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ARTICLE

Agomelatine prevents indomethacin-induced gastric ulcer in rats

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Abstract

Background Gastric ulcer is a very common gastrointestinal disease that may be dangerous and even may lead to death. The current study was conducted to detect the prophylactic effects of agomelatine on indomethacin-induced gastric ulcer.

Methods In this study, a total of 5 groups were created as the sham, ulcer, omeprazole, agomelatine 1 mg/kg and agomelatine 5 mg/kg groups. The effects of agomelatine on indomethacin-induced gastric injury were investigated. Total antioxidant and oxidant levels; the oxidant parameters like oxidative stress index and the inflammation markers such as tumor necrosis factor-α, interleukin-1β, interleukin-6 and interleukin-10 levels in stomach tissue were determined by ELISA. In addition, the gastric mucosal injury occurred in stomach wall was examined with histopathological methods.

Results While the levels of the inflammatory markers, total oxidant status and oxidative stress index increased at an obvious level especially in the indomethacin group, the total antioxidant status levels decreased. It was observed that these parameters were improved at a significant level in agomelatine 1 mg/kg and agomelatine 5 mg/kg groups when compared to ulcer group; and the results were similar to omeprazole group. It was also observed that our histopathological findings were consistent with all our other results.

Conclusions The results of this study showed that agomelatine usage in indomethacin-induced gastric ulcer model provides beneficial results.

Keywords Agomelatine · Ulcer · Indomethacin · Inflammation

Abbreviations

HCl	Hydrochloric acid
NSAID	Nonsteroidal anti-inflammatory drug
ROS	Reactive oxygen species
MT1 and MT2	Melatonin receptor 1 and 2
AGO	Agomelatine
GI	Gastrointestinal
DMOS	Dimethyl sulfoxide
TAS	Total antioxidant status
TOS	Total oxidant status
IL-1β	Interleukin 1-beta
IL-6	Interleukin-6
IL-10	Interleukin-10
TNF-α	Tumor necrosis factor-alfa
OSI	Oxidative stress index
NF-κB	Nuclear factor kappa B

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Introduction

The stomach is one of the organs which are affected by stress at the highest level. It was reported that some biological and psychosocial situations may increase stomach ulcer formation [1]. A total of 200,000 people are hospitalized on an annual scale with the diagnosis of ulcer; 3 million people are admitted to polyclinics; and the financial cost of the treatment of this disease reaches about 4 billion dollars [2]. Various factors like *Helicobacter pylori* infection, smoking [3], indomethacin and nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ethanol, stress, free radicals, bile acids, protease, exercise, hunger, cold and immobility cause the formation of ulcers in the gastrointestinal (GI) system [4, 5]. While indomethacin

causes increase in acid release and pepsin activity, and decrease in the mucus layer and bicarbonate release; it also causes increases in the lipid peroxidation and reactive oxygen species (ROS) formation in the gastric mucosa [6]. Indomethacin-induced gastric ulcer model is one of the most common methods to induce gastric ulcer in rats [7].

Unlike conventional drugs, as a melatonin receptor (MT1 and MT2) agonist and a serotonin (5-HT2C) receptor antagonist with a unique receptor profile, agomelatine (AGO) is an atypical antidepressant [8, 9]. Studies over past 5 years have suggested that AGO is a drug that can be used to treat symptoms of depression and anxiety [10] and to improve neuropathic pain caused by diabetes mellitus [11]. In addition, in several ischemia reperfusion models, in brain [12] and ovaries [13] AGO decreased injury by increasing the antioxidant properties and inhibiting apoptosis, and also it was found to reduce dose-dependent injury in heart [14]. AGO, a naphthalene analogue of melatonin, is a new melatonergic drug with a longer half-life (approximately 2.3 h) and has greater affinity for melatonin receptors (MT1 and MT2) than melatonin [15]. The receptors for melatonin were identified and characterized in the gastrointestinal tract of various species [16]. Melatonin has been shown to be a potent reactive oxygen metabolite scavenger and antioxidant that affects many physiological functions including secretion, motility, digestion and absorption of the gastrointestinal pathway [17]. In addition, melatonin has anti-inflammatory effect that may contribute to the protection of the gastrointestinal mucosa [18, 19].

In the present study, the purpose was to evaluate the antioxidant, anti-inflammatory and anti-apoptotic properties of AGO, a melatonin derivative, in indomethacin-induced gastric ulcer treatment.

Materials and methods

Ethics and animals

The ethical COMMITTEE approval for the study was received from Atatürk University Experimental Animals Local Ethics Committee (Protocol no. 19.04.2016/45). All the experimental procedures applied to animals were performed in line with the Ethics Committee Protocol.

A total of 40 Wistar Albino male rats, which weighed 230–260 g were obtained from Atatürk University Experimental Animals Laboratory. They were left hungry for 1 day before the study commenced; however, the rats were allowed to reach water freely. The medium in which the rats were placed was adjusted as 55% humidity, 20–22 °C temperature, and 12 h light/dark period.

Drugs

Agomelatine (Sigma Aldrich, Germany) was dissolved in Dimethyl sulfoxide (DMSO) (Sigma Aldrich, Germany) and then was administered to the rats as intraperitoneally. Omeprazole and indomethacin (Sigma Aldrich, Germany) were dissolved in DMSO and were administered to the animals by oral gavage.

The experimental groups

The rats were divided to 5 groups ($n = 8$) randomly and 8 animals were placed in each group. The sham group was applied diluted DMSO (750 µl/250 g bw) only. After 90 min, the animals were sacrificed. Group 2, omeprazole (30 mg/kg) was dissolved in DMSO and was orally administered to the animals. Group 3, indomethacin (100 mg/kg) was dissolved in DMSO and was orally administered to rats to induce a maximum level of acute ulcer. Groups 4 and 5 comprised ulcerated rats pretreated with AGO 1 mg/kg, AGO 5 mg/kg, respectively. All AGO doses were applied as intraperitoneally. After 90 min, animals were sacrificed by an overdose of general anesthetic and stomach tissues were collected.

Sacrificing of the animals and collecting the tissues

The surgical procedures were applied to the rats when they were under general anesthesia by intramuscular administration of ketamine 60 mg/kg (Ketalar, Pfizer, Istanbul, Turkey), xylazine hydrochloride 10 mg/kg (Rompun, Bayer, Istanbul, Turkey). Gastric contents were washed with normal saline and then divided into two parts. One part was stored at 10% formaldehyde for histopathological procedures and the other part was stored at − 80 °C for biochemical analyzes.

Tissue homogenization and determining the biochemical parameters

The stomach samples (100 mg) were homogenized with phosphate buffer (2 ml). The homogenized gastric tissues were centrifuged at 5000 rpm at + 4 °C for 20 min; and the supernatants obtained by this way were transferred to microcentrifuge tubes. The total antioxidant status (TAS) (Rel Assay Diagnostics, Gaziantep, Turkey), total oxidant status (TOS) (Rel Assay Diagnostics, Gaziantep, Turkey), interleukin 1-beta (IL-1β) (Elabscience, Wuhan, China), interleukin-6 (IL-6) (Elabscience, Wuhan, China), interleukin-10 (IL-10) (Elabscience, Wuhan, China) and tumor necrosis factor-alfa (TNF-α) (Elabscience, Wuhan, China) levels were measured from the supernatants by employing

rat-specific ELISA kits. The measurements were made according to the protocols of the kits. Oxidative Stress Index (OSI) was calculated with the OSI = [TOS, mmol H_2O_2 equivalent/L]/[TAS, mmol Trolox equivalent/L] × 10 formula [20].

Histopathologic examination

The inflammation and apoptotic features in the groups were investigated in an immunohistopathological manner by employing caspase-3 (Novus Biological, USA) and nuclear factor kappa B (NF-κB) (Abcam, UK). The tissue damage levels were determined by hematoxylin–eosin staining. The hematoxylin–eosin (H&E) and immunohistochemical staining procedures for gastric tissue were performed according to the protocols reported in the study of Aksak Karamese et al. [21].

Statistical analyses

The TAS, TOS, OSI, IL-1β, IL-6, IL-10 and TNF-α results were analyzed via IBM SPSS 20.0 Package Program. Following the One-Way ANOVA test for statistical measurements, the Tukey test was employed for multiple comparisons of the groups. The data was shown as mean ± standard deviation (SD). In the analyses, $p < 0.05$ and $p < 0.01$ values were considered as statistically significant.

Results

Biochemical examination

The stomach tissue levels of TAS, TOS and OSI are indicated in Table 1. Although TOS and OSI levels were not statistically significant in AGO 1 mg/kg Group, these values decreased in omeprazole and AGO 5 mg/kg groups at a significant level compared to ulcer group. Even if not at a statistically significant level, TAS level increased in omeprazole, AGO 1 mg/kg and AGO 5 mg/kg groups compared to ulcer group.

Gastric TNF-α, IL-1β, IL-6 and IL-10 were significantly increased in indomethacin group when compared with normal control. Pretreatment with either omeprazole or AGO (1 mg/kg and 5 mg/kg) decreased gastric TNF-α, IL-1β, IL-6 and IL-10 levels significantly when compared with ulcer control group (as shown in Fig. 1).

Histopathologic and immunohistochemical findings

It was observed in sham group that gastric mucosa layer was compatible with normal histological structure. It consisted of a single-layered prismatic epithelium. Lamina propria was filled with gastric glands, and mucosa layer ended with a thin muscularis mucosa layer. In ulcer group, a huge amount of single-layer prismatic epithelial cells were degenerated; and there were deteriorations in epithelial integrity. It was also determined that gastric glands under the mucosa had dilatation, they showed irregular placement, and there were hemorrhagic areas among them. Hemorrhagic foci were determined in submucosa as well. Gastric surface epithelium was in pieces and gastric crypts were damaged. Leukocyte infiltration with polymorph-nuclei was observed in lamina propria. It was noted that there was extensive edema in lamina propria, submucosa and disruption in vessel wall. In immunohistochemical staining, intense (++++) immunopositivities of NF-κB and caspase-3 were observed. In omeprazole group, in surface mucosal cells, it was observed that there were decreased injury, decreased degeneration in gastric pit and glandular cells, mucosal hemorrhage, and a decrease in lesions compared to ulcer group. In immunohistochemical staining, there were decrease in NF-κB (++) and caspase 3 (+++) immunopositivities. In AGO 1 mg/kg group, the injury occurred in the apical part of gastric mucosa decreased partially. The amount of damaged structures that stretched towards lumen and which were observed clearly in ulcer and omeprazole groups, were less in AGO 1 mg/kg group. However, there were moderate dilatation and mild congestion in gastric glands in this group, which is similar to ulcer groups. In AGO 1 mg/kg group, NF-κB and caspase 3 immunopositivities (+++) were observed at similar levels with omeprazole group. Mucosal damage was observed in AGO 5 mg/kg Group; however, there was less damage when compared to AGO 1 mg/kg group. No inflammatory cell infiltration and edema were observed. The NF-κB (+) and caspase-3 (++) immunopositivities still

Table 1 The effects of agomelatine on TAS, TOS and OSI values in gastric injury rats

Groups/parameters	Sham	Ulcer	Omeprazole	AGO 1 mg/kg	AGO 5 mg/kg
TAS	2.44 ± 0.40	3.59 ± 0.70	3.12 ± 0.86	3.27 ± 1.15	3.68 ± 1.75
TOS	11.91 ± 5.87	30.50 ± 26.04	10.45 ± 1.32*	11.66 ± 2.50	10.72 ± 3.56*
OSI	0.35 ± 0.17	0.20 ± 0.40	0.28 ± 0.47*	0.37 ± 0.11	0.32 ± 0.13*

The TOS, and OSI levels were decreased at a significant level in the AGO 5 mg/kg and omeprazole groups compared to the ulcer group (*$p < 0.05$). The TAS level increased in the omeprazole, AGO 1 mg/kg and AGO 5 mg/kg groups, although it was not at a statistically significant level compared to the ulcer group

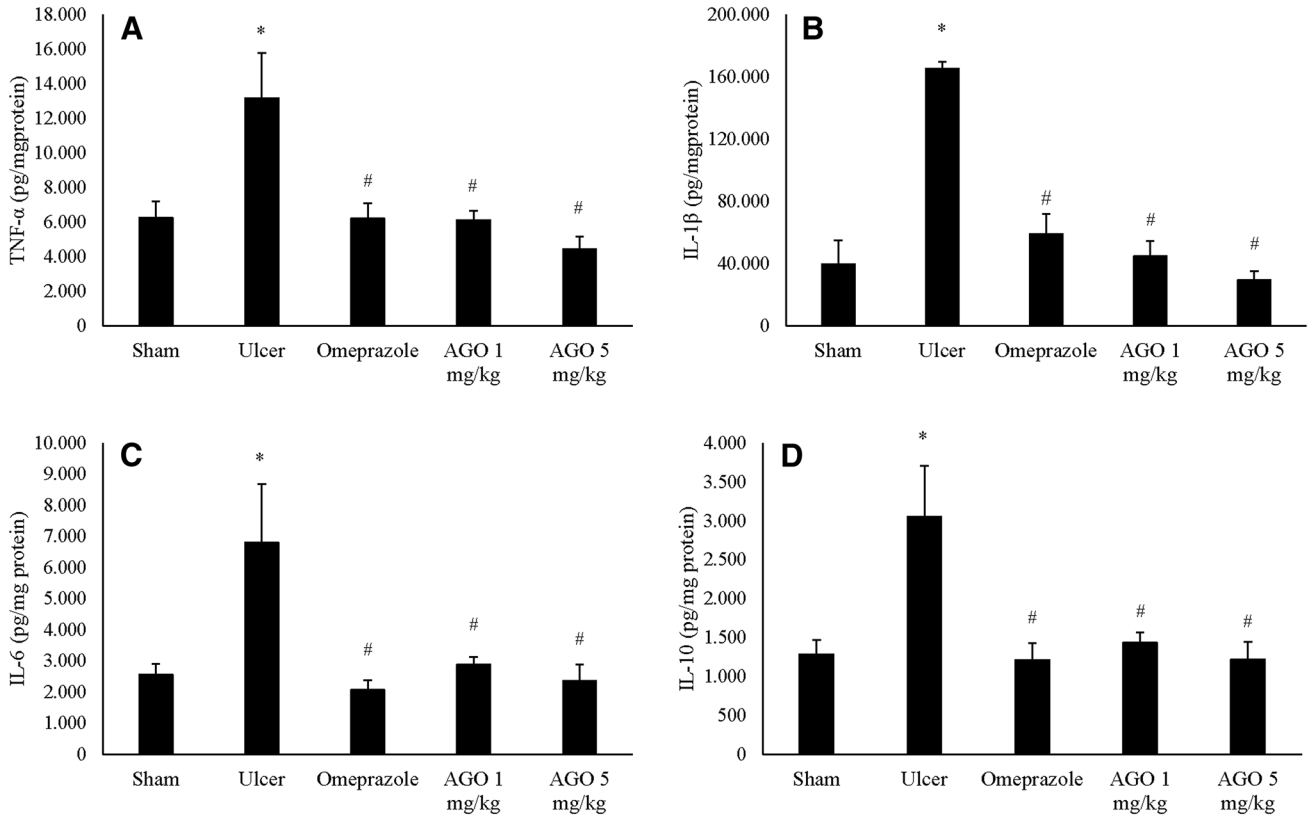

Fig. 1 Effect of Agomelatine on TNF-α (**a**), IL-1β (**b**), IL-6 (**c**) and IL-10 (**d**) levels in the stomach tissue of rats with or without indomethacin. $^{*}p < 0.01$ Significantly different from the value in the control group. $^{\#}p < 0.001$ Significantly different from the value in the ulcer group

existed compared to other groups despite a decrease. Histopathologic results were presented in Fig. 2 and in Table 2.

Discussion

Gastric ulcer is one of the most frequent diseases seen in the gastrointestinal tract, and affects 5% of the population of the world: Its prevention and management is very difficult [22]. The main problem in our present day is hemorrhage and mucosal lesions due to consumption of NSAIDs, which are known as the second common cause of peptic ulcer after *Helicobacter pylori* [23]. It is already known that NSAIDs-induced gastric injury is the most common and dangerous side effect of these drugs, which accounts for a total of 25% of gastric ulcer cases [24].

Different pathogenic mechanisms were proposed for the purpose of explaining the pathophysiology of the indomethacin-induced gastric damage like overproduction of ROS [25] and inflammatory cytokines [26]. It was proven that indomethacin causes serious gastrointestinal injury and oxidative stress [27, 28]. The development of indomethacin-induced gastric mucosal lesions occurs basically via the formation of oxygen free radicals [29]. Oxidative stress is a systemic phenomenon in peptic ulcer disease [30]. Antioxidant scavenging of the ROS is a novel strategy for the prevention and treatment of chronic and degenerative diseases like peptic ulcer [31]. In addition, indomethacin induces apoptosis via the ROS formation and caspase-3 activation [32].

Gastric ulcers are usually treated with histamine-2 receptor blockers or proton pump inhibitors (omeprazole, etc.). The aim of such treatments is to reduce the secretion of hydrochloric acid (HCl), facilitate the mucosal regeneration and healing of ulcers. Although these drugs work, their long-term use causes several side effects. Therefore, substances with high antioxidant and gastroprotective properties are used in ulcer investigations. Current studies show that melatonin protects against gastric ulceration in a variety of ulcer models and gastric injuries [33–35]. Against indomethacin-induced gastric ulcer damage, AGO has gastroprotective effects with its antioxidant, anti-inflammatory and antiapoptotic features.

The highlights of this study are; AGO reduces dose-dependent gastric mucosal damage induced by indomethacin in rats and it is a better gastroprotective agent at some points than omeprazole, the proton pump inhibitor, used in the pharmacology market. Indomethacin-induced ulcer model demonstrates ROS production during gastric ulceration by

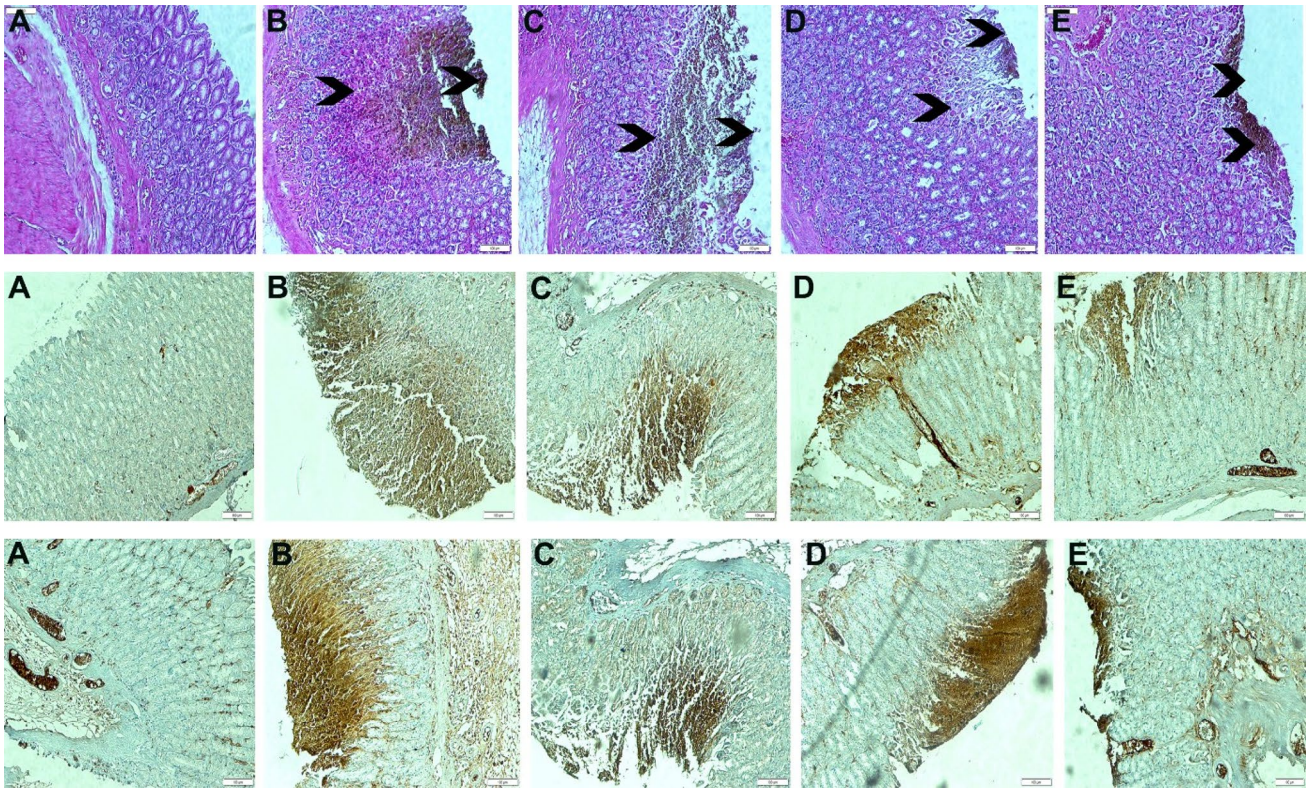

Fig. 2 Rat gastric tissue morphology after ulcer induction by indomethacin and protection via AGO. Histological sections were stained with H&E (line 1) and photographs were taken at 20X. In line 2, caspase-3 immunopositivity and in line 3 NF-κB immunopositivity were presented

Table 2 The histopathological findings in gastric tissues; epithelial damage, edema in the epithelium, leukocyte infiltration, hemorrhagic areas, Caspase-3 and NF-κB immunopositivity

Groups/Parameters	Sham (A)	Ulcer (B)	Omeprazole (C)	AGO 1 mg/kg (D)	AGO 5 mg/kg (E)
Epithelial damage	–	+++++	+++	+++	+
Epithelial edema	–	++++	++	+++	–
Leukocyte infiltration	–	++++	++	++	++
Hemorrhagic areas	–	+++++	++	++	+
Caspase-3 immunopositivity	–	++++	+++	+++	++
NF-κB immunopositivity	–	++++	++	+++	+

Damage levels; – (none), + (less), ++ (mild), +++ (moderate), ++++ (severe), +++++ (very severe)

leading to oxidation of cellular proteins and depletion of antioxidants [36]. ROS causes oxidative damage with the disruption of the antioxidant defense system [37]. Excessive ROS production causes oxidation of lipids, proteins and nucleic acids, and disrupts the functioning of cells [38]. The measurement of the TAS provides much more information than other antioxidant paramaters measurement; and it is widely used to determine the total antioxidant capacity [39]. OSI is an indicator that can show the lipid and protein oxidation with DNA damage that are caused by non-compensated free radicals which are produced due to increased oxidants or decreased antioxidants. For the oxidative stress and antioxidant situation evaluation, recently, OSI is detected via dividing total oxidants by total antioxidants [40]. We demonstrated that TOS decreased with AGO treatment in indomethacin-induced ulcer injuries. This documents the AGO's ability to act as a gastroprotective agent. AGO application did not affect TAS level but suppressed TOS level. AGO, like melatonin, was found to affect changes in the activities of the key antioxidant enzymes of the stomach [35].

NF-κB plays an active role in ulcer healing process [41] and NF-κB activation in tissues increases during gastric ulcer [42]. NF-κB plays a centric role in regulating the immune response [43, 44]; and triggers the transcriptional

up-regulation of proinflammatory cytokines such as IL-6, IL1-β and TNF-α [45]. It is already known that the TNF plays a key role in gastric damage that is caused by NSAIDs, and triggers the inflammation [46]. IL-1, NF-κB, TNF-α and IL-6 levels increase in studies conducted on jejunoileitis [47], indomethacin-induced gastric ulcer [48–50], small intestine injury [51] and gastric damage [52, 53]. In a previously study, AGO decreased the levels of cytokines like TNF-α, IL-6; and performed protective effects in the liver [54]. The neuroprotective effects of AGO against contrast-induced nephrotoxicity in rats were shown in rats by decreasing the TNF-α, NF-κB and IL-6 levels [55]. TNF-α levels decreased in diabetic rat testis [56], major depressive patients [57], sepsis-induced acute renal injury [58] and psychosis-related behavioral models [59] via AGO application. IL-10 is one of the main regulatory cytokines suppressing the inflammatory pathways [60]. It also inhibits IL-1β, TNF-α, IL-6 and other proinflammatory cytokine production [61]. In current study, AGO decreased the indomethacin-induced gastric ulcer damage by decreasing the proinflammatory cytokine levels through NF-κB signal pathway, which is consistent with previous studies. We considered that the decreasing IL-10 levels might be associated with the decrease in the need for activation of anti-inflammatory mechanisms due to AGO reduces proinflammatory cytokine levels.

Apoptosis is a quite regulated process for removing the unwanted cells in the normal cell cycle during the development and functioning of the immune system and in the re-modeling of the tissues. Apoptosis regulation disorder is associated with various diseases [62]. Caspase-3 is called as the "death-walker protease"; and it is located in the center of the apoptosis [63]. It is also the primary mediator of the apoptosis [64]. It was shown in studies conducted on indomethacin-induced peptic ulcer [65] and jejunal injury [66] that the caspase-3 levels increased. AGO administration in a cerebral ischemia reperfusion study demonstrated a neuroprotective effect via antiapoptotic properties by decreasing the caspase-3 level [12]. In a rat stress model, increased caspase-3 levels in hippocampus were decreased with AGO [67]. AGO administration in neuronal PC12 cells decreased the caspase-3 levels [68]. In another research, melatonin decreased the mitochondrial DNA damage in the *substantia nigra*, reduced the free radical production, and inhibited the apoptosis [69]. In the present study, caspase-3 level increased in the ulcer group compared to the sham group, but it was decreased by AGO and omeprazole administration; and the gastroprotective effect of AGO was shown by preventing cell-tissue death via anti-apoptotic effect.

As a result, in current study, AGO reduced ROS production and regulated cytokine formation by suppressing NF-κB expression in indomethacin-induced gastric ulcer. AGO also reduced cell damage by exhibiting similar effects to omeprazole. In order to better understand the effects of AGO on gastric ulcer, further clinical studies are needed by using different doses of AGO in different ulcer models supported by molecular studies.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no actual or potential conflict of interest in relation to this article.

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