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Protective effect of an aqueous extract of *Hedera helix* (English ivy) on indomethacin-induced ulceration in mice

Hebah Majed HAMOOD[1*], Aya F. AL-QRIMLI[1], Hadeel Yousif NOORI [2]

[1] Department of Pharmacology, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.
[2] Medicolegal Directorate, Ministry of Health and Environment, Baghdad, Iraq

* Corresponding Author. E-mail: hebarafal@yahoo.com (H.M.H.); Tel. +964 770 606 3084.

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ABSTRACT: The most common cause of stomach ulcer illness affecting people globally is the long-term usage of anti-inflammatory medicines. Synthetic antiulcer treatments like antacids, cytoprotective drugs, and antibiotics have negative effects like ulcer recurrence and poor healing, imposing financial strain on patients and public health systems. Therefore, there is an urgent demand for non-toxic, easily accessible antiulcer medication. The current study sought to assess the gastroprotective properties of *Hedera helix* (English ivy) extract against indomethacin-induced gastric ulcers in mice. Forty male mice weighing between 25 and 30 grams were split up into four groups, each with ten mice. One group served as a control, and the other three received oral doses to produce various types of stomach ulcers, and treatments were applied. The stomach contents were collected for analysis. The acid output and ulcer content were determined. Compared to the indomethacin-induced ulcer group, the *Hedera helix* extract significantly ($p < 0.05$) lowered the gastric ulcer index and improved the stomach acid volume, acidity, and pH as well as the malondialdehyde (MDA) and superoxide dismutase (SOD) levels. *Hedera helix* extract reduces oxidative stress and lipid peroxidation, which may have therapeutic benefits in stomach injuries caused by indomethacin which indicate that *Hedera helix* extract have the ability to reduce indomethacin's adverse effects on the stomach and possibility of using it as a potential treatment to prevent ulceration and also as a potent antioxidant.

KEYWORDS: Peptic ulcer; Indomethacin; English ivy; *Hedera helix*

1. INTRODUCTION

Peptic ulcers represent a major health concern, impacting over 14.5 million individuals globally [1]. An imbalance between defensive (blood supply, mucus barrier, prostaglandins) and offensive (HCl, stress, non-steroidal anti-inflammatory drugs, smoking, alcohol, and *H. pylori*) variables have been linked to the pathogenesis [2]. The pathological signs include bleeding, erosion, ulcers, and damage to the stomach mucosa. Patients frequently complain of dull discomfort, nausea, vomiting, regurgitation of acid, and epigastric pain. Acute consequences from the disease's long-term progression, such as gastrointestinal bleeding, and stomach perforations, can have a major negative impact on patients' health [3]. Antacids, cytoprotective drugs, proton pump inhibitors, H2 histamine receptor antagonists, and a combination of antibiotics are among the various synthetic antiulcer treatments currently on the market [4]. However, these medications have several negative effects, such as ulcer recurrence and poor healing, which place a significant financial strain on patients and public health systems [5]. As a result, there is a need to find non-toxic, readily available antiulcer medication [6].

Indigenous people have been using natural resources for healing and illness cures since ancient times. Folk medicine and herbal remedies have also used as a rich source of medication discovery for numerous researchers worldwide throughout modern history [7]. Recently, populations become more attracted to the concept of herbal medicine as they think that the natural treatments prevent the negative effects of synthetic medications, and lessen the number of chemicals in their systems, and for all these reasons, patients worldwide have recently begun to favour natural alternatives [8]. *Hedera helix* (English ivy) is one of the plants that is grown extensively worldwide. Plant phytochemical screening revealed the existence of numerous significant bioactive substances, including sterols, tannins, flavonoids, and saponins, among

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many others [9]. Ivy leaves have long been used to treat upper respiratory tract infections and relieve the symptoms of the common cold. Because of the extract's broncho dilating, expectorant, and antitussive properties, it has been used in numerous cough treatments and formulations [10]. Furthermore, ivy's anti-inflammatory, antioxidant, anti-arthritic, and anticancer properties have been documented [11-13]. Remarkably, prior studies have documented the gastroprotective properties of ivy extract, demonstrating antispasmodic and stomach ulcer prevention capabilities [14-16]. This study aimed to investigate the *in vitro* gastroprotective effect of aqueous ivy extract on ulceration caused by indomethacin.

2. RESULTS and DISCUSSION

The stomach wall was thinner than typical in the control group of mice given indomethacin, and the glandular area had obvious mucosal lesions ranging from spot ulcers to hemorrhagic lesions coated in coagulated blood (ulcer index: 119.35). Gross examination of the stomachs of the mice revealed that the glandular part appeared pink and the gastric mucosa had a whitish colour (ulcer index: 119.35). The linear lesions manifested as extended bands running parallel to the stomach's long axis. When compared to the induction group (indomethacin), glandular areas showed somewhat hyperaemic with sporadic lesions, and treatment with famotidine inhibited indomethacin-induced mucosal lesions (ulcer index: 33.75). The *Hedera helix* extract recipients were also prevented (ulcer index 38.18). The glandular mucosa showed hyperaemia in the presence of indomethacin-induced mucosal lesions, however, there were no hemorrhagic lesions (Table 1).

Table 1. Ulcer index of the experimental mice

Group	Ulcer index%
Control	0
Indomethacin	119.35
Famotidine + Indomethacin	33.75
Hedera helix extract+ Indomethacin	38.18

In comparison to the control group, indomethacin administration resulted in a significant ($p < 0.05$) decrease in pH, a corresponding significant ($p < 0.05$) increase in stomach volume of gastric content, and increased acidity. Pre-treatment with famotidine and *Hedera helix* extract resulted in a significant decrease ($p < 0.05$) in gastric juice volume, and acidity, and a significant increase ($p < 0.05$) in gastric pH compared to animals given indomethacin. However, there was no statistically significant ($p < 0.05$) difference when compared to the control group (Table 2).

Table 2. Acid output, pH, and gastric volume in experimental groups

Group	Gastric volume (ml/100g)	Gastric pH	Acid output (Acidity) (mEq/L/100g)
Control	1.12±0.65	2.45±0.15	53.42±0.91
Indomethacin	2.54±0.81#	1.01±0.22#	131.25±0.26#
Famotidine+ Indomethacin	1.66±0.27*	1.98±0.76*	66.36±0.88*
Hedera helix extract+ Indomethacin	1.83±0.09*	1.92±0.33*	62.45±0.09*

n = 10; #: Statistically significant ($p < 0.05$) compared with control; *: Statistically significant ($p < 0.05$) compared with indomethacin.

The indomethacin-induced animals showed a significant increase ($p < 0.05$) in malondialdehyde (MDA) levels and a significant decrease ($p < 0.05$) in superoxide dismutase (SOD) activity compared to control groups. However, both famotidine and extract produced a significant ($p < 0.05$) improvement in these parameters compared to the control group (Table 3).

Table 3. Malondialdehyde (MDA) and superoxide dismutase (SOD) levels in experimental groups

Group	MDA (umol/mg)	SOD (umol/mg)
Control	18.41 ±0.38	4.56±0.58
Indomethacin	93.41±0.03#	1.27±0.29#
Famotidine+ Indomethacin	28.05± 0.95*	3.86±0.02*
Hedera helix extract+ Indomethacin	34.45 ±0.67*	3.57±0.11*

n = 10; #: Statistically significant ($p < 0.05$) compared with control; *: Statistically significant ($p < 0.05$) compared with indomethacin.

Stomach mucosal integrity and gastric secretions (pH, volume) are biochemically analyzed to determine the stomach's condition after exposure to pharmacological agents. The volume of stomach secretions and the degree of acidity are indicated by the pH.A lower concentration of hydrogen ions in gastric juice is indicated by a low pH value. According to Inas *et al*. [17], this has been connected to the pathophysiology of ulcers and suffering from gastrointestinal injury. Any erosion of the mucosa is caused by aggressive agents attacking the mucosal epithelia from the outside, such as chemicals and drugs, as well as from within, such as oxidants produced in the gastric lumen. Non-steroidal anti-inflammatory drugs (NSAIDs), like indomethacin, can affect antioxidant and oxidant pathways, as well as disrupt and prevent the formation of prostaglandins. Prostaglandins have a dual function in the stomach by reducing acid output and raising gastric mucus. It has been suggested that gastric ulcer pathogenesis involves important biochemical events, such as the inhibitory action of indomethacin on prostaglandin synthesis in conjunction with the creation of free radicals, which trigger apoptosis and lipid peroxidation [18]. Since oxidative stress may influence ulcers, this model is also widely regarded as oxidative stress-induced stomach disease. The current study suggests that the formation of free radicals and gastric ulceration may be the cause of the substantial increases in ulcer index and gastric volume, increased MDA levels (a lipid peroxidation indicator) [19], and decreased SOD antioxidant activity after oral administration of indomethacin in the ulcerated induced mice. This is consistent with the findings of Muhammad *et al*. [20] and Sabiu *et al*. [6]. Gastric ulcers brought on by indomethacin may be treated with antioxidants. The administration of famotidine was shown to reverse the rise in mucosal oxidants and decrease enzymatic and nonenzymatic antioxidants caused by indomethacin [21].

Because *Hedera helix* extracts include a variety of bioactive substances, such as triterpene saponins, quercetin, rutin flavonoids, coumarins, polyacetylenes, anthocyanins, sterols, alkaloids, amino acids, vitamins, carbohydrates, and volatile oils, they have been utilized medicinally [22]. This is due to several pharmacological activities, such as anti-inflammatory, antispasmodic, antioxidant, and antiallergic [23]. The study into the antioxidant activities of *H. helix* extract in indomethacin-induced ulcers was prompted by reports of the antioxidant properties of quercetin, rutin, and saponins [24]. The state of the body's natural antioxidant enzyme, SOD, is enhanced in oxidative stress conditions and protects against free radical damage. In pretreated mice, *Hederahelix* extract exhibited gastroprotective benefits against indomethacin-induced ulcers as demonstrated by a reduction in ulcer index, the prevention of hemorrhagic mucosal lesions, and a decrease in lipid peroxidation. These effects may be caused by the gastroprotective action of the *Hedera helix* extracts, that is owned to its antioxidative effect that obtained from its ability to cause an elevation in the SOD levels and the ability to scavenge free radicals, which reduces the amount of lipid peroxide in the stomach mucosa [25].

3. CONCLUSION

The results of the current study showed that the aqueous *Hedera helix* extracts can cause reduction in the adverse effects of indomethacin administration on the stomach when the extract administered with a dose of 300 mg/kg bw. The results also showed that the *Hedera helix* extracts has an antioxidative effect in addition to its gastroprotective properties.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1. Plant collection and extract preparation

The fresh *Hedera helix* leaves were obtained form the from the pharmacognosy garden of the College of Pharmacy, AL-Nahrain University. After the leaves were washed thoroughly, they were allowed to air dry. An electric grinder was used to grind the dried leaves into a fine powder. With slight modifications, the extraction was performed [26]. After a week of macerating the dried, ground leaves in distilled water, the aqueous extract was produced by refluxing the mixture for three hours in a 70% hydroalcoholic solvent. Then, Whatman filter paper was used to filter the extract. After the extract was dried, a rotary evaporator was employed to evaporate the solvent, and the extract was used.

4.1.2 Source of animals

Forty male mice weighing between 25 and 30 grams were purchased from the animal center market, Bagdad, Iraq. The animals were kept in regular laboratory settings at 20 to 22°C. The drugs/chemicals employed in this investigation include thiopental sodium, famotidine, and indomethacin (Turkey).

4.2. Methods

4.2.1 Induction of gastric ulcer

To prevent coprophagy, which could have an impact on the development of stomach ulcers, mice were housed in cages with high floors and large mesh. The animals were fasted for a whole day to facilitate gastric damage from indomethacin treatment by increasing the amount of gastric acid in the stomach and clearing it of food. They had free access to water but were denied food. A single oral dosage of indomethacin (30 mg/kg body weight [bw]) caused a gastric ulcer. Four hours after indomethacin was administered, ulcerations of varying degrees appeared. Ten animals were divided into four groups at random. Group 1 was the control group and was given the distilled water. Group 2 was the ulcer-induction group; they were administered a single oral dosage of indomethacin at a dose of 40 mg/kg bw. After receiving 30 mg/kg of fomotidine and 300 mg/kg of *Hedera helix* extract, respectively, groups 3 and 4 received gastric gavages of indomethacin five minutes later. All mice were euthanized by diethyl ether inhalation four hours after the indomethacin was administered [27]. The stomachs were sliced open along the larger curvature, and the contents were collected in small vessels for biochemical analysis. After that, these stomach contents were centrifuged for five minutes at 3000 rpm. After being separated, the supernatant's volume was expressed as ml/100 g of body weight. Then carefully rinsed in ice-cold buffered saline phosphate (PBS; pH 7.2–7.4) to get rid of any blood clots or contents. Subsequently, the stomachs were positioned with their mucosal surface facing upwards on a wax plate protected by filter paper. The stomachs were digitally photographed for macroscopic examination.

4.2.2. Acid output

The acid output was estimated by titrating the stomach supernatant fluid with 0.05N NaOH. Acidity was measured in mEq/L/100 g of body weight [28].

$$\text{Acidity} = \frac{\text{Volume of NaOH} * \text{normality} * 100}{0.1}$$

4.2.3. Ulcer index

As previously described [29], a scoring system was used to classify the quantity and severity of gastric mucosal lesions. The ulcers were rated as follows: 0 for a stomach with a normal colour; 0.5 for hyperaemia; 1 for spot ulcers; 1.5 for haemorrhagic streaks; 2 for deep ulcers; and 3 for perforations. The ulcer score was calculated using the mean number of ulcers in each group. The ulcer score was then multiplied by 100 to get the ulcer index (UI). The stomachs were sectioned and prepared for histological and biochemical evaluation following the completion of ulcer scoring. The tissues were cut into tiny pieces and then homogenized with a homogenizer on ice in a specific volume of PBS (about 1 gram of tissue to 9 milliliters). Two freeze-thaw cycles were applied to the resultant suspension to further disrupt the cell membranes. Following that, the homogenate was centrifuged at 5000 rpm for 15 minutes [30]. Then, following the manufacturer's instructions, homogenized tissue stomach was utilized to measure oxidative stress indices (levels of MDA and SOD1) using enzyme-linked immunosorbent assay (ELISA; Elabscience).

4.2.4. Statistical analysis

The data were expressed as mean values ± standard error of means. The means of two groups were compared using the unpaired t-test, and the means of three groups or more were compared using an ANOVA (analysis of variance). Statistical Package for the Social Sciences (SPSS; version 23) was used to analyze the results. A p-value of less than 0.05 was considered significant.

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