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Orignal

Gastroprotective effect of *Hypericum perforatum* extract on indomethacin induced gastric ulcer in rats

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ABSTRACT

Objective. *Hypericum perforatum* L. (*H. perforatum*) is a traditional herb used in the treatment of many diseases. *H. perforatum* extract has antimicrobial, antioxidant, anti-inflammatory, antiulcerative and antidepressant effects. Indomethacin is a nonsteroidal anti-inflammatory drug. It may cause oxidative damage in stomach tissue. For this purpose, the protective efficacy of *H. perforatum* extract was investigated in indomethacin-induced gastric ulcer model. **Materials and Methods.** Sixty *Wistar albino* male rats were used and the rats were divided into 6 groups as negative control, positive control, *H. perforatum*, *H. perforatum* extract + indomethacin 10/25, *H. perforatum* extract + indomethacin 25/25, *H. perforatum* extract + indomethacin 50/25. Different doses of *H. perforatum* extract were administered orally by gavage, and after 5 minutes gastric ulcer was induced with indomethacin (25 mg/kg). After 6 hours, the rats were sacrificed. Ulcer indices were measured for each stomach. Superoxide dismutase (SOD) activity, malondialdehyde (MDA), glutathione (GSH) and catalase (CAT) levels were analyzed in stomach tissue. **Results.** *H. perforatum* extract + indomethacin 50/25 increased SOD activity and GSH level and decreased MDA levels in indomethacin-induced gastric ulcer. However, the ulcer index significantly was lower only in the *H. perforatum* extract given group. **Conclusions.** This study indicate that *H. perforatum* extract may have as a potential alternative agent in treating indomethacin-induced gastric ulcer.

Keywords: Antioxidant; gastric ulcer; *Hypericum perforatum*; indomethacin; oxidant (*Source: CAB Thesaurus*).

RESUMEN

Objetivo. *Hypericum perforatum* L. (*H. perforatum*) es una hierba tradicional utilizada en el tratamiento de muchas enfermedades. El extracto de *H. perforatum* tiene efectos antimicrobianos, antioxidantes, antiinflamatorios, antiulcerosos y antidepresivos. La indometacina es un fármaco antiinflamatorio no esteroideo. Puede causar daño oxidativo en el tejido del estómago. Para este

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propósito, se investigó la eficacia protectora del extracto de *H. perforatum* en un modelo de úlcera gástrica inducida por indometacina. **Materiales y métodos.** Se utilizaron sesenta ratas macho *albinas Wistar* y las ratas se dividieron en 6 grupos como control negativo, control positivo, *H. perforatum*, extracto de *H. perforatum*+indometacina 10/25, extracto de *H. perforatum*+indometacina 25/25, extracto de *H. perforatum*+indometacina 50/25. Se administraron por vía oral diferentes dosis de extracto de *H. perforatum* por sonda, y después de 5 minutos se indujo la úlcera gástrica con indometacina (25 mg/kg). Después de 6 horas, se sacrificaron las ratas. Se midieron los índices de úlceras para cada estómago. Se analizaron los niveles de glutatión (GSH), catalasa (CAT), malondialdehído (MDA) y superóxido dismutasa (SOD) en el tejido del estómago. **Resultados.** El extracto de *H. perforatum*+indometacina 50/25 aumentó la actividad de SOD y el nivel de GSH y disminuyó los niveles de MDA en la úlcera gástrica inducida por indometacina. Sin embargo, el índice de úlcera fue significativamente menor solo en el grupo que recibió el extracto de *H. perforatum*. **Conclusiones.** Este estudio indica que el extracto de *H. perforatum* puede tener como potencial agente alternativo en el tratamiento de la úlcera gástrica inducida por indometacina.

Palabras clave: Antioxidante; úlcera gástrica; *Hypericum perforatum*; indometacina; oxidante (*Fuente: CAB Thesaurus*).

INTRODUCTION

Ulcer is one of the most common diseases of digestive system caused by superficial erosions in the mucosa occurring due to disruption of the balance between the protective layer in the stomach and acid release. There are numerous causes of ulcers, nonsteroidal anti-inflammatory drugs (NSAIDs) being the most widely known following *Helicobacter pylori* (1). The drugs in question have analgesic, antipyretic, and anti-inflammatory effects (2). Undesirable side effects mostly occur in the gastrointestinal tissue as ulcers if NSAIDs are used for a long time and at high doses. Ulcer happen due to the inhibition of prostaglandin E (PGE) synthesis that protects the gastric mucosa (3).

Indomethacin is a methylated indole acetic acid derivative NSAID. It is a drug with strong analgesic, antipyretic, and anti-inflammatory effects, widely used in the world. Indomethacin strongly inhibits PG synthesis. Indomethacin induce gastric damage through inhibition of cyclooxygenase-1 (COX-1), which leads to a reduction of PG secretion in the gastrointestinal tract. Cyclooxygenase-2 (COX-2) enzyme inhibition play a role in the mucosal injury (4). Indomethacin has many side effects on the digestive system thus causing nausea, vomiting, exile, abdominal pain, ulcer, and bleeding (2).

Hypericum genus belongs to *Clusiaceae* family and *Hypericaceae* subfamily. There are more than 500 species worldwide belonging to this genus (5). *Hypericum perforatum* L. is the most common species among *Hypericum* species. Known as St. John's Wort in the world, this herb is widely used as antispasmotic, calming, antiseptic, and wound and burn healing. Pharmacological studies involving St. John's Wort and its extracts have shown the antimicrobial, antiviral, antidepressant, anti-inflammatory, antioxidant, cytotoxic, analgesic, antinociceptive, wound healing, and anti-neurodegenerative effects (6). A large number of secondary metabolites are found in *Hypericum* species, such as organic acids, tannins, amino acids, essential oils, and other water-soluble components, primarily naphrodiantrons, flurogluconols, and flavonoids (7). *Hypericum* species have many different medicinal effects thanks to their secondary metabolites. Many experiments have proven the pharmacological effects of *Hypericum perforatum (H. perforatum)* extracts. Alcoholic extract prepared from the upper sections of the plant has shown antiulcer and wound healing (8) effects. Topical preparations of *H. perforatum* extracts alone or in combination with other plant extracts have demonstrated that *H. perforatum* shows wound healing properties by enhancing the fibroblastic activity and collagen synthesis in mice (8,9,10).

Previous studies have shown the antiulcer activity of *H. perforatum* against cold stress (11), ethanol (12) and pylorus ligation (13) induced experimental ulcer models. Therefore, our aims in this study were to evaluate the effects of *H. perforatum* extract in indomethacin-induced gastric ulcer in terms of oxidative/antioxidative status in the gastric tissue in rats.

MATERIALS AND METHODS

The rats were maintained and used in accordance with the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines. The ethical clearance for experimental study was provided by Animal Ethics Committee of the University of Aydin Adnan Menderes (2016/114).

Chemicals. Indomethacin (Endol, Deva, Turkey) and *H. perforatum* extract (Sigma 05295001, Kuelzheim, Germany) were also used in the biological assays.

Animals and Experimental Protocol. In our study, 60 male 3 months old *Wistar albino* rats (weighing 250-350 g) were used. The rats were obtained from the experimental animal production facility of Faculty of Medicine, Aydın Adnan Menderes University, Turkey. The animals were raised in individual cages, standard temperature, humidity, and lighting conditions. The rats received standard pelleted diet. *Ad libitum* feed and water was offered during the experiment. The rats were subjected to a 2-week adaptation period followed by random allocation to six experimental groups each consisting of 10 rats as follows: negative control group (healthy and untreated rats), positive control group (25 mg/kg indomethacin), *H. perforatum* group (25 mg/kg *H. perforatum* extract), *H. perforatum extract* + indomethacin 10/25 group (10 mg/kg *H. perforatum* extract + 25 mg/kg indomethacin), *H. perforatum extract* + indomethacin 25/25 group (25 mg/kg *H. perforatum* extract + 25 mg/kg indomethacin), and *H. perforatum* extract + indomethacin 50/25 group (50 mg/kg *H. perforatum* extract + 25 mg/kg indomethacin). *H. perforatum* extract was obtained commercially. *H. perforatum* extract is a dry extract and contains hypericin (0.30 mg/g) and pseudohypericin (0.99 mg/g). Since it is in the form of dry extract, it was dissolved in 2% ethanol and administered to rats. Indomethacin was dissolved in 5% NaOH (Sigma 06203, Czech Republic). *H. perforatum* extract was dissolved in 2% ethanol (Sigma 32221, Germany). The dose of indomethacin was selected from previously studies (14,15). The extract and indomethacin were administered in a single dose through oral gavage following 24-h fasting period. Indomethacin was administered to the animals 5 minutes after the *H. perforatum* extract. All the animals were euthanized by cervical dislocation under general anesthesia induced by intraperitoneal administration of xylazine (Xylazinbio, Bioveta, Czech Republic) 5 mg/kg and ketamine (Ketasol, Richter Pharma AG, Austria) 50 mg/kg.

Macroscopic Evaluation of Gastric Ulcers. After euthanazia, the stomach tissues were removed for macroscopic evaluation. Each stomach was washed with 0.9% NaCl and the lesions were macroscopically examined (14). The stomach samples were photographed. Ulcer areas of each sample were measured one by one using the LabSens computer program (LabSens version 1.1) for photographs using millimetric paper as a scale. The stomach tissues were immediately stored at -80°C (Nuaire, NU 9668E, Japan) for further analysis.

Stomach Tissue Oxidant/Antioxidant Analysis. In order to determine oxidant and antioxidant parameters, stomach tissues were homogenized at 2000 rpm for 1 min by adding 10% 150 mM phosphate-buffered saline (PBS) (pH 7.4) (Yellowline, OST basic, US). The homogenates were centrifuged at +4°C at 12000 rpm for 10 min (Hettich Zentrifugen, Tuttlingen, Germany) and stored at -80°C (Nuaire, NU 9668E, Japan) until the supernatants were analyzed. SOD activity (16), CAT activity (17), GSH levels (18), and MDA levels (19) were analyzed using a UV spectrophotometer (Shimadzu U1601, Kyoto, Japan).

Statistical analysis. SPSS (version 22.0, NY, US) program was used for statistical analysis. The compliance of the parameters to the normal distribution was determined using the Shapiro-Wilk's test. Kruskall-Wallis test was used according to the type of data to normal distribution in order to make statistical evaluation between the groups. A probability of $p<0.05$ was considered significantly.

RESULTS

Ulcer Index. The ulcer index for each rat was taken as the mean ulcer score. The percentage of inhibition (% I) was calculated as described by Ngueleface et al (20).

%I= (Ulcer surface area of control - Ulcer surface area of test animal) × 100 / Ulcer surface area of the control

Ulcer indices of groups are shown in Table 1. Results showed that *H. perforatum* extract + indomethacin 50/25 group had higher ulcer index compared with other groups whereas,

positive control, *H. perforatum* extract + indomethacin 10/25, and *H. perforatum* extract + indomethacin 25/25 group had greater ulcer index than negative control and *H. perforatum* groups (p=0.001).

Table 1. Gastric ulcer index of indomethacin induced experimental groups.

Groups	Ulcer Index
negative control	0.00 ±0.00[c]
positive control	33.87 ±2.44[b]
H. perforatum	0.00 ±0.00[c]
H. perforatum extract + indomethacin 10/25	27.45 ±3.61[b]
H. perforatum extract + indomethacin 25/25	33.29 ±3.86[b]
H. perforatum extract + indomethacin 50/25	43.09 ±2.79[a]
p	0.001

[a,b,c]; Different superscripts within the same column indicate statistically significant differences.

Oxidative Stress Markers. The results of oxidant/antioxidant stress parameters are shown in Table 2. Positive control group had lower SOD levels in comparison with other groups whereas, *H. perforatum* extract + indomethacin 50/25 group had greater SOD levels than other groups except *H. perforatum* extract + indomethacin 10/25 group (p=0.001). SOD levels increased in groups receiving *H. perforatum*, *H. perforatum* extract + indomethacin 10/25, and *H. perforatum* extract + indomethacin 25/25 than positive and negative control groups (p=0.001).

CAT levels were greater in positive control, *H. perforatum* extract + indomethacin 50/25 groups than negative control, *H. perforatum* extract + indomethacin 25/25, and *H. perforatum* groups (p=0.009).

GSH levels were greater in *H. perforatum* group in comparison with other treatments whereas, positive control and *H. perforatum* extract + indomethacin 25/25 had lower GSH levels than other groups (p=0.001). In addition, *H. perforatum* extract + indomethacin 10/25 had greater GSH levels than negative control and *H. perforatum* extract + indomethacin 50/25 groups (p=0.001).

Table 2. Oxidative stress parameter levels of rat stomach tissue.

Groups	SOD (U/mg protein)	CAT (k/mg protein)	GSH (mg/g protein)	MDA (nmol/mg protein)
Negative control	9.37 ± 0.49[c]	1.14 ± 0.27[b]	9.20 ± 0.33[c]	100.06 ± 14.24[b]
Positive control	6.74 ± 0.26[d]	1.72 ± 0.25[a]	6.66 ± 0.39[d]	146.38 ± 11.67[a]
H. perforatum	12.07 ± 0.40[b]	0.79 ± 0.06[bc]	28.52 ± 2.31[a]	63.48 ± 8.71[c]
***H. perforatum extract* + indomethacin 10/25**	14.50 ± 1.34[ab]	1.09 ± 0.19[ab]	18.02 ± 1.66[b]	112.55 ± 7.87[b]
***H. perforatum extract* + indomethacin 25/25**	12.16 ± 0.99[b]	0.72 ± 0.10[bc]	9.59 ± 1.15[d]	95.22 ± 5.76[b]
***H. perforatum extract* + indomethacin 50/25**	16.04 ± 1.40[a]	1.46 ± 0.22[a]	9.24 ± 0.67[c]	115.74 ± 9.53[b]
p	0.001	0.009	0.001	0.001

[a,b,c,d] ; In each column, figures bearing different superscripts are significantly different.

Positive control group exhibited greater MDA levels than other groups whereas, negative control, *H. perforatum* extract + indomethacin 10/25, *H. perforatum* extract + indomethacin 25/25, and *H. perforatum* extract + indomethacin 50/25 groups had greater MDA levels than *H. perforatum* group (p=0.001).

DISCUSSION

Gastric ulcer is a common disease affecting the digestive system, seen in 10% of the world population (21). In a healthy stomach, protective factors such as bicarbonate release, cell renewal, and the amount of acid and pepsin in the stomach are in balance with each other. When this balance is disturbed, gastric ulcer occurs (22).

Due to the antipyretic, analgesic and anti-inflammatory activities of NSAIDs, these are recognized as the most favored drug category in the world. Gastric mucosa damage is the most common adverse effect in this drugs (23). The decrease in the amount of PGE is one of the basic mechanisms of NSAIDs in gastric damage. In addition, gastric damage can be caused by the rise in the amount of reactive oxygen radicals (ROS). The organism undergoes oxidative stress when oxidants are increased, or antioxidants are insufficient. Consequently, cellular metabolism is impaired followed by molecular degradation, and tissue damage (24).

Nowadays, the importance of gastroprotective plants is increasing due to the increasing problem of gastric ulcer. *H. perforatum* is a widely used traditional herb because of its positive effects against many diseases such as bronchitis, diabetes, depression, bile disorders, hemorrhoids, migraine, urogenital system diseases, and ulcers (25). The wound healing property of *H. perforatum* is its best known feature. At the same time, hepatoprotective, anti-ulcerogenic, and antioxidant properties have been reported (8). A study reported that *H. perforatum* provides gastroprotective effect against ethanol-induced gastric ulcer in mice (25). In a study, in which gastric ulcer was induced by indomethacin, it was reported that the oil of *H. perforatum* showed antioxidant properties (26). In different study, it was determined that *H. perforatum* exhibited gastroprotective effect against gastric ulcer caused by cold stress in Wistar rats (11). Also, it was observed that *H. perforatum* extract improved gastric lesions in Wistar rats exposed to hypothermic restraint stress (27). There are limited studies on the protective effect of *H. perforatum* extract on indomethacin-induced gastric ulcers. Therefore, effect of *H. perforatum* extract on indomethacin-induced gastric ulcer in rats was our aim of the study.

NSAIDs are one of the leading causes of gastric ulcer formation after *H. pylori*. In the ulcer model created with NSAIDs, it has been reported that the substance named isobrucein B reduces the effects of leukocytes and thus provides a protective effect (28). In the gastric ulcer model created with ethyl alcohol, it was observed that *Caesalpinia pyramidalis* plant grown in Brazil provides a gastroprotective effect (29).

Indomethacin, one of the most commonly used NSAIDs, initiates oxygen-induced free radicals and lipid peroxidation with ROS production, thus playing an important role in gastric mucosal damage (30). In indomethacin-induced ulcer models, it has been reported that the oily extract of the potion of pomegranate fruit (*Momodica charantia* L.) provides a gastroprotective effect (31). A study investigating the gastroprotective effect of selenium against indomethacin-induced ulcer model has reported to reduce ulcer formation, lipid peroxidation, and ROS production (32).

SOD is an antioxidant that plays a role in preventing oxidative damage caused by free radicals in aerobic organisms. It reduces the amount of superoxide radicals in the cell and converts superoxide anion ($O_2^{\cdot-}$) to a weaker reactive H_2O_2. Studies have reported that *Nigella sativa* (black seed) plant and selenium increase SOD and GSHPx (*Glutathione peroxidase*) activities in stomach tissue (32,33). In this study, when the indomethacin group and the other experimental groups were compared, the SOD activity of the indomethacin group was found to be significantly higher. The increase in SOD activity may be caused by the excess production of $O_2^{\cdot-}$ due to oxidative stress caused by indomethacin.

CAT is an antioxidant enzyme that converts H_2O_2 to H_2O. In the experimental ulcer model induced by indomethacin, it has been reported that lycopene, an antioxidant substance found in tomato (*Solanum lycopersicum*), decreased the CAT enzyme level at a dose of 100 mg/kg (34). In this study we conducted, similar

to the literature findings, it was determined that the CAT enzyme level was high in ulcer groups. The CAT level of *H. perforatum* 25 mg/kg + indomethacin group was statistically lower when compared to the indomethacin group.

GSH plays an important role in the detoxification of H_2O_2. Increased GSH activity to protect the cell may be caused by excessive $O_2^{\cdot-}$ production or peroxides. The data obtained from the studies carried out indicate that the level of GSH in the gastric tissue decreases due to ulcers. It has been observed that antioxidants and plant extracts play an important role in gastric tissue GSH values (35, 36). Indomethacin-induced oxidant / antioxidant balance disruption may be the reason for the decrease in GSH level. In our study, while there was no difference between positive control and *H. perforatum* extract + indomethacin 25/25, GSH level was found to be significantly higher in *H. perforatum* extract + indomethacin 10/25 and *H. perforatum* extract + indomethacin 50/25.

MDA is a toxic product of lipid peroxidation. Increasing MDA level indicates the increased tissue damage. There was an increase in lipid peroxidation products associated with the damage caused by NSAIDs to stomach tissue (37). For this reason, MDA values are expected to increase in the gastric tissue in the ulcer model created with indomethacin in studies. Compared with the control group, it was observed that the MDA level increased statistically in the indomethacin group. It was observed that the levels of MDA decreased in the groups given *H. perforatum* extract compared to the indomethacin group.

In conclusion, in this study, when the macroscopic findings were evaluated, no decrease was observed in the groups in which *H. perforatum* was administered for protective purposes against gastric ulcer. If the dose of indomethacin had been reduced or the duration of administration of *H. perforatum* extract had been longer, macroscopic improvement could have been observed. However, considering the oxidant and antioxidant levels, *H. perforatum* extract may have a gastroprotective effect. Both the increase in SOD activities and the decrease in MDA levels in all three treatment groups showed that the *H. perforatum* extract had a protective effect. It was determined that *H. perforatum* extract has a protective effect, but this effect occurs depending on the dose. New studies with different doses are needed to better understand the protective and therapeutic properties of *H. perforatum* extract on gastric ulcer.

Conflict of Interest

The authors declare that they have no conflict of interest.

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