Gastroprotective effect of water extract of Muscari neglectum on indomethacin-induced gastric ulcer in rats

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Abstract

Muscari Mill. is used raw and cooked for gastric diseases, expectorants, wart treatment, and urine enhancers. No scientific study has been found on the effect of *Muscari neglectum* (MN) water extract on stomach diseases. Here, the effect of water extract of MN plant on some biochemical and histopathological parameters on indomethacin-induced gastric ulcer in rats was examined. In this study, 60 male Sprague Dawley rats were used for 24 acute toxicity and 36 gastric ulcer models (n = 6). They were divided into 6 groups as intact; indomethacin, famotidine, indomethacin and MN (100, 200, 400 mg/kg). The results of the gastric tissues examined biochemically, macroscopically and histopathologically showed that all doses of MN extracts prevented indomethacin-induced gastric mucosal damage and the 400 mg/kg dose had the strongest antiulcer effect with 69%. When SOD, GSH, CAT and MDA levels were investigated, the indomethacin-induced gastric ulcer group had a decrease in SOD, CAT and GSH levels and an increase in MDA levels. Additionally, LC-MS/MS analysis of the water extract of MN was performed and 14 phenolic compounds were determined. Biochemical analyses and histopathological examinations revealed that the water extract of MN has a good protective effect against gastric ulcer due to its high antioxidant content.

1. Introduction

Gastric ulcer is a disease affecting 10% of the world's population, caused by inappropriate diets, stress, anxiety, alcohol and cigarette addiction, *Helicobacter pylori* infection and overuse of nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (IND). This disease is caused by a disruption of the integrity of the mucosal layer, affecting the superficial or deeper muscularis mucosa of the gastric, which reduces the quality of life and productivity of many people [1].

Medicinal Aromatic Plants (MAPs) are therapeutic for many diseases and have not been sufficiently explored to provide scientific evidence of their claimed biological activity. It is necessary to reveal their potential by chemical analysis of the constituents of MAPs, determining their biological, pharmacological and toxic effects [2, 3]

Studies on many plants related to alternative medicine have been reported to be generally protective [4, 5]. It has been reported that leaves, tubers, flowers and flower buds of *Muscari* species are consumed raw and cooked and used as a gastricic, expectorant, wart treatment, urine enhancer [6]. *Muscari neglectum* (MN), popularly known as grape hyacinth; studies on its chemical composition and biological activities showed that it contains homo-isoflavanones [7], flavonoid, saponin, cardiotonic glycoside, alkaloid, terpenoid and steroid compounds [8, 9], uracil, succinic acid [10] Lim, 2014), caffeic, aconitic, gentisic, quinic and fumaric acid, kaempferol and apigenin [11, 12] and is rich in antioxidant content.

In the present study, the effects of antioxidant *Muscari neglectum* plant grown in Sakarya region on the protection from gastric damage in rats were investigated using biochemical parameters [Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Malondialdehyde (MDA)] and histopathological observations.

2. Materials and Methods

2.1. *Plant and Animal Material*

Muscari neglectum (MN) plant was collected from Sakarya province (40°44'41.2 "N 30°19'47.0 "E) in April 2022 and the identification procedures of MN were carried out by Sakarya University Faculty of Science, Department of Biology, and the sample material (M. Sağiroğlu 7068) was recorded in Sakarya University Herbarium (SAKU). The plant was dried in the shade at room temperature and stored under appropriate conditions until the study.

The animal material was conducted with the approval of Atatürk University Animal Experiments Local Ethics Committee (numbered 2022/213). 60 male *Sprague dawley* rats weighing 250–300 g were obtained from Atatürk University, Medical Experimental Application and Research Center Laboratories and fed ad libitum with standard rat food and water at room temperature (25 ± 1°C), 12 hours light/12 hours dark light period, 55–60% humidity.

2.2. *Phytochemical Screening and Antioxidant Activity Measurement*

2.2.1. *Plant Extraction and LC-MS/MS Analysis*

The above-ground parts of dried *Muscari neglectum* were crushed and extracted with water at 60°C in 30 minutes. Then, the extract was lyophilized (Bıobase-BK- FD10P) and the % yield value was calculated [8]. Phenolic content was determined by LC-MS/MS device (Shimadzu-8030) [13]

2.3. *Acute toxicity test of crude extracts*

In this study, four separate study groups (Intact group, 500 mg/kg, 1000 mg/kg and 2000 mg/kg *Muscari neglectum* extracts groups) consisting of six rats each were established using a total of 24 rats. The rats were fasted overnight before the treatments and the toxicity and mortality-related behaviors of the rats were observed with periodic monitoring for 24 hours for 14 days after the administration of *Muscari neglectum* doses [14].

2.4. *Experimental Protocol and Indomethacin-Induced Ulcer Model*

Animals were randomly divided into 6 groups, each consisting of 6 rats, as intact, control (Indomethacin; Endol® capsules, 25 mg/capsule; Deva Drug, Istanbul, Turkey), reference (Famotidine; Famodin® tablets, 20mg/tablet; Sandoz Drug, Istanbul, Turkey) and treatment groups (100, 200 and 400 mg/kg *Muscari neglectum*). Following 24 hours of fasting for ulcer formation, rats in the treatment groups were applied *Muscari neglectum* at doses of 100, 200 and 400 mg/kg and rats in the reference group were given 40 mg/kg famotidine orally. Five minutes after the application, 25 mg/kg dose of indomethacin was given by gastric gavage to all rats except the intact group [15]. After the six hours application, the rats were

decapitated under mild sevofluorane anesthesia. The gastrics of all animals were opened along the large quartur, washed with saline and examined macroscopically. Afterwards, the gastric tissues of all rats in the experimental groups were stored in a deep freezer at -20℃ for biochemical examinations and in 10% formalin solution for histopathological examinations.

2.5. *Biochemical Examination of Gasric Tissues*

Gastric tissues were lysed, diluted 1/9 with phosphate buffer (pH:7.4) and homogenized (TissueLyser LT (Qiagen) 50 hz, 1 min.). Homogenates were centrifuged at 5000xg for 5 min at + 4°C and supernatants were separated. SOD, CAT enzyme activities, GSH, MDA levels in the supernatants were analyzed by ELISA method (sandwich enzyme-linked immunoassay) using commercial kits (BT Lab, Zhejiang, China).

2.5.1. Macroscopic Examination of Gastric Tissues

The gastric tissues of the tested rats were opened and images were taken, ulcer areas and anti-ulcer effect (%) were calculated with the Stereo Investigator program [16].

2.5.2 *Histopathologic Examination of Gastric Tissues*

Tissue samples taken at the end of the evaluation were fixed in 10% formaldehyde solution for 48 hours and embedded in paraffin blocks after routine tissue follow-up procedures. 4 µm thick sections were taken from each block and the tissues prepared for histopathologic examination were stained with hematoxylin-eosin (HE) and examined by light microscopy (Olympus BX 51, JAPAN). The sections were evaluated as absent (-), mild (+), moderate (++) and severe (++++) according to histopathologic features.

2.6. *Statistical Analysis*

SPSS 13.0 package program was used in the analysis of all the data obtained and the data were evaluated by accepting $p < 0.05$ as significant. Statistical changes and significance levels in biochemical measurements were explained with the "One-way Analysis of Variance (ANOVA)" test. Tukey hoc test was applied for multiple comparisons. Duncan test was used for comparison between groups in histopathologic examinations. Non-parametric Kruskal-Wallis test was used to determine group interaction and Mann Whitney U test was used to determine differences between groups.

3. Results

3.1 *Result of acute toxicity study of the water extract of Muscari neglectum in rats*

An observational acute toxicity study showed that *Muscari neglectum* (MN) administered to test rats was relatively safe, and no toxic effects and deaths were recorded for 14 days. This showed that the LD_{50} of MN extract exceeded 2000 mg/kg

3.2. *Macroscopic Findings of Gastric Tissues*

In this study, the anti-ulcer effects of different doses of *Muscari neglectum* plant (100, 200 and 400 mg/kg) extracts and 20 mg/kg dose of famotidine (as a reference drug) on indomethacin (IND) induced experimental ulcer were investigated. It was found that all doses of MN extracts prevented indomethacin-induced gastric mucosal damage ($p < 0.001$), and 400 mg/kg dose showed the strongest antiulcer effect with 69% (Table 1). The same effect was similar to famotidine used as a reference (Fig. 1). All values in tables and figures are shown as mean ± standard deviation.

Table 1
Ulcer area and anti-ulcer effect in experimental groups.

Groups	Dose (mg/kg)	n	Ulcer Area (mm^2) (X ± SD)	Anti-ulcer effect (%)
INTACT		6	0,0 ± 0,0 ^{d}	-
IND	25	6	23,16 ± 3,76 ^{a}	-
IND + FAM	40	6	7,0 ± 0,07 ^{c}	67%
IND + 100MN	100	6	14,84 ± 6,5 ^{b,c}	36%
IND + 200MN	200	6	10,52 ± 3,83 ^{b,c}	54%
IND + 400MN	400	6	7,08 ± 1,97 ^{c}	69%

Different letters $^{a, b, c, d}$ indicate statistically significant differences ($p < 0.001$) in the same column. IND: Indomethacine; IND + FAM: Indomethacine + Famotidine, IND + 100MN: Indomethacine + 100mg/kg *Muscari neglectum*; IND + 200MN: Indomethacine + 200mg/kg *Muscari neglectum*; IND + 400MN: Indomethacine + 400mg/kg *Muscari neglectum*

3.3. *Histopathologic Findings of Gastric Tissues*

Intact group: Histopathologic examination of gastric tissues revealed normal histologic structure (Fig. 1B. a)

Indomethacin group: Histopathologic examination of gastric tissues revealed severe erosion and ulceration of the lamina epithelialis in the tunica mucosa, which progressed to the lamina propria. Severe degeneration and necrosis of the epithelium, cell infiltration and hemorrhage foci were observed in the interstitial spaces. Severe edema in the submucosa and severe hyperemia in the vessels were observed (Fig. 1B. b).

Reference group: Histopathologic examination of gastric tissues revealed very mild erosion of the lamina epithelialis, degeneration and necrosis of the epithelium, and moderate hyperemia of the vessels in the lamina propria (Fig. 1B. c).

IND + 100MN group: In histopathologic examination of gastric tissues, moderate erosions in the lamina epithelialis up to the lamina propria, moderate degeneration and necrosis in the epithelium were determined. Severe edema was observed in the submucosa (Fig. 1B. d).

IND + 200MN group: Histopathologic examination of gastric tissues revealed mild erosion of the lamina epithelialis, degeneration and necrosis of the epithelium and moderate hyperemia in the vessels. Mild edema was observed in the submucosa (Fig. 1B. e). A statistically significant difference (p□0.05) was detected when compared with the indomethacin group.

IND + 400MN group: Histopathologic examination of gastric tissues revealed very mild erosion of the lamina epithelialis, degeneration and necrosis of the epithelium, and mild hyperemia of the vessels (Fig. 1B. f). A statistically significant difference (p□0.05) was found when compared with the indomethacin group. Histopathologic findings were summarized in Table 2.

Table 2
The scoring of histopathologic findings in gastric tissues

	Erosion ulceration of the lamina epithelium	Epithelium degeneration and necrosis	Hyperemia of the veins	Edema in submucosal
INTACT	-	-	-	-
IND	++++	++++	++++	++++
IND + FAM	+	++	++	-
IND + 100MN	+++	+++	++++	++++
IND + 200MN	++	++	+++	+
IND + 400MN	+	+	++	-

IND: Indomethacine; IND + FAM: Indomethacine + Famotidine, IND + 100MN: Indomethacine + 100mg/kg *Muscari neglectum*; IND + 200MN: Indomethacine + 200mg/kg *Muscari neglectum*; IND + 400MN: Indomethacine + 400mg/kg *Muscari neglectum*

3.4. *Antioxidant enzymes and oxidative stress markers*

Free oxygen radicals have an important effect on the formation of indomethacin-induced ulcers. For this reason, in the present study, the effect of indomethacin on the antioxidant defense mechanism of MN was determined by GSH, MDA analysis and SOD and CAT enzyme activities (Table 3).

Table 3
SOD, CAT enzyme activities and GSH and MDA levels analyzed in rats gastric tissues.

Groups	Dose (mg/kg)	n	SOD (ng/mg wet tissue)	CAT (ng/ mg wet tissue)	GSH (mg/ g wet tissue)	MDA (nmol/mg wet tissue)
INTACT		6	1,26 ± 0,09 [b]	16,83 ± 0,14[c]	113,16 ± 0,01 [b]	0,59 ± 0,11[b]
IND	25	6	0,58 ± 0,2[a]	6,61 ± 0,01 [a]	49,81 ± 0,01 [a]	1,13 ± 0,11[a]
IND + FAM	40	6	2,15 ± 0,1 [c]	33,99 ± 0,1 [e]	137,5 ± 0,1[b]	0,55 ± 0,08 [b,c]
IND + 100MN	100	6	1,05 ± 0,15 [a,b]	14,71 ± 0,4 [b,c]	71,46 ± 0,14[a]	1,05 ± 0,17 [a]
IND + 200MN	200	6	1,13 ± 0,05 [a,b]	18,21 ± 0,14[c]	124,52 ± 0,11 [b]	0,56 ± 0,07[b,c]
IND + 400MN	400	6	3,22 ± 0,20 [d]	26,49 ± 0,08 [d]	134,51 ± 0,13 [b]	0,31 ± 0,09 [c]

Different letters [a, b, c, d] indicate statistically significant differences ($p < 0.001$) in the same column. IND: İndometazin, FAM: Famotidin, MN: *Muscari neglectum*, SOD: Süperoksit dismutase, CAT: Catalase, GSH: Glutathione, MDA: Malonaldehyde

SOD, CAT enzyme activities, GSH and MDA levels analyzed in rat gastric tissues are given in Table 3 and Fig. 3.

While SOD and CAT activities of the intact group did not show a significant difference when compared with IND + 100MN and IND + 200MN groups ($p > 0.05$), a significant difference was observed when compared with IND, IND + FAM, IND + 400MN groups ($p < 0.0001$).

There was no significant difference in the SOD activities of the IND group when compared with IND + 100MN and IND + 200MN groups ($p > 0.05$), while a significant difference was observed when compared with IND + FAM, IND + 400MN groups ($p < 0.0001$). When the CAT activities of the IND group were compared with the other groups, a significant difference was observed ($p < 0.0001$). This showed that the most effective MN dose was 400 mg/kg ($p < 0.0001$).

No significant difference was observed in the GSH level of the intact group when compared with IND + FAM, IND + 200MN, IND + 400MN ($p > 0.05$), whereas this difference was significant in IND, IND + 100MN ($p < 0.0001$). Compared to IND group, IND + FAM IND + 200MN, IND + 400MN increased GSH levels. While 200 and 400 mg/kg doses of MN, whose effect we tested, increased GSH levels ($p < 0.0001$), it gave similar results with the reference group ($p > 0.05$).

While there was no significant difference between the MDA levels of the intact group and IND + FAM and IND + 200MN groups ($p > 0.05$), this difference increased MDA levels in IND, IND + 100MN groups and decreased in IND + 400MN group ($p < 0.0001$). The 100 mg/kg dose of MN, which we tested the effect of, had no effect on MDA levels, while the 200 and 400 mg/kg doses showed a similar effect with the reference group and decreased MDA levels ($p < 0.0001$).

SOD and CAT activities showed a significant difference when IND group, IND + FAM, IND + 400MN groups were compared ($p < 0.0001$).

LC- MS/MS analysis of phenolic compounds of water extracts of above-ground parts of Muscari neglectum used in the experiment is shown in Table 4 and Fig. 4. In the analysis, Fumaric Acid, Protocatechuic Acid, Phloridzindyhrate and Myricetin compounds were found intensively, while Acetohydroxamic Acid, Syringic Acid, Resveratrol, Kaempferol, Gallic Acid, 4-Hydroxybenzoic Acid, Caffeic Acid, Salicylic Acid, Quercetin and Luteolin compounds were also found in certain amounts. It was observed that some of the components found in the studies of MN using different solvents were the same as in our study and some were different. [11, 12].

3.4. *LC- MS/MS Analysis*

Table 4
Quantitative analysis of phytochemicals in water extract of *Muscari neglectum* aeriel part by LC-MS/MS

	Analytes	RT	m/z	Compound Content (mg/g of dry extract)
1	Acetohydroxamic Acid (+)	0,460	76,15 > 58,00	0,0066244
2	Catechinhyrate (+)	2,482	291,00 > 139,00	ND
3	Vanilic Acid (+)	2,853	168,95 > 65,00	ND
4	Syringic Acid (+)	2,973	199,00 > 140,00	0,00575919
5	Thymoquinone (+)	3,338	165,00 > 137,10	ND
6	Resveratrol (+)	3,632	229,00 > 135,00	0,00322667
7	Myricetin (+)	3,725	319,00 > 153,00	0,01061646
8	Kaempferol (+)	4,222	287,00 > 153,00	0,00270288
9	Fumaric Acid (-)	0,859	115,30 > 71,20	0,30281874
10	Gallic Acid (-)	1,483	169,10 > 124,90	0,00455979
11	Protocatechuik Acid (-)	2,060	152,80 > 108,00	0,16369329
12	4-Hydroxybenzoic Acid (-)	2,550	137,20 > 93,00	0,00475702
13	Caffeic Acid (-)	2,850	178,80 > 134,90	0,00280178
14	Salisilik Acid (-)	3,480	137,20 > 93,10	0,0015531
15	Phloridzindyhrate (-)	3,523	435,30 > 272,90	0,02561198
16	2-Hydoxycinamic Acid (-)	3,553	162,80 > 118,90	ND
17	Oleuropein (-)	3,587	539,20 > 275,10	ND
18	2-hyroxy1,4 naphthaquinone (-)	3,654	172,80 > 144,90	ND
19	Naringenin (-)	3,960	271,20 > 150,90	ND
20	Silymarin (-)	4,000	481,10 > 124,90	ND
21	Quercetin (-)	4,000	300,80 > 150,80	0,00627964
22	Luteolin (-)	4,122	285,20 > 133,00	0,00278937
23	Alizarin (-)	4,552	238,80 > 211,00	ND
24	Curmin (-)	4,605	367,00 > 216,90	ND

This difference is thought to be due to the use of different parts of the plant and different solvents. Phenolic compounds have anti-cancer, anti-inflammatory, antiulcer and antioxidant effects in terms of health [17].

4. Discussion

Previous studies have shown that Protocatechuic Acid (PCA) has a protective effect against gastrointestinal oxidative damage, protects against neurotoxicity, nephrotoxicity and hepatotoxicity, and is therefore a good antioxidant and anti-radical protector [3, 18].

Park et al. [19] investigated the protective effect of Myricetin, a flavanoid, against gastric damage and observed that it increased total glutathione (GSSG/GSH) and prostaglandin E levels, increased SOD and cyclooxygenase-1 (COX-1) activity and decreased MDA levels in gastric tissues.

The therapeutic properties of fumaric acid (FA) against Cd-induced toxicity in rats were determined by a significant decrease in lipid peroxidation and an increase in GSH levels, GPx, SOD and CAT activities. It was stated that this effect can protect cellular membrane integrity against free radicals and lipid peroxidation products due to the antioxidant properties of FA [20].

Patel et al. [21] found in their study that locally applied dimethyl fumarate can be used in the treatment of gastric ulcers caused by ethanol.

Shakya et al. [22] reported that monomethyl fumarate, a component of methanolic extracts of fumaria indica, is a potent gastro-protector against gastric ulcers and other pathologies triggered by chronic and severe stress.

It has been reported that caffeic acid improves gastric mucosal damage [23] and kaempferol and apigenin have anti-ulcer effects [25, 26]. It has been stated that these effects are due to its antioxidant properties.

Previous studies have reported that Resveratrol inhibits the growth of Helicobacter pylori, which causes the development of ulcers, gastritis and cancer in the gastric, and the growth of breast cancer cells [26].

The studies mentioned above have generally reported that phenolic compounds are protective against gastric damage. Similarly, in our study, it was determined that the water extract of the above-ground parts of the MN plant, which contains these phenolic components, increased the level of antioxidant enzymes by synergistic effect and decreased the oxidant factor and showed a protective effect against gastric damage.

Eroğlu and Dogan [11] investigated the protective effects of ethanolic extract of aerial parts and bulb of MN at a dose of 400 mg/kg on carbon tetrachloride (CCl_4) induced gastric damage in rats and found that MN caused a statistically significant decrease in MDA levels and an increase in antioxidant parameters. In our study, the effects of three different doses of MN water extract (100, 200, 400 mg/kg)

against gastric damage caused by indomethacin were tested, the most effective dose was determined to be 400 mg/kg and showed similar effects to famotidine (reference). Compared to this 35-day study of Eroglu et.al., in our study, it was observed that water extract of MN aerial parts had a protective effect against indomethacin-induced gastric damage by decreasing MDA levels and increasing antioxidant parameters within 6 hours. Our results are compatible with Eroğlu and Doğan [11], however, it was also determined in our study that the water extract obtained showed a protective effect against indomethacin-induced gastric ulcers in rats in a very short period of 6 hours, and was also supported by pathological observations.

5. Conclusion

In conclusion, the findings of our research showed that the lethal dose of the water extract of the Muscari neglectum plant in rats was more than 2000 mg/kg. Biochemical and histopathological observations showed that the 100 mg/kg dose of Muscari neglectum was not very protective against gastric ulcers, but the 200 and 400 mg/kg doses showed a protective effect by reducing MDA levels and increasing SOD, CAT and GSH levels. It can be concluded that this effect is partly due to the phenolic components contained in MN. In the light of these studies, further toxicological and clinical studies to be conducted in the future will reveal the safety profile of MN more clearly and allow it to be used as an alternative treatment.

Abbreviations

MN, Muscari neglectum; FAM, Famotidin; IND, Indomethacin; SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde; COX-1, cyclooxygenase-1; CCl_4, carbon tetrachloride; FA, fumaric acid; PCA, Protocatechuic Acid

Declarations

Authorship contribution statement

Menekse Soydan: Investigation, Conceptualization, Methodology, Analysis of biochemistry, Formal analysis, Writing - original draft. **Gulnur Arabaci:** Investigation, Methodology, Writing - review & editing original draft, Supervision, Project administration. **Necati Utlu:** Investigation, Writing – review & editing, original draft. **Mesut Bunyamin Halici:** Formal analysis. **Esra Aktas Senocak:** Analysis of biochemistry. **Metin Kiliclioglu:** Investigation, Analysis of pathology

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Conflict of interest The authors confrm that they have no confict of interest to declare.

Ethical approval All animals were treated with humane conditions, following the institutional guidelines for the care and use of animals.

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Figures

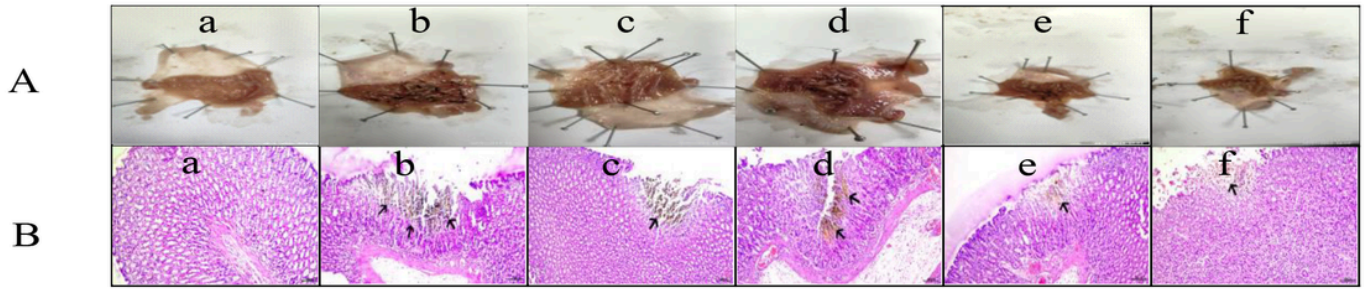

Figure 1

Results of gastric morphology and H&E staining. A, picture of rat gastric tissue; B, results of gastric H&E staining section 100μm. a, intact group; b, indomethacin group; c, reference group (20mg/tablet b.w. of famotidine); d, e, f, treatment groups (100, 200 and 400 mg/kg b.w. of *Muscari neglectum* respectively).

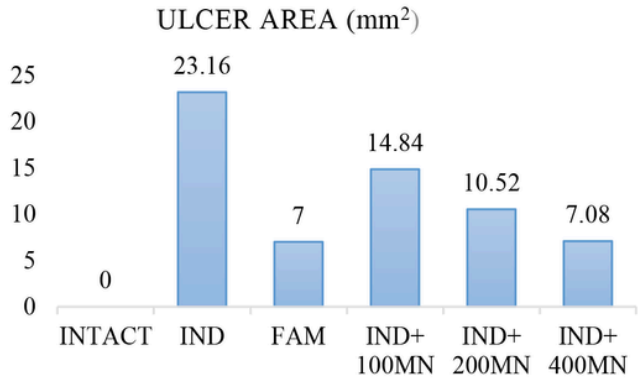

Fig. 2. Comparison of ulcer areas in the experimental group

Figure 2

See image above for figure legend.

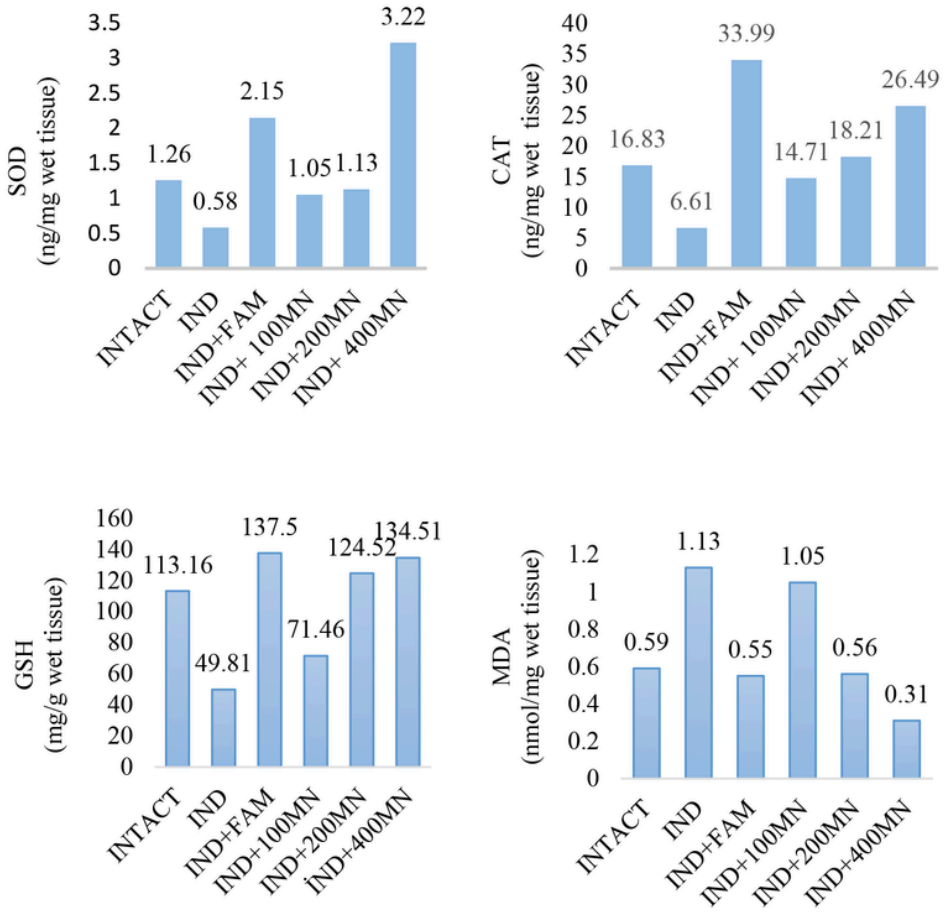

Fig. 3. SOD, CAT enzyme activities and GSH and MDA levels analyzed in rat gastric tissues IND: Indomethacin, FAM: Famotidine, MN: Muscari neglectum, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, MDA: Malonaldehyde

Figure 3

See image above for figure legend.

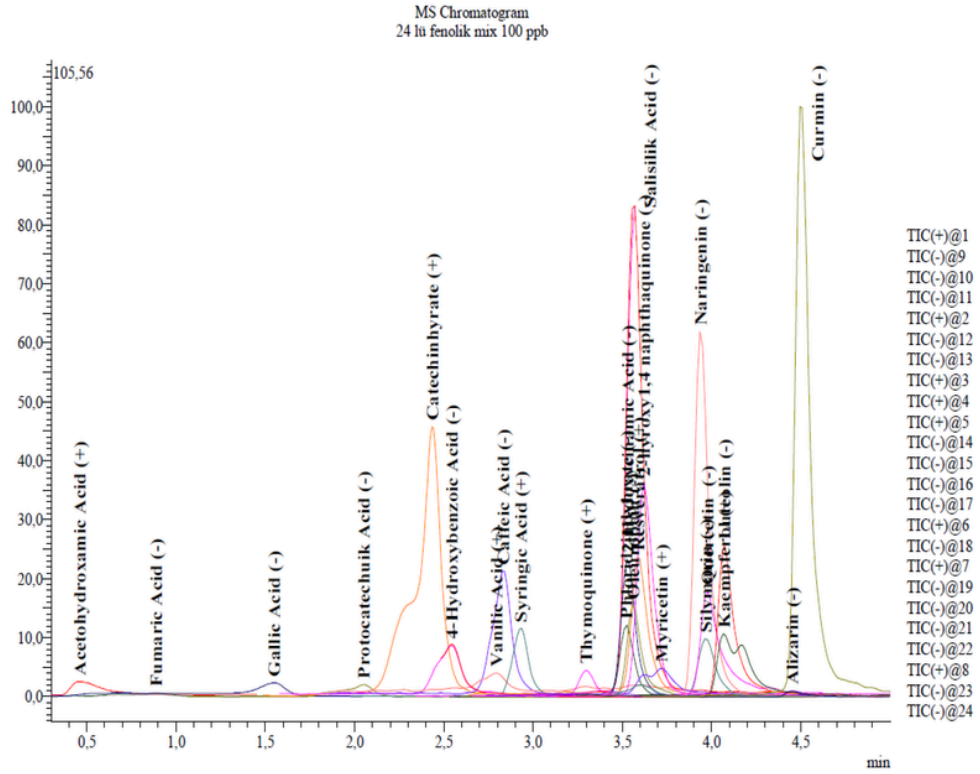

Fig. 4. LC-MS/MS chromatogram of water extract of *Muscari neglectum aerial part*

Figure 4

See image above for figure legend.