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THE EFFECT OF HESPERIDEN & DIOSMIN MIXTURE ON GASTRIC ULCERS INDUCED BY INDOMETHACIN IN MICE

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ABSTRACT

Peptic ulcer is a lesion on the stomach lining known as a gastric ulcer or on the duodenum lining referred to as duodenal ulcers. This leads to a disruption of mucosal integrity. Non-Steroidal AntiInflammatory Drugs (NSAIDs) are primarily responsible for most cases of the disease. Flavonoids provide cytoprotective and restorative effects by enhancing protective factors such as mucus and prostaglandins, also by shielding against potentially harmful elements through their antioxidative, anti-inflammatory, and antibacterial activities. Therefore, this study aims to investigate the effect of Hesperidin and Diosmin Mixture (Hes&Dio) on gastric ulcers induced by Indomethacin in mice. Animals were divided into 4 groups (n= 6 in each): group 1 (Control, CMC), group 2 (Indomethacin), group 3 (Famotidine 8.3 mg/kg), group 4 (Hesperidin and Diosmin 615 mg/kg). Drugs were given orally for 15 days, and then gastric ulcers were induced by a single oral dose of Indomethacin (275 mg/kg) in the last day of the experiment. Histological findings revealed that the group of animals that received Indomethacin experienced ulceration of the entire mucosa, while pre-treatment with Hesperidin and Diosmin reduced ulceration and the depth of lesions in comparison to the Indomethacin group. There was no statistically significant difference observed between the Hes&Dio mixture group and the Famotidine group Our study was the only and the first study that evaluated the effectiveness of mixture of two components (Hesperidin and Diosmin) in Indomethacin induced gastric ulcers, in light of many researches that studied the effect of only one component of the mixture.

KEYWORDS: Hesperidin & Diosmin, Gastric Ulcers, NSAIDs, Famotidine, Mice.

INTRODUCTION

Gastric ulcers are a prevalent health issue that impacts many individuals globally. This condition involves injury to the mucosal layer that extends into the muscularis mucosae, resulting from an imbalance between harmful and protective elements in the stomach.[1,2] Several factors can contribute to this imbalance, but Helicobacter pylori and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are primarily responsible for most cases of the disease.[1,2]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage pain, fever, and inflammation.[3] The primary mechanism by which NSAIDs lead to gastric ulcers is through the systemic inhibition of cyclooxygenase-1 (COX-1), which is continuously expressed and is essential for producing prostaglandins. These prostaglandins are crucial for preserving the integrity of the gastric mucosa by stimulating the production and release of mucus and bicarbonate, ensuring proper blood flow to the mucosa, and encouraging the proliferation of epithelial cells.[3,4]

In the clinical setting, the current approach for the management of gastric ulcers focuses on the use of proton pump inhibitors and H2 receptor antagonists as the

mainstay treatment. However, administration of these drugs has been associated with several adverse effects, such as nausea, constipation, gynecomastia, and impotence that limit their use. Thus, the search for effective agents with fewer side effects has been regarded as an effective strategy for the management of gastric ulcers.[5]

Recently, there has been a growing interest in research focusing on natural compounds that possess gastroprotective effect such as flavonoids, which are one of the most prevalent polyphenols in plants and constitute a significant category of natural products with various pharmacological effects, such as antioxidative, anti-inflammatory, anticancer, antiviral, and anti-diabetic properties. Numerous studies have shown the protective benefits of flavonoids on the intestinal epithelium, including the preservation of intestinal barrier function, the absorption of lipids and carbohydrates, modulation of enzyme activities, regulation of gastric secretions, immune system control, and interactions with pathogenic microorganisms.[6] In our study, we use Hesperiden & Diosmin mixture (Hes & Dio) which belongs to flavanones glycosides. Hes & Dio largely present in citrus fruits (Rutaceae) in addition to their presence in pharmaceutical forms marketed in a non-separate mixture such as Daflon (Servier, Orleans, France 500 mg) and its local similarities (Dovien, Unipharma company, Damascus, Syria / Hemoflavon, Barakat company, Aleppo, Syria).[7,8]

The marketed mixture enhanced blood vessels as hemostatic drug by lengthening the Vasoconstrictor effect of adrenaline. The mixture is in the form of a micronized purified flavonoid fraction (MPFF) with a 90 % diosmin 10 % hesperidin.[7,8]

Therefore, the purpose of our study was to investigate the effect of Hes & Dio mixture on gastric ulcers induced by Indomethacin in mice.

MATERIALS AND METHODS

Drugs and chemicals

Hes&Dio were obtained from Unipharma Company (Syria), Indomethacin was obtained from Barakat Company (Syria), and famotidine was obtained from Alpha Company (Syria). All drugs were suspended in Carboxymethyl cellulose (CMC), Indomethacin in CMC 1% while Hes & Dio mixture and Famotidin in CMC 0.5%. All drugs were given orally.

Animals

In this study, 24 Male Balb/c mice with a weight ranging from 25 to 30 g were used. The mice were kept in a room maintained at a temperature of 25 ℃, adhering to a 12-hour light/dark schedule, and allowed unrestricted access to food and water. All procedures related to the experiment complied with the regulatory standards for the treatment and utilization of laboratory animals.

Experimental design

The duration of the experiment spanned 15 days, during which all medications were given at the same time each day to prevent variations caused by diurnal rhythms of potential gastric function regulators.

The mice were classified into 4 groups, with 6 members included in each group:

1. Control group: in which animals received CMC (0.5%).
2. Indomethacin group: in which animals received CMC (0.5%).
3. Famotidine+ Indomethacin group: in which animals were previously treated with 8.3 mg/kg of Famotidine.
4. Hes&Dio mixture + Indomethacin group: in which animals received 615 mg/kg of the mixture.

In all groups (except for the Control), gastric ulcers were induced by a single dose of Indomethacin (275 mg/kg) on the last day of the experiment (day 15). The mice underwent a 16-hour fasting period while housed in mesh-bottom cages to reduce the likelihood of coprophagia, and were allowed to drink water freely except for the final 6 hours prior to the anatomical procedure.

After 6 hours of inducing ulcers, animals of all groups were killed with an overdose of ether; their stomachs were removed, opened along the greater curvature, and washed with saline to remove gastric contents and blood clots. The stomachs were fixed in 10% formalin for subsequent histological evaluation.

Histological evaluation

The stomachs were dehydrated in ascending alcohol series and embedded in paraffin. For each animal, 5µm-thick sections were obtained and stained with hematoxylin and eosin (H&E) prior to the study under alight microscope. All tissue sections were examined by an experienced histologist who was unaware of the treatment groups. Based on the depths of lesions, the gastric injury was measured using a scale ranging from 0 to 5 (Table1).

The severity of gastric injury was assessed based on the lesion depths, utilizing a scale from 0 to 5 (refer to Table 1).

Table 1: Measuring system.

score	Changes in the gastric mucosa
0	Normal gastric mucosa
1	Superficial erosions of mucosa

2	Medium depth lesions of mucosa
3	Deep lesions of mucosa with muscularis mucosa intact
4	Deep lesions of the mucosa with damage to the muscularis mucosa but without penetration
5	Ulceration of the entire mucosa (there is penetration of the muscularis mucosa into the submucosa)

Assessment of ulcer index and protective ratio

Ulcer index (UI) was used to asses gastric mucosal lesions according to the method of Khallouf *et al.*[9] and was estimated according to the formula:

Ulcer Index (UI) = mean of intensity in a group + [number of ulcer positive animals/total number of animals] × 2

The percentage protective ratio was estimated according to the formula[9] Protective ratio = 1- (UI pretreated group/ UI indo group) × 100

Statistical analysis

The results are presented as mean ±SD. The data were analyzed by ANOVA (one-way analysis of variance). The statistical analysis was performed using LSD multiple comparison tests for all parameters. The values were considered significant at the levels of $p < 0.05$.

RESULTS

Effect of Hes&Dio Mixture on Histopathological changes of Stomach

The Control group exhibited no gastric ulcers or lesions, with normal epithelial tissue in the gastric mucosa (Figure 1A). Conversely, the group of animals treated with Indomethacin displayed extensive ulceration throughout the mucosa, featuring multiple severe ulcers accompanied by significant inflammatory infiltrates (Figure 1B). In contrast, the Famotidine group showed few signs of ulceration, and any lesions present were minimal and localized. Inflammatory infiltrates were either absent or only mildly present if they occurred (Figure 1C).

The results in the Hes&Dio mixture group were similar to the Famotidine group whereas pre-treatment with Hes&Dio decreased ulceration the depth of lesions compared to the Indomethacin group. The lesions were also few and focal, and the inflammatory infiltrates, if present, were mild (Figure 1D).

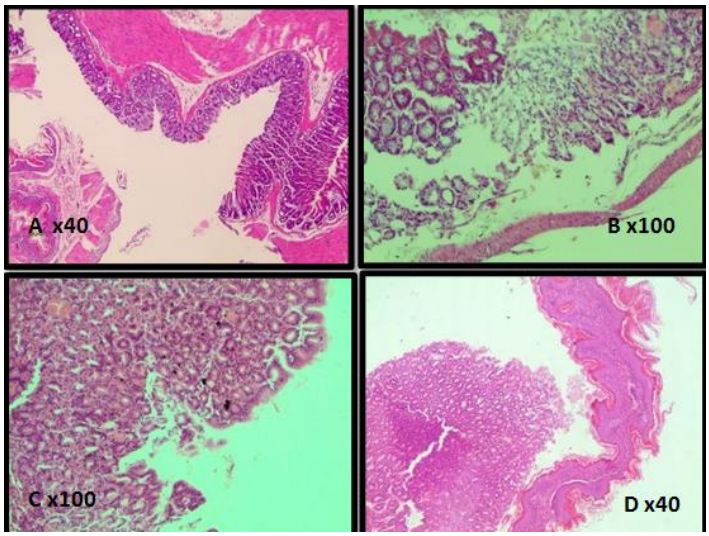

Figure 1: Histological assessment of gastric tissues using H&E stain. (A) Control group showed normal mucosa without lesions or ulcers. (B) Indomethacin group showed ulceration of the entire mucosa. (C) Famotidine group, (D) Hes &Dio group showed shallow mucosal lesions without any ulcer.

Effect of Hes&Dio Mixture on ulcer score, ulcer index and protective ratio

Pre-treatment with His&Dio significantly decreased ulcer score compared to Indomethacin group, and no statistically significant difference was measured in ulcer score between Hes&Dio group and Famotidine group.

Table 2: Effect of Hes&Dio Mixture on Indomethacin- induced gastric ulcer in mice.

Parameter / Group	Ulcer Score	Ulcer index	Protective ratio
Control	0.00 ±0.000	0.00	100
Indomethacin	5 ± 0.000	7	0
Famotidine	2 ± 0.63	2	71.42
Hes&Dio	1.83 ± 0.75	1.83	73.80

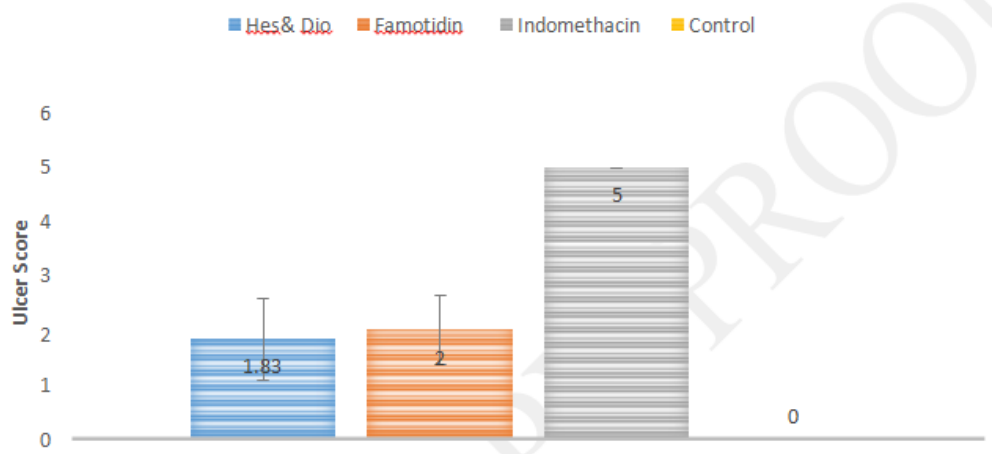

Figure 2 Effect of Hes&Dio Mixture on the ulcer score in an Indomethacin-induced gastric ulcer model. p<0.05*compared to the Control group, p<0.05¤ compared to the Indomethacin group, p<0.05† compared to the Famotidine group, p<0.05‡ compared to the Hes&Dio group.

DISCUSSION

Gastric ulcer is an injury to the mucosal layer that extends into the muscularis mucosae, resulting from an imbalance between harmful and protective elements in the stomach.[1,2]

Medicinal plants have been utilized effectively with minimal side effects since ancient times and can serve as a viable option for treating various health issues. Medical studies indicated that these natural plant compounds do not present any substantial risk. The presence of various active components, particularly flavonoids, is linked to antiulcer properties.[5,10]

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are a major contributor to the onset of gastric ulcers since they block the synthesis of prostaglandins, which are essential for preserving the health of the gastric mucosa, thus limiting their clinical application in managing pain, inflammation, and fever.[3,4]

In the present study, we studied the effect of Hesperidin & Diosmin Mixture on an Indomethacin-induced gastric ulcer model. The histopathological examination revealed that Indomethacin caused ulceration of the entire mucosa, and pre-treatment with Hes&Dio Mixture (at the 615 mg/kg dose) for 15 days decreased ulceration and the depth of lesions that are caused by Indomethacin.

Arab *et al.*[5] reported that pre-treatment with Diosmin (100 mg/kg, orally for 7 days) attenuated the severity of ethanol (5ml/kg, intragastic) gastric mucosal damage by lowering the Ulcer Index (UI) and area of gastric lesions. Arab *et al* demonstrated that this effect is mediated by decrease Myeloperoxidase (MPO) and Tumor necrosis factor- α (TNF-α) level. Also, Diosmin showed anti-oxidative effect via enhancement of glutathione (GSH), glutathione peroxidase (GPx).

In our study, Hes&Dio mixture achieved decrease in Ulcer index (UI) greater than the standard (Famotidine), and this has resulted in the differences between our study and the study of Arab (the material used to induce ulcers and difference in the period of the protocol). As in our study we used a mixture of two substances with a high dose of diosmin equivalent to 553.5 mg, which is 5 times the dose used in Arab study.

In another study, Selmi *et al.*[11] studied the effect of Hesperidin in peptic ulcer induced by Alcohol in rats. They found that 50 mg of Hes for 15 days before ulcer induction showed a percentage of protection =73.60% in comparison with Omeprazole (78.16%). Also, Hesperidin achieved a decrease in Ulcer Index (UI) (26.42) in comparison with ethanol (98,50).

The percentage of the prevention achieved by the hesperidin in the study of selmi was great approach to the degree we achieved in our study (73.80) despite the differences between the two studies (the animal of the experiment, the material used to induce the ulcers). Also, in our study we used a mixture of two substances in which the percentage of hesperidin was 61.5 mg.

In our study, we administered a dose of Hesperidin & Diosmin Mixture equivalent to the maximum daily dose typically used in humans.

CONCLUSION

Our Mixture of Hesperidin & Diosmin provides protection against Indomethacin- induced gastric ulcers in mice. As a result, it could be safely given at therapeutic doses combined with medications that may cause gastric ulcers as a side effect. We recommend preforming biochemical analysis to determine the mechanisms beyond this effect.

REFERENCES

1. DE LIRA MOTA, Kelly Samara, et al. Flavonoids with gastroprotective activity. Molecules, 2009; 14(3): 979-1012.
2. RAVIKKUMAR, V. R., et al. A

comprehensive review on ulcer and their treatment. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2023; 39: e20230006.

3. VENERITO, Marino; MALFERTHEINER, Peter. Interaction of Helicobacter pylori infection and nonsteroidal anti-inflammatory drugs in gastric and duodenal ulcers. Helicobacter, 2010; 15(4): 239-250.
4. MELCARNE, Luigi; GARCÍA-IGLESIAS, Pilar; CALVET, Xavier. Management of NSAID-associated peptic ulcer disease. Expert Review of Gastroenterology & Hepatology, 2016; 10(6): 723-733.
5. ARAB, Hany H., et al. Diosmin protects against ethanol-induced gastric injury in rats: novel anti-ulcer actions. PloS one, 2015; 10(3): e0122417.
6. SERAFIM, Catarina, et al. A review of the role of flavonoids in peptic ulcer (2010– 2020). Molecules, 2020; 25(22): 5431.
7. TORABI, MEHDI; ESFAHANI, A.; BANESHI, M. A new method of epistaxis management using nasal gel: a single centre, randomized clinical trial. Asian J Pharm Clin Res, 2018; 11(3): 104-8.
8. MUKKERJEE, DHRUBOJYOTHI. Hesperidin and diosmin-a novel drugs. International Journal of Pharmacy Research & Technology (IJPRT), 2020; 10(2): 25-33.
9. KHALLOUF, Sara; MAKHOUS, Rana; ISSA, Rana. Efficacy of olmesartan, irbesartan and telmisartan against acute indomethacin-induced gastric ulcers in mice. Bulletin of Pharmaceutical Sciences Assiut University, 2022; 45(2): 1005-1012.
10. KHANPARA, Pooja; MAVANI, Parth. A review on herbal plants used in peptic ulcer. Journal of Medicinal Plants, 2022; 10(5): 122-30.
11. SELMI, Slimen, et al. Protective effects of orange (Citrus sinensis L.) peel aqueous extract and hesperidin on oxidative stress and peptic ulcer induced by alcohol in rat. Lipids in health and disease, 2017; 16(1): 152.