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Effects of taxifolin on aspirin-induced gastric damage in rats: macroscopic and biochemical evaluation

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Running Head: TAXIFOLIN ON GASTRIC DAMAGE IN RATS

Abstract

Taxifolin (dihydroquercetin), is a flavanonol isolated from various plants and has antioxidant effect. The aim of our study is to macroscopically and biochemically investigate the effect of taxifolin on aspirin-induced oxidative gastric damage in rats and to evaluate this effect by comparing taxifolin with famotidine. Rats were divided into four drug administration groups: a healthy control group (HCG), an aspirin-alone group (ASG), a taxifolin + aspirin group (TASG), and a famotidine + aspirin group (FASG). In conclusion, in light of the results that we obtained, 50 mg/kg taxifolin was revealed to have anti-ulcer effects. At this dose, taxifolin was able to bring COX-1 activities to a level close to those seen in healthy rats with appropriate macroscopic, oxidant/antioxidant, and biochemical parameters. Based on these results, it can be said that taxifolin may be successfully used as a more potent alternative to famotidine, which is the currently accepted treatment for aspirin-induced ulcers.

Key words: aspirin; biochemical; gastric damage; rat; taxifolin.

INTRODUCTION

Aspirin, an O-acetyl derivative of salicylic acid, is a nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic effects [1]. Today, aspirin is successfully used in the chronic treatment of acute coronary heart diseases, cerebral sinovenous thrombosis, and inflammatory diseases such as arthritis, carditis, and rheumatoid fever [2]. Like other NSAIDs, aspirin inhibits cyclooxygenase (COX) activity, which is responsible for the synthesis of prostaglandins (PGs) that cause inflammation, edema, pain, and fever [3]. As is known, the COX enzyme has structural COX-1 and inducible COX-2 isoforms [4]. COX-1 is responsible for the protection of the stomach and many other organs and tissues, while COX-2 plays a role in inducing pathological processes such as inflammation [5]. The inhibitory effect of aspirin on COX-1 is greater than its effect on COX-2 [6]. The gastrointestinal toxic effects of NSAIDs are known to result from the inhibition of COX-1 production [7]. Aspirin is a drug that has gained worldwide acceptance in the treatment of various cardiovascular and inflammatory diseases. However, aspirin therapy causes toxic effects, especially on the stomach and liver [8]. Even low-dose aspirin administration leads to an increased risk of gastroduodenal ulcers and upper gastrointestinal bleeding [9]. It has been reported that aspirin causes damage by increasing the oxidant status level in the stomach tissue while decreasing the antioxidant and COX-1 levels [10]. In addition, it has been documented that aspirin increases the production of malondialdehyde (MDA), the toxic end-product of lipid peroxidation in gastric tissues, and decreases the level of glutathione (GSH), which is known as an endogenous antioxidant parameter [11]. All this information suggests that drugs that inhibit the increase in oxidant levels and the decrease in antioxidant and COX-1 levels may be beneficial in the treatment of the gastrototoxic effects of aspirin.

Taxifolin (dihydroquercetin), is a flavanonol isolated from various plants such as thistle, onion, and tamarind and the bark of French maritime pine [12]. Recent studies have documented the

antioxidant effects of taxifolin [13]. Taxifolin has been shown to create an antioxidant effect by inhibiting the production of radical oxygen species (ROS) [14]. It has also been reported that taxifolin protects stomach tissues from oxidative stress with this antioxidant effect [15]. However, no studies have been found in the literature exploring whether the gastroprotective effect of taxifolin is associated with the COX-1 enzyme. The aim of our study is to macroscopically and biochemically investigate the effect of taxifolin on aspirin-induced oxidative gastric damage in rats and to evaluate this effect by comparing taxifolin with famotidine.

MATERIALS AND METHODS

Animals

This research involved a total of 24, 3 months old, male albino Wistar rats with weights ranging from 275 to 287 grams. All of these animals were obtained from the Erzincan Binali Yildirim University Medical Experimental Application and Research Center. Before the experiment, animals were housed in groups in an animal room with antimicrobial fan system for one week at normal room temperature ($22\pm 2^{\circ}\text{C}$) and $55\pm 5\%$ relative humidity in 12 hours of light and 12 hours of darkness. They were fed ad libitum with standard rat chow (Bayramoglu feed/Bayramoğlu Trade Company- Turkey)) and tap water. The compliance of the research with ethical principles was confirmed by the Erzincan Binali Yildirim University Animal Experiments Local Ethics Committee (dated 07.02.2022 and No. 22/01).

Chemicals

The thiopental sodium (CAS no: 76-75-5; 0.5gr/20ml; lot no: (01)08699508270385) used in this research was obtained from Ulagay (Turkey), taxifolin (CAS no: 480-18-2; 25mg/tablet; lot no: 74034) from Evalar (Russia), aspirin (CAS No: 50-78-2; 100mg/tablet lot no:

(01)08699514040019) from Abdi İbrahim Pharmaceuticals (Turkey), and famotidine (CAS No: 76824-35-6; 20mg/tablet; lot no: (21)A0000004347152) from Sandoz Drugs, Turkey.

Experimental groups

Rats were divided into four drug administration groups: a healthy control group (HCG), an aspirin-alone group (ASG), a taxifolin + aspirin group (TASG), and a famotidine + aspirin group (FASG).

Experimental procedure

Experimental applications were carried out in the laboratories of Erzincan Binali Yildirim University Medical Experimental Application and Research Center. The animal rooms in the laboratory are closed to the outside and ventilated with a HEPA filtered ventilation system. In addition, animal rooms and cages are periodically washed with high pressure hot water. In this way, the experimental environment is protected from microbial pathogens. Surgical materials, gloves and personnel clothes used during the experiment were cleaned from pathogens with the necessary disinfection and sterilization methods. Sterilization processes were carried out using autoclave.

Pharmaceutical tablets, each containing 25 mg of taxifolin were ground into powder in a mortar and mixed in distilled water. The drugs to be administered to the rats were suspended in distilled water in the laboratory environment and made suitable for oral use. A solution containing taxifolin at a concentration of 10 mg/ml (w/v) was prepared. Pharmaceutical tablets of 100 mg containing 20 mg of famotidine were ground into powder in a mortar and mixed in distilled water. A solution containing famotidine at a concentration of 30 mg/ml (w/v) was prepared. To conduct the planned experiment, the animals were fasted for 12 hours (rats were allowed to drink water). Taxifolin 50 mg/kg was given with an oral gavage to the rats in the TASG group. This dose was chosen because, in a previous study, administration of 50 mg/kg of taxifolin

prevented liver damage by reducing pazopanib-induced oxidative stress [16]. Famotidine 20 mg/kg were administered through oral gavage to the FASG group. This dose was chosen because famotidine showed antiulcer activity at a dose of 20 mg/kg in a previous study [17]. As a solvent, normal saline (0.9% NaCl) at the same volume was administered orally to the HCG and ASG groups. 130 mg pharmaceutical tablets containing 100 mg aspirin were ground into powder in a mortar and mixed in distilled water. A solution containing aspirin at a concentration of 30 mg/ml (w/v) was prepared. One hour after the administration of taxifolin, famotidine and 0.9% NaCl solution, aspirin were administered at a dose of 100 mg/kg through oral gavage directly into the stomachs of rats in the TASG, FASG, and ASG groups. This dose of aspirin is the dose that causes damage to animal stomachs [18]. Six hours after aspirin administration, all animals were killed with high-dose thiopental anesthesia (50 mg/kg). After macroscopic evaluation of the inner surfaces of the removed stomachs, the gastric tissues were biochemically examined.

Macroscopic analyses

Stomach tissue was evaluated according to the ulcerative areas (black colored bleeding areas) and the degree of erosion. The ratio of ulcerative areas on the mucosa to the entire stomach surface was calculated by using squared paper and measured one by one. The protective efficacy of taxifolin on gastric ulcer was compared with the results obtained from ASG group. Mean total ulcer area (mm²) of each rat was determined and the percent anti-ulcerative effect (%) was calculated according to the following formula:

$$\text{Antiulcerative effect (\%)} = (\text{Control Group mean total ulcer area} - \text{treatment group mean total ulcer area}) / \text{Control Group mean total ulcer area} * 100$$

Biochemical analyses

144 ***Determination of tissue MDA and total Glutathion (tGSH)***

145 The MDA measurements were based on the technique used by Ohkawa et al., which involves
146 the spectrophotometric measurement of the absorbance of the pink-colored complex formed by
147 thiobarbituric acid (TBA) and MDA [19]. The total Glutathion (tGSH) measurements, on the
148 other hand, were based on the method presented by Sedlak and Lindsay [20].

149 ***Determination of tissue TOS and TAS***

150 Total oxidant status (TOS) and total antioxidant status (TAS) levels of tissue homogenates were
151 determined using a novel automated measurement method and commercially available kits (Rel
152 Assay Diagnostics, Turkey), both developed by Erel [21, 22].

153 ***Measurement of COX activity***

154 We measured the COX activity in rat stomachs in this series of experiments using a COX
155 activity assay kit (Item No. 760151, Cayman Chemical, USA). Stomach tissue was removed
156 from stomach membranes and washed thoroughly with ice-cold Tris buffer (pH 7.4) containing
157 0.16 mg/mL of heparin to remove any red blood cells and clots, then stored at -80 °C until
158 assayed. For each rat, a sample of stomach tissue was homogenized in 5 mL of cold buffer (0.1
159 M Tris-HCl, pH 7.8, containing 1 mM EDTA) per gram of tissue and centrifuged at 10000 × g
160 for 15 minutes at 4 °C. The supernatant was removed for the assay and stored on ice. We then
161 measured the protein concentration in the supernatant using the Bradford method [23]. The
162 COX activity assay kit measures the peroxidase activity of COX. This is assayed
163 colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-p-
164 phenylenediamine at 590 nm. We measured COX-2 activity using the COX-1-specific inhibitor
165 [24]. Results for COX-1 and COX-2 activity are given as units per milligram of protein. The
166 activity of COX in the tissue was expressed as nmol/min/mg protein (U/mg protein).

Statistical analysis

For statistical analysis, IBM SPSS Statistics for Windows 22 (IBM Corp., USA) was used. The results were presented as mean $\pm$ standard error of the mean (SEM). For comparisons of groups, one-way ANOVA was used. After ANOVA, according to the homogeneity of variances, the post hoc LSD (least significant difference) test was used. The statistical level of significance for all tests was considered to be 0.05.

RESULTS

Macroscopic findings of gastric tissue

As seen in Figure 1, no hyperemia, edema, or damage to the gastric mucosa of healthy animals was observed. However, the gastric mucosa tissue of the animals who were given aspirin alone had foci of damage (ulcers) in different numbers and of different diameters. The ulcers consisted of round-oval and irregular mucosal defects with different depths, and the edges of the ulcers were edematous. The boundaries of the ulcers were clear. However, the gastric tissues had mild hyperemia and edema, while the ulcer foci were less numerous and smaller in the TASG and FASG samples. The mean total ulcer area in the gastric tissue of ASG rats was $83.33 \pm 1.45 \text{ mm}^2$ (Table 1). However, the mean total ulcer area was $1.33 \pm 0.42 \text{ mm}^2$ (Table 1) in the TASG rats. In the FASG rats, the mean total ulcer area was calculated as $5.00 \pm 0.57 \text{ mm}^2$ (Table 1).

Biochemical findings

The mean $\pm$ standard error values of biochemical findings are shown in Table 2. tGSH levels were found to be significantly higher in the HCG, TASG, and FASG groups than in the ASG group ($P < 0.001$). MDA levels were significantly lower in the HCG, TASG, and FASG groups than in the ASG group ($P < 0.001$). The MDA and tGSH levels of the TASG group were similar to those of the HCG group.

The measured TOS levels were quite close to the MDA levels, while the TAS levels were quite close to the tGSH levels. The highest TOS value was found to be 4.09 nmol H₂O₂/mg protein in the ASG group, while the lowest TOS value was 2.25 nmol H₂O₂/mg protein in the HCG group. TOS values in the TASG and FASG groups were significantly lower compared to those in the ASG group ($P<0.001$). The highest TAS value was measured as 5.18 μ mol Trolox equiv./mg protein in the HCG group, while the lowest TAS value was 2.70 μ mol Trolox equiv./mg protein in the ASG group. TAS values in the TASG and FASG groups were significantly higher compared to those in the ASG group ($P<0.001$).

COX enzyme findings

COX enzyme findings are shown in Figure 2. Compared to the ASG group, COX-1 showed significantly higher levels of activity in the HCG, TASG, and FASG groups ($P<0.001$). Considering the COX-2 activity results, it is seen that the HCG and FASG groups showed significantly higher levels of activity ($P<0.001$) compared to the ASG group, while the TASG group was not different in this regard.

DISCUSSION

This study investigated the effects of taxifolin (dihydroquercetin), a flavonol, on aspirin-induced gastric ulcer damage in rats. The potential effect of taxifolin on aspirin-induced oxidative gastric injury in rats was macroscopically and biochemically investigated, and this effect was evaluated by comparing taxifolin with famotidine.

Aspirin is a very widely used NSAID. Gastric ulcer formation, a well-known side effect of aspirin, is used experimentally in animals to create ulcer models in pharmacology. In our study, significant total ulcer areas were formed in the group given aspirin at a dose of 100 mg/kg. Total ulcer area was smaller in the aspirin+famotidine group than in the aspirin group. Taxifolin, the substance that we tested, showed a stronger anti-ulcer effect than famotidine. The anti-ulcer effect of taxifolin against aspirin was calculated to be 98.4%, while the effect of

famotidine was calculated to be 93.9%. When we reviewed the literature for studies related to the effects of taxifolin on the gastrointestinal tract, we found many studies related to the protective effects of taxifolin. For example, a fairly recent study conducted by Xie et al. showed that taxifolin suppresses malignancy in gastric cancers [25]. In another recent study, taxifolin supported gastric recovery in vivo and was able to inhibit *Helicobacter pylori* infection in vitro [26]. In a celiac artery ligation model in rats, taxifolin prevented oxidative gastric damage [15]. Similarly, the protective effects of taxifolin were shown in many other studies in the literature [16, 27]

Flavonoids constitute one of the main groups of plant phenolic antioxidants, and they have high chelating properties. Taxifolin (3,5,7,3,4-pentahydroxy flavanone or dihydroquercetin) is a flavonoid commonly found in onion, milk thistle, and the bark of French maritime pine and Douglas fir [28]. Taxifolin has exhibited promising pharmacological activities in the management of inflammation, tumors, microbial infections, oxidative stress, and cardiovascular and liver disorders [28]. Among these, the antioxidant effects of taxifolin particularly draw attention [29]. Taxifolin has been shown to exert antioxidant effects by inhibiting ROS production [14]. It has been reported that taxifolin protects stomach tissues from oxidative stress with those antioxidant effects [15].

In our study, it was observed that taxifolin inhibited the decrease of COX-1 level in gastric tissue with aspirin. As is known, the COX-1 enzyme plays a critical role in maintaining the integrity of the GI mucosa. [2] In the literature, it has been reported that aspirin causes damage by lowering the COX-1 level. [30] This literature information indicates that the gastroprotective effect of taxifolin is due to its protective effect on COX-1. COX-2 inhibition is responsible for the therapeutic effects of aspirin and other NSAIDs, and COX-1 enzyme inhibition is responsible for its side effects. [3] As can be understood from our experimental results, aspirin, an anti-inflammatory drug, significantly reduced the activity of the COX-2 enzyme. Taxifolin

241 did not significantly alter the inhibitory effect of aspirin on COX-2. This information shows
242 that the anti-inflammatory effect of aspirin will not be suppressed by taxifolin. It is even known
243 that taxifolin inhibits COX-2 and creates an anti-inflammatory effect. [31]

244 A study conducted by Naito et al. showed that ROSs have a role in the etiopathogenesis of
245 gastric damages induced by NSAIDs [32]. In tissues, enzymatic and nonenzymatic defense
246 mechanisms (or antioxidant mechanisms) develop against these damaging free oxygen radicals
247 (FORs) [33, 34]. Tissue damage begins with the formation of lipid radicals on the cell
248 membrane. These radicals then complete the damage by first turning into lipid hydroperoxides,
249 and finally into toxic products such as aldehydes, alkanes, and MDA [32, 35].

250 GSH is a tripeptide that can be synthesized from the amino acids glycine, cysteine, and
251 glutamate. It is a very important antioxidant that protects cells from oxidative damage by
252 reacting with free radicals and peroxides. GSH and other antioxidant substances (e.g., melatonin
253 or vitamins) prevent tissue damage by ensuring that the amounts of FORs in cells remain at
254 certain concentrations and low levels [36]. MDA, another biomarker of oxidative damage,
255 appears in the blood and urine, and it is the end-product of lipid peroxidation. Therefore, the
256 amount of MDA in biological material is an indicator of lipid peroxidation. Nonenzymatic lipid
257 peroxidation is a very harmful chain reaction. It directly damages the membrane structure and
258 indirectly damages other cell components through the reactive aldehydes that it produces. In
259 this way, it causes tissue damage and many diseases [37]. Toxic oxygen radicals formed in
260 excess amounts in tissues exposed to oxidative stress are known to stimulate lipid peroxidation,
261 which leads to the formation of MDA [38]. High MDA levels in damaged gastric tissues have
262 also been shown in a previous study [39]. In our study, measurements showed low GSH and
263 high MDA levels in the ASG group, while taxifolin increased the GSH levels and reduced the
264 MDA levels. Measurements of TOS and TAS showed similar results for MDA and GSH levels.

Taxifolin showed an anti-ulcer effect by protecting the stomach from aspirin-induced oxidative stress.

In conclusion, in light of the results that we obtained, 50 mg/kg taxifolin was revealed to have anti-ulcer effects. At this dose, taxifolin was able to bring COX-1 activities to a level close to those seen in healthy rats with appropriate macroscopic, oxidant/antioxidant, and biochemical parameters. Based on these results, it can be said that taxifolin may be successfully used as a more potent alternative to famotidine, which is the currently accepted treatment for aspirin-induced ulcers.

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FIGURE LEGENDS

Figure 1. Effects of taxifolin on aspirin-induced ulcers in rats. HCG: Healthy control group, ASG: 100 mg/kg aspirin group, TASG: 50 mg/kg taxifolin +100 mg/kg aspirin group, FASG: 20 mg/kg famotidine + 100 mg/kg aspirin group. Arrows indicate ulcer foci, starss indicate mucosal edema.

Figure 2. Cyclooxygenase enzyme activity results for aspirin-induced ulcers in rats. HCG: Healthy control group, ASG: 100 mg/kg aspirin group, TASG: 50 mg/kg taxifolin + 100 mg/kg aspirin group, FASG: 20 mg/kg famotidine + 100 mg/kg aspirin group.
* means $p<0.001$ when compared to ASG group.

TABLES

Table 1. Effects of taxifolin on aspirin-induced ulcers in rats.

Group	Number of animals	Total Ulcer area (mm ²)	Anti- ulcer effect (%)
HCG	6	0	-
ASG	6	83.33±1.45*	0
TASG	6	1.33±0.42**	98.4
FASG	6	5±0.57**	93.9

HCG: Healthy control group, ASG: 100 mg/kg aspirin group, TASG: 50 mg/kg taxifolin + 100 mg/kg aspirin group, FASG: 20 mg/kg famotidine + 100 mg/kg aspirin group. The results were presented as mean ± standard error of the mean (SEM). For comparisons of groups, one-way ANOVA was used. After ANOVA, according to the homogeneity of variances, the post hoc LSD (least significant difference) test was used. *means $p < 0.001$, compared to HCG group. ** means $p < 0.001$, compared to ASG group.

Table 2. Glutathione (GSH), malondialdehyde (MDA), total oxidant capacity (TOS), and total antioxidant capacity (TAS) measurement results for aspirin-induced ulcers in rats.

Group	tGSH (nmol/g protein)			MDA (μ mol/g protein)			TOS (nmol H ₂ O ₂ /mg protein)			TAS (μ mol Trolox equiv./mg protein)		
HCG	6.25	±	0.06	3.20	±	0.04	2.25	±	0.05	5.18	±	0.05
ASG	3.48*	±	0.11	5.47*	±	0.09	4.09*	±	0.16	2.70*	±	0.23
TASG	5.60**	±	0.09	3.37**	±	0.06	2.47**	±	0.16	4.61**	±	0.13
FASG	5.77**	±	0.04	3.27**	±	0.03	2.36**	±	0.08	4.86**	±	0.08

HCG: Healthy control group, ASG: 100 mg/kg aspirin group, TASG: 50 mg/kg taxifolin + 100 mg/kg aspirin group, FASG: 20 mg/kg famotidine + 100 mg/kg aspirin group. The results were presented as mean $\pm$ standard error of the mean (SEM). For comparisons of groups, one-way ANOVA was used. After ANOVA, according to the homogeneity of variances, the post hoc LSD (least significant difference) test was used. * means $p < 0.001$ compared to HCG group. ** means $p < 0.001$ compared to ASG group.

FIGURES and FIGURE LEGENDS

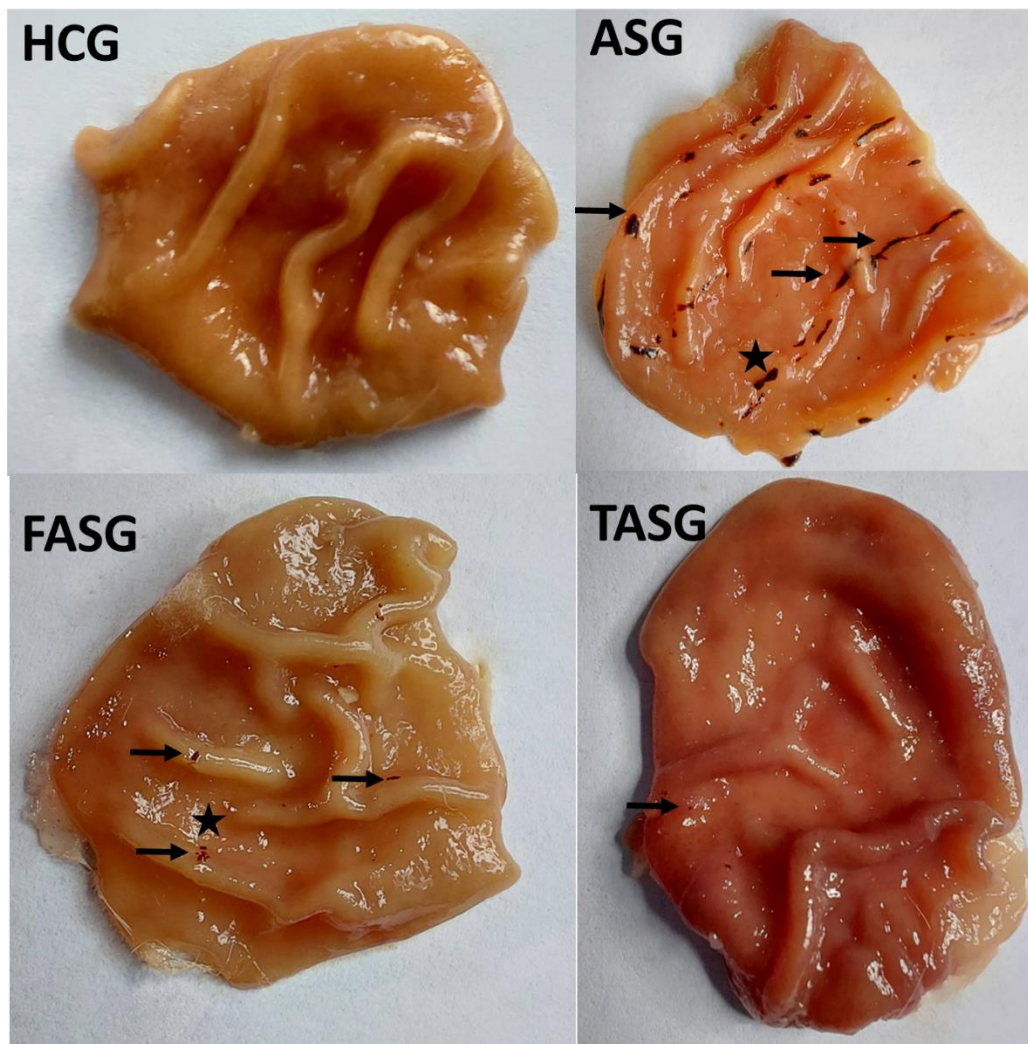


Figure 1. Effects of taxifolin on aspirin-induced ulcers in rats. HCG: Healthy control group, ASG: 100 mg/kg aspirin group, TASG: 50 mg/kg taxifolin +100 mg/kg aspirin group, FASG: 20 mg/kg famotidine + 100 mg/kg aspirin group. Arrows indicate ulcer foci, stars indicate mucosal edema.

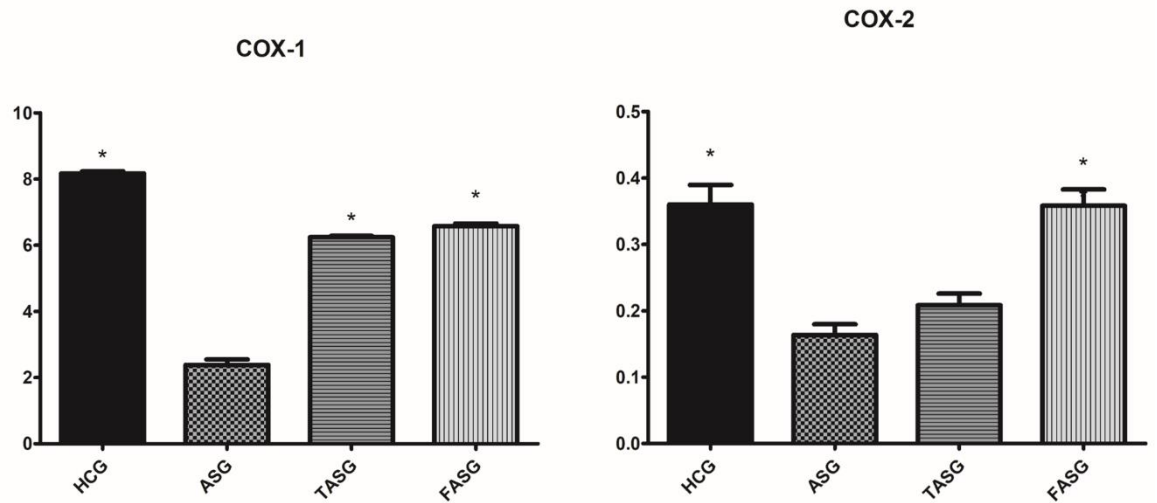


Figure 2. Cyclooxygenase enzyme activity results for aspirin-induced ulcers in rats.

HCG: Healthy control group, ASG: 100 mg/kg aspirin group, TASG: 50 mg/kg taxifolin + 100 mg/kg aspirin group, FASG: 20 mg/kg famotidine + 100 mg/kg aspirin group. * means $p < 0.001$ when compared to ASG group.