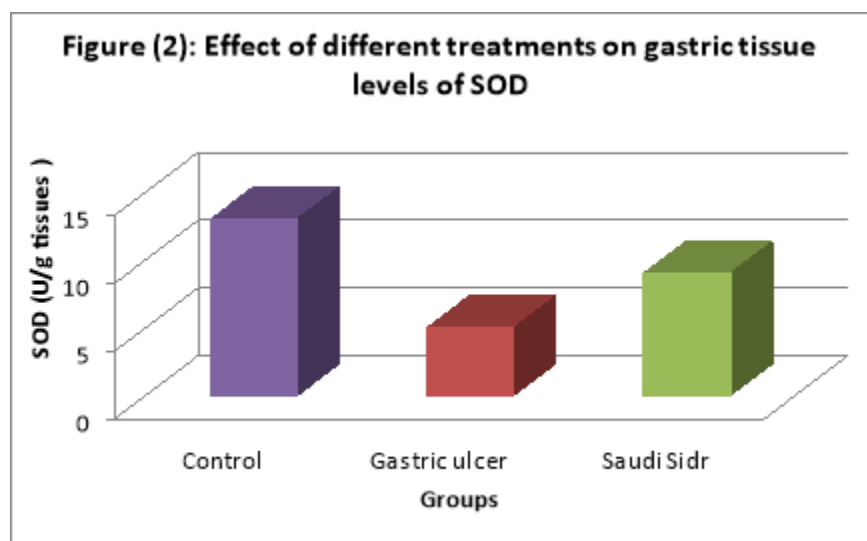
Data were statistically analyzed using the statistical software, SPSS. The differences between mean values were determined by analysis of variance (ANOVA test),  $p < 0.001$  was considered significant.



### 3. RESULTS

Figure 1 shows the effect of different treatments on the ulcer index. The administration of aspirin significantly increased the ulcer index ( $p \leq 0.001$ ) in the GU group. Treatment with Saudi Sidr honey reduced ulcer index significantly ( $p \leq 0.001$ ) compared to the gastric ulcer rats.

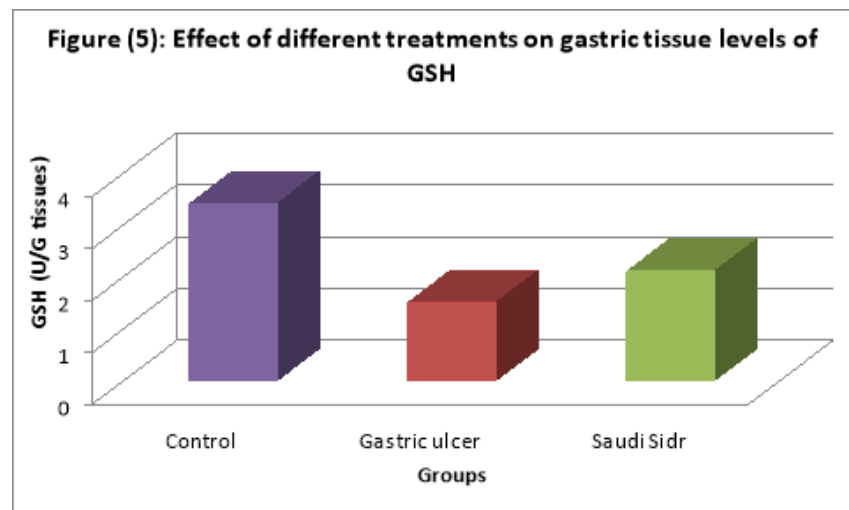
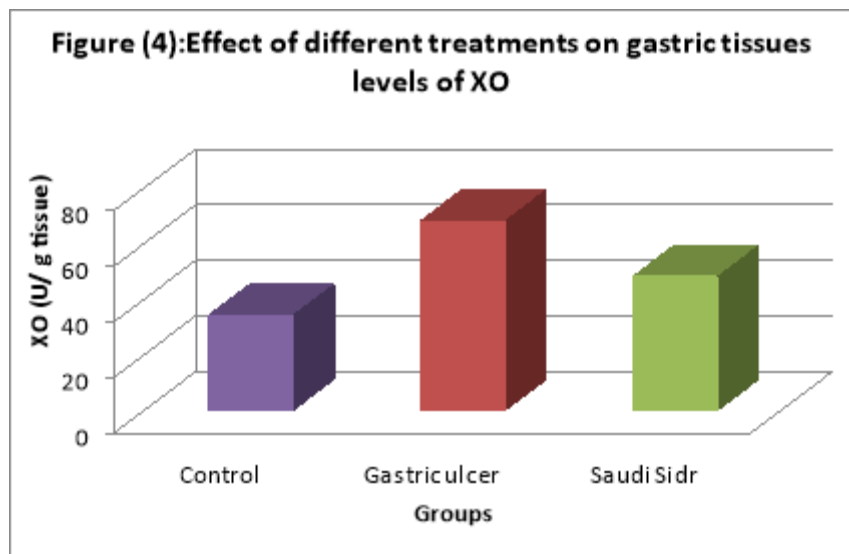
Oxidative stress is a critical pathogenic factor in the process of gastric ulceration. Figures (2, 3, 4 and 5) represented the effect of different treatments on gastric tissue oxidative stress markers. Aspirin-induced oxidative stress as indicated by a significant reduction ( $p \leq 0.001$ ) in gastric SOD and GSH enzyme levels and a significant elevation ( $p \leq 0.001$ ) in lipid peroxidation, MDA and XO levels compared to the control rat group. On the other hand, treatment by Saudi Sidr honey, significantly attenuated ( $p \leq 0.001$ ) the level of oxidative stress markers in all groups.



### 4. DISCUSSION

Gastric ulcer GU is considered as a big burden worldwide and as the most important common disease, affects millions of individuals (Awaad *et al.*, 2013) and the healing process plays a critical role in the treatment of gastric ulcer. For many decades gastric ulcer was identified as a lesion of the gastric mucous wall, and it was the most frequent cause of surgery, with high morbidity and mortality rates (Alimi *et al.*, 2010). In this study we used Aspirin as to induce peptic ulcer, this peptic ulcer model in the animals is preferable than those caused by other types because it is easier to administer and control. Moreover, aspirin-induced gastric ulceration is an experimental model used widely to investigate the beneficial effects of such therapeutic strategies (Shahrokhi *et al.*, 2015). This study confirms that aspirin damages the gastric mucosa as shown previously in animals (and humans, also it agrees with different studies

which demonstrated that the use of aspirin is associated with an elevated risk of symptomatic peptic ulcer. Moreover, the risk of peptic ulcer was elevated throughout treatment independently of its duration, was elevated with doses as low as 75 mg/d, and was no different from that with doses of 150 mg/d and 300 mg/d (Ishikawa et al., 2008). Reactive oxygen species ROS could promote marked cell damage by interacting with cellular components proteins, lipids and DNA, thus promoting lipid peroxidation (Viana et al., 2013). These mechanisms causes epithelial cells erosion, expansion of the inter-glandular area and the increased inflammation, promoting the installation of hemorrhagic gastric ulcer which is sometimes due to over excretion of acidic gastric juice (Li et al., 2008).



Oxidative stress has been well linked to the pathological process of gastric ulcer. Another inducer of gastric ulcer is ethanol which causes a disruption of optimum oxidant/antioxidant balance, which can lead to gastric mucosa injury via oxidative stress (Fahmy et al., 2020). It is well known that oxygen-derived free radicals can react with lipids to form lipid peroxides, for the main components of cellular membranes are lipids, which do extensive damage. If the processes were not neutralized by sufficient antioxidant molecules, their peroxidation can lead to cell death and/or apoptosis (Souza et al., 2019). In the present study, increased production of free radicals may be responsible for the membrane damage observed in pathological experiment as also evidenced by the raised lipid peroxidation namely TBARS. Oxygen-derived free radicals can be scavenged by the antioxidant defense system of the body, such as GSH, SOD and CAT. Reduction in cellular GSH and SOD and CAT activities could weaken retrieval after brief period of ethanol induced gastric oxidative injury (Badr et al., 2019). Results revealed that Aspirin increased the oxidative damage and disturbs antioxidant parameters levels. Aspirin induces acute mucosal damage within an hour which could be visualized as haemorrhage and erosion. In an acidic medium of stomach aspirin, penetrates the epithelial cells where it gets trapped causing cellular injury, uncouples mitochondrial oxidative phosphorylation, alters mucosal barrier function including physicochemical

characteristics of mucus and reduces epithelial surface hydrophobicity (Sokolova and Naumann, 2019). Results showed that, Aspirin increased MDA levels as well as decreased activities of SOD and GSH in the stomach of aspirin-ulcerated rats as a result of lipid peroxidation and over production of free radicals resulting in mucosal damage (Saadaoui et al., 2020). In addition, superoxide radicals are converted into H<sub>2</sub>O<sub>2</sub> and peroxy radicals at acidic pH (4.8) levels. MDA is the main end product of lipid peroxidation. Thus, measurement of gastric MDA level can estimate indirectly the level of lipid peroxidation (Sidahmed et al., 2015). Subsequently, the low gastric GSH level increased the rate of lipid peroxidation, which mediates gastric tissue damage.

One of the most important detoxification pathways of xenobiotics is their conjugation with GSH. GSH plays a major role for detoxification of H<sub>2</sub>O<sub>2</sub>. Increased GSH activity might have been sustained to counteract fast generating O<sub>2</sub> or may have protected the cells from reactive free radicals and peroxides (Van Bladeren, 2000). GSH-reductase mediates the reduction of oxidised glutathione to GSH, and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) is an electron donor in this reaction. It is possible that NADPH is lower in indomethacin-induced rats. Previous study have showed that ROS, lipid peroxidation and exhaustion of glutathione had an important part in the pathogenesis of acute lesions induced by Aspirin and are responsible for various types of oxidative damage in gastric mucosal cells (Park et al., 2013).

Results showed that treatment with manuka honey and Saudi Sidr honey improved the oxidative status even were administered as therapeutic or protective agent. Both gastric mucus and GSH serve as protective molecules against gastric mucosal injury (Cnubben et al., 2001). Manuka was able to protect gastric mucosa by reducing MDA level. This could be because it reserved gastric mucosal GSH contents and increased the formation of gastric mucosal nitric oxide NO. Natural honey prevented gastric mucosal lesions induced by ethanol through the production of nonprotein sulfhydryls and endogenous NO (Al-Waili, 2003).

Sidr honey modulated the 1928 aemor-expression of mitochondrial associated protein and plays a considerable effect against gastric ulcer. Future study is required to explore its potential clinical usage (Taha et al., 2015). Saudi Sidr Honey has been suggested to protect against lipid peroxidation by reducing the production of lipid hydroperoxides (Alvarez-Suarez et al., 2012). The antioxidant property of honey may be due to its phenolic or non-phenolic antioxidant contents, such as vitamin C, vitamin E and β-carotene.

## 5. CONCLUSION

This study showed that Saudi Sidr honey showed the most potent gastroprotective effect and confirms that it has a promising role in protection against the development of gastric ulcers.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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