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Gastroprotective and Antioxidant Effects of the *Cinnamon, Cumin, Sumac* on Indomethacin Induced Gastric Ulcer in Rats

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Abstract

The gastro-protective effect of water extracts spice of cinnamon, cumin and sumac in indomethacin induced ulcer model in rats was investigated, in vivo and biochemical mechanism of spice extracts were monitored by measuring their antioxidant. For each cumin, cinnamon and sumach extracts 50, 100, 200 and 400 mg/kg, positive control famotidine (FAM) 25 mg/kg and negative control indomethacin (IND) 25 mg/kg dosage form was orally administered. After that catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPx) enzyme activities and glutathione (GSH), lipid perxidase (LPO) amounts were measured. In IND induced ulcer group, It is determined that each four dosage form of spices and positive control FAM group significantly decreased the ulcer area ($p<0.05$). In addition to this, CAT, SOD, MPx enzyme activities and GSH, LPO amounts were measured in spice extract administered gastric damaged rat stomach tissues in order to explain the effects of antioxidant defense system on antiulcerogenic activity. In IND induced tissues, while LPO, MPx and CAT increased GSH and SOD decreased with respect to the (healthy) control group. It is claimed by the measured enzyme activity differences that these spices have antiulcerogenic activity.

Keywords: Antioxidant, cinnamon, cumin, sumac, rat

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INTRODUCTION

The use of nonsteroidal anti-inflammatory (NSAID) drugs is very common in cases of pain and fever. However, these drugs have some side effects, especially on the gastrointestinal tract. Reactive oxygen species (ROS) play an important role in gastric lesions resulting from inhibition of prostaglandin synthesis. (Elliott and Wallace, 1997; Kaplan et al., 2012; Tanas et al., 2010). Indomethacin-induced ulcer models are a good source of reactive oxygen species. These play a creative role in gastric injury, neurodegeneration and arthritis through various processes, cancer, atherosclerosis (Miura et al., 2012; Halici et al., 2008; Kandaz et al., 2009; Karaca et al., 2009; Kumtepe et al., 2010).

Indomethacin is a commonly used analgesic in humans but in many studies, showed pro-oxidant activity and provided the production of ROS. Thus leading to the onset of lipid peroxidation and the confrontation of the mucosal cells with antioxidant systems (Miura et al., 2012; Halici et al., 2008; Kandaz et al., 2009; Karaca et al., 2009; Kumtepe et al., 2010; Takeuchi et al., 1991; Odabasoglu et al., 2006a; Odabasoglu et al., 2006b; Albayrak et al., 2010). Especially, ROS such as hydrogen peroxide (H_2O_2) can be seen as the cause of many illnesses, especially gastric damage. (Karaca 2013; Kumtepe et al., 2010) So, researchers have concentrated more on oxygen-derived free radicals in recent years (Mates et al., 1999; Cadirci et al., 2010a and b) ROS attacks unsaturated fatty acids, destroy the structure of membrane lipids and initiate lipid peroxidation. The enzyme activity, permeability, and cell activation are reduced in the damaged membrane proteins. Therefore, antioxidant defense systems are important to prevent the toxic effects of free radicals which cause many diseases. Oxygen-handling cells contain antioxidant enzymes, the enzymatic and non-enzymatic, that can protect them against oxidative stress (Odabasoglu, 2006; Koc et al.,2008). These antioxidants such as glutathione (GSH), vitamin-A and C, α-tocopherol, β-carotene also play an important role in the prevention of gastric damage.

Plants are used, all over the world for hundreds of years, in improving the taste and aroma of foods, (Shelef, 1983) eliminating unwanted odors in foods (Giese, 1994) and most importantly for therapeutical reasons. Spices that are known as aromatic herbal products are used for different purposes in food and beverages. Some parts of plants can be used as spices. Root, tuber, rhizome, onions, straw, bark, leaf, flower, fruit, seeds and secretion are parts of plants that can be used as a spice (Pruthi, 1980; Aran, 1988; Akgül, 1997). Cinnamon is genus of evergreen trees aromatic odor where is mostly found in South and Southeast Asia (Gunther, 1959). Due to its antioxidant properties provides a wide usage to cinnamon as a food product (Shan et al., 2005). Cinnamon bark extract has been reported that it has a good free radical scavenging effect and also depending to dose, it inhibits superoxide radicals in different models *in vitro* (Mathew and Abraham, 2006). The Motherland of cumin is Eastern Mediterranean and the Middle East. It is also found in northern and central Europe. Cumin has been reported that it is used for gas removed, milk enhancer, retarding the periodic bleeding, relieve diarrhea, muscle pain reliever (analgesic and myorelaksan effect), treatment of rheumatism (anti-inflammatory effect), dental pain (analgesic and anti-inflammatory action), pharyngitis (anti-inflammatory effect), abdominal pain (antispasmodic effect), the diuretic and urinary tract congestion on (anti-inflammatory effect) (Baytop, 1983; Pamuk, 1998). Sumac (*Rhus coriaria* L.) is called genus *Rhus* in the family of Anacardiaceae. *Rhus coriaria* is common in Turkey which is grown in different regions of the world around 150 types (Davis, 1967). Sumac is commonly used as spice in Turkey and the Middle East. Chemical compounds contained in sumac plant have antioxidant and antimicrobial properties (Wildman, 2001; Kosar et al., 2007).

Active oxygen forms are occurred with the encouragement of some factors during the normal process of oxygen usage in human metabolism. If active oxygen forms are not inhibited, they may cause structural corruption in DNA, protein, carbohydrates and lipids. Thus, Active oxygen forms bring about degenerative disease by corrupting both structure and functions of cell membrane (Katiyar ve Mukhtar, 1997; Sivritepe, 2000). Antioxidants inhibit degenerative disease formation and oxidation induced cell damages by either inhibiting active oxygen formation or inactivate the active oxygens E and C vitamins, karetenoids and phenolic compounds are the most important antioxidants for human health (Baublis ve ark., 2000; Sivritepe, 2000).

Their many beneficial effects emerge when many diseases are presented to related with oxidative stress (Luximon-Ramna et al., 2003, Toyokuni et al., 2003, Caia et al., 2004 and Romani et al., 2004). The antioxidants taken as daily nutrients can be said to be more effective in preventing diseases caused by oxidative stress. (Ames et al., 1995; Kaur and Kapoor, 2001). There is increasingly growing market for nutraceuticals and functional food. Products containing nutraceuticals have reached a worldwide estimated value of $65 billion (Lachance, 2002).

MATERIALS and METHODS

Chemicals

All chemical materials used in the experiments Sigma Chemicals Company (Germany) were obtained from.

Plant material

Spices (cinnamon, cumin and sumac) was used as working material in this research were obtained from the country's leading industrial companies and one of the most important spice producers ‘Bagdat Baharat’.

Extraction of plant materials

Examples of spices were pulverized with treated liquid nitrogen in a mortar. 100 g of species sample was weighed and was placed in a Soxalet device flask. They were extracted in a shaker water bath for two days. The extracts were filtered and the solvent content is also removed in rotary evaporator (the evaporator) by low pressure and low temperature. The extracts were lyophilized in a 5 mm-Hg pressure. % yields of extracts (g lyophilizate / 100 g of spices) was determined by weigh.

Antioxidant activity assays

The antioxidant activities of spice samples were measured by thiocyanate method. (Mitsuda et al., 1996). Briefly, each species sample (1mg) in 1 ml distilled water was mixed with 5 ml linoleic acid emulsion (0.02M, pH 7.0) and 5ml phosphate buffer (0.2M, pH 7.0). Linoleic acid emulsion was prepared by mixing 0.5608 g of linoleic acid with 0.5608 g of Tween 20 as emulsifier, and 100 ml phosphate buffer (0.2M, pH 7.0). The obtained mixture was homogenized and was incubated at 37 ^{0}C.

According to the thiocyanate method, the degree of oxidation was measured by sequentially adding 4.7 ml ethanol (75%), 0.1 ml ammonium thiocyanate (30 %), 0.1 ml sample solution, and 0.1 ml ferrous chloride (0.02 M, in 3.5 % HCl). After three minutes of waiting, the peroxide value was assigned by reading the absorbance at using a UV (ThermoSpectronic-HEλIOS β). Trolox and ascorbic acid solutions were used as positive control. While a separate linoleic acid was measured as a control group without extracts (Tables 2,3 and 4). Inhibition % was calculated by using the equation:

I = (1-absorbance of sample at 500 nm/absorbance of control at 500 nm) x 100

Table 2. The antioxidant activities of cinnamon species extract in different doses

Extract	Dose	Total Antioxidant Activitiy	Inh.	Reducing The Amount of Power	Phenolic Compounds	C Vitamin 1mg/ml (Inhibition %)	Trolox 1mg/ml (inhibition %)	Control (Water)
	mg/ml	Avg. Absorbance	%	Avg. Absorbance	mg GAE/g lyophilizate	Avg. Absorbance	Avg. Absorbance	Avg. Absorbance
WECN	1	0.423±0.001**d**	80.0	0.417±0.014**a**	0.746±0.008**a**	0.951±0.001**g** (55.0)	0.165±0.02**b** (92.2)	2.114±0.01**e**
	5	0.260±0.004**d**	87.7	1.500±0.012**b**	1.393±0.001**b**			
	10	0.204±0.007**b**	90.4	2.371±0.002**c**	1.790±0.003**c**			
EWECN	1	0.654±0.005**d**	43.4	0.391±0.003**a**	0.555±0.001**a**	0.413±0.008**e** (64.3)	0.109±0.001**c** (90.6)	1.160±0.014**f**
	5	0.321±0.002**c**	72.2	1.305±0.002**b**	1.293±0.001**b**			
	10	0.183±0.001**a**	84.1	2.194±0.001**c**	1.944±0.009**c**			
MECN	1	0.913±0.001**f**	45.4	0.079±0.002**a**	0.252±0.008**a**	0.570±0.006**e** (66.0)	0.184±0.003**b,c** (89.0)	1.671±0.001**d**
	5	0.830±0.003**e**	50.4	0.125±0.001**b**	0.351±0.001**b**			
	10	0.831±0.003**d**	50.3	0.151±0.002**c**	0.650±0.006**c**			
CECN	1	1.268±0.005**h**	26.7	0.089±0.008**a**	0.250±0.005**a**	0.631±0.01**g** (63.6)	0.308±0.006**b,c** (82.2)	1.731±0.003**h**
	5	0.988±0.002**f**	42.9	0.102±0.009**a**	0.352±0.001**b**			
	10	0.995±0.005**g**	42.5	0.142±0.008**b**	0.501±0.002**c**			

Table 3. The antioxidant activities of cumin species extract in different doses

Extract	Dose	Total Antioxidant Activitiy	Inh.	Reducing The Amount of Power	Phenolic Compounds	C Vitamin 1mg/ml (Inhibition %)	Trolox 1mg/ml (inhibition %)	Control (Water)
	mg/ml	Avg. Absorbance	%	Avg. Absorbance	mg GAE/g lyophilizate	Avg. Absorbance	Avg. Absorbance	Avg. Absorbance
WECM	5	0.226±0.001**b**	89.3	0.499±0.002**a**	0.652±0.001**a**	0.951±0.001**g** (50.0)	0.165±0.002**b** (92.2)	2.114±0.001**e**
	7.5	0.203±0.003**b**	90.4	0.948±0.003**b**	0.979±0.001**b**			
	10	0.184±0.002**b**	91.3	1.667±0.001**c**	1.509±0.002**c**			
EWECM	1	0.310±0.002**d**	74.2	0.243±0.001**a**	0.699±0.008**a**	0.227±0.003**c** (81.1)	0.111±0.002**a** (90.1)	1.201±0.001**g**
	5	0.208±0.002**a**	82.7	0.673±0.001**b**	1.401±0.009**b**			
	10	0.17±0.002**a,b**	85.9	1.023±0.001**c**	2.316±0.004**c**			
MECM	1	0.444±0.005**d**	73.4	0.273±0.005**a**	0.421±0.009**a**	0.568±0.006**e** (66.0)	0.184±0.003**b,c** (90.0)	1.671±0.001**d**
	5	0.33±0.003**c,d**	80.5	0.306±0.002**b**	0.680±0.001**b**			
	10	0.260±0.002**a**	84.5	0.384±0.002**c**	0.850±0.005**c**			
CECM	1	1.244±0.003**i**	28.2	0.129±0.008**a**	0.181±0.001**a**	0.631±0.001**g** (63.6)	0.308±0.006**b,c** (82.2)	1.731±0.004**h**
	5	1.244±0.003**i**	28.2	0.152±0.003**a**	0.203±0.008**b**			
	10	1.112±0.003**g**	35.7	0.132±0.001**a**	0.240±0.005**c**			

Table 4. The antioxidant activities of sumac species extract in different doses

Extract	Dose	Total Antioxidant Activitiy	Inh.	Reducing The Amount of Power	Phenolic Compounds	C Vitamin 1mg/ml (Inhibition %)	Trolox 1mg/ml (inhibition %)	Control (Water)
	mg/ml	Avg. Absorbance	%	Avg. Absorbance	mg GAE/g lyophilizate	Avg. Absorbance	Avg. Absorbance	Avg. Absorbance
WES	5	1.075±0.008**e**	49.2	0.516±0.003**a**	0.844±0.001**a**	0.951±0.001**g** (55.0)	0.165±0.002**b** (92.2)	2.114±0.001**e**
	7.5	0.958±0.006**f**	54.7	0.992±0.001**b**	1.442±0.008**b**			
	10	0.596±0.006**d**	67.1	1.344±0.01**c**	2.203±0.009**c**			
EWES	2.5	0.22±0.003**d,e**	82.6	0.641±0.005**a**	1.403±0.003**a**	0.300±0.001**e** (76.1)	0.124±0.001**b** (90.1)	1.255±0.01**g**
	10	0.186±0.001**e**	85.2	1.741±0.008**b**	2.401±0.001**b**			
	20	0.183±0.001**d**	85.4	2.561±0.008**c**	2.733±0.001**c**			
MES	2.5	0.598±0.006**e**	64.2	1.076±0.001**a**	1.602±0.001**a**	0.566±0.006**e** (66.0)	0.194±0.001**c** (88.4)	1.671±0.001**d**
	10	0.410±0.005**e**	76.0	2.618±0.009**b**	2.901±0.005**b**			
	20	0.323±0.001**c**	80.7	3.750±0.002**c**	3.402±0.008**b**			
CES	2.5	1.279±0.002**f**	26.2	0.174±0.008**a**	0.424±0.002**a**	0.631±0.001**g** (63.6)	0.308±0.006**b,c** (82.2)	1.731±0.003**h**
	10	1.190±0.004**h**	31.3	0.184±0.009**a**	0.531±0.001**b**			
	20	1.00±0.003**e,f**	42.0	0.221±0.001**b**	0.608±0.002**c**			

Animals

The 90 Wistar rats, weighing 180–200 g, were obtained from Experimental Animal Laboratory of Ataturk University, Experimental Animal Teaching and Researcher Center. The animals were kept under the same conditions (Care 1993). The experiment protocol of the Ethics Committee on Experimental Animal Use and Care was approved throughout the research (B.30.2.ATA.0.23.85-9/58).

Indomethacin-induced gastric damage

The gastroprotective effect of species was determined in comparison with famotidine. The animals were fasted for 24 hours. They are divided into groups to be practiced. 50, 100, 200, 400 mg/kg doses of cinnamon, cumin, sumac and 20 mg / kg doses of famotidine orally administrated to the rats. After five minutes, indomethacin was administered to induce damage. It was waited for 6 hours. At the end of 6 hours, the animals were sacrificed and the stomachs removed. The stomaches was washed and ulcer areas were identified on the millimeter paper (Halici et al., 2008; Karakus et al., 2009).

Biochemical investigation of stomach tissues

The biochemical enzymes such as catalase, myeloperoxidase, superoxide dismutase and the amounts of GSH, LPO were determined after the macroscopic analysis. The stomach tissues were ground to prepare the tissue homogenates with liquid nitrogen in a mortar. Then, 0.5 g tissue was kept under 4.5 ml of appropriate buffer. Ultra-turraks homogenizer were used to homogenize the stomach tissues. Filtration and homogenization process were carried out at 4°C. Then, these supernatants were used in order to determine enzymatic activities and amounts of GSH, LPO. All biochemichal assays were analyzed by using a UV–VIS spectrophotometer.

Catalase (CAT) activity

Decomposition of H_2O_2 in presence of catalase was at 240 nm (Aebi, 1984). Catalase activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25°C and pH 7.8. Results were expressed as mmol/min/mg tissue.

Myeloperoxidase (MPx) activity

According to the modified method of myeloperoxidase activity was measured (Bradley et al., 1982). The homogenized samples were frozen and thawed three times, and centrifuged at 1500 g for 10 min at 4°C. Myeloperoxidase activity in the supernatant was determined by adding 100 ml of the supernatant to 1.9 ml of 10 mmol/l phosphate buffers (pH 6.0) and 1 ml of 1.5 mol/l o-dianisidine hydrochloride containing 0.0005% (w/v) hydrogen peroxide. The changes in absorbance at 450 nm of each sample were recorded on a UV–VIS spectrophotometer. Myeloperoxidase activity in tissues was expressed as μmol/min/mg tissue.

Superoxide dismutase (SOD) activity

SOD activity was measured according to the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium (NTB) to form formazan dye (Sun et al., 1988). SOD activity was then measured at 560 nm by the degree of inhibition of this reaction.

Total glutathione (GSH) determination

The amount of GSH was measured in the appropriate method (Sedlak and Lindsay, 1968) with minor changes. The stomach tissues were homogenized in 2 ml of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 M sucrose (pH 7.5). The homogenate was centrifuge at 4200 rpm for 40 min at 4°C. The supernatant was used to determine GSH using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). Absorbance was measured at 412 nm. The results of the GSH level in the gastric mucosa were expressed as nmol/g tissue.

Lipid peroxidation (LPO) determination

The level of LPO was determined by estimating MDA using the thiobarbituric acid test (Ohkawa et al., 1979). The stomach weighed and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added with a solution containing 0.2 mL of 80 g/L sodium laurylsulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate and 0.3 mL distilled water. The mixture was incubated at 98°C for 1 h. Upon cooling, 5 mL of n-butanol: pyridine (15:l) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 1875 x g. The absorbance was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. The results were expressed as nanomol MDA per gram tissue (nmol/g tissue).

Statistical analyses

Statistical calculations were done by using SPSS 12.0 software. To be able to determine the statistical significance of AA, one-way variance analyses (ANOVA) was applied, which showed that there was a statistically significant difference ($p<0.05$) Multiple comparisons were performed Duncan test.

RESULTS

Gastroprotective effect of cinnamon, cumin and sumac on indomethacin-induced gastric damage

The gastroprotective effect of 50, 100, 200 and 400 mg/kg doses of species on IND-induced gastric damage in rats was macroscopically determined. Their inhibitory effects are showed in Table 1 and Figure 1. There was a significant improvement in treatment and FAM groups when there was a strong injury in the IND applied rats. As indicated in the table, when the ulcer areas were compared with indomethacin, the treatment groups were as effective as the famotidine group and provided the necessary healing. According to these results, FAM and all doses of species significantly protective against gastric damage caused by IND.

Table 1. Effects of different doses of species extracts and single dose of famotidine (FAM) on indomethacin (IND)-induced gastric damage in rats.

Treatment	N	Ulcer index (mm^2/rat)[a]	% Inhibition[b]
IND+Cinnamon (50mg/kg)	6	21.4±0.7i	48
IND+Cinnamon (100mg/kg)	6	20.3±0.3ı	50.6
IND+Cinnamon (200mg/kg)	6	19.1±0.1h	54
IND+Cinnamon (400mg/kg)	6	15.1±0.1d	63
IND+Cumin (50mg/kg)	6	20.1±0.1ı	51
IND+Cumin (100mg/kg)	6	18.1±0.1g	56
IND+Cumin (200mg/kg)	6	17.2±0.1f	58
IND+Cumin (400mg/kg)	6	14.1±0.1c	66
IND+Sumac (50mg/kg)	6	21±0.2i	49
IND+Sumac (100mg/kg)	6	19.1±0.1h	54
IND+Sumac (200mg/kg)	6	17.2±0.1f	58
IND+Sumac (400mg/kg)	6	16.1±0.1e	61
FAM (25 mg/kg)	6	7.1±0.1b	83
IND (25 mg/kg)	6	41.1±0.04j	0
Healthy[c]	6	0±0a	-

Means in the same column by the same letter are not significantly different to the Duncan test p<0.05).
a Mean damage index ± SE of six animals in each group.
b % Inhibition in ulcer index in relation to indomethacin group.
c Nothing administrated. N: The number of rats.

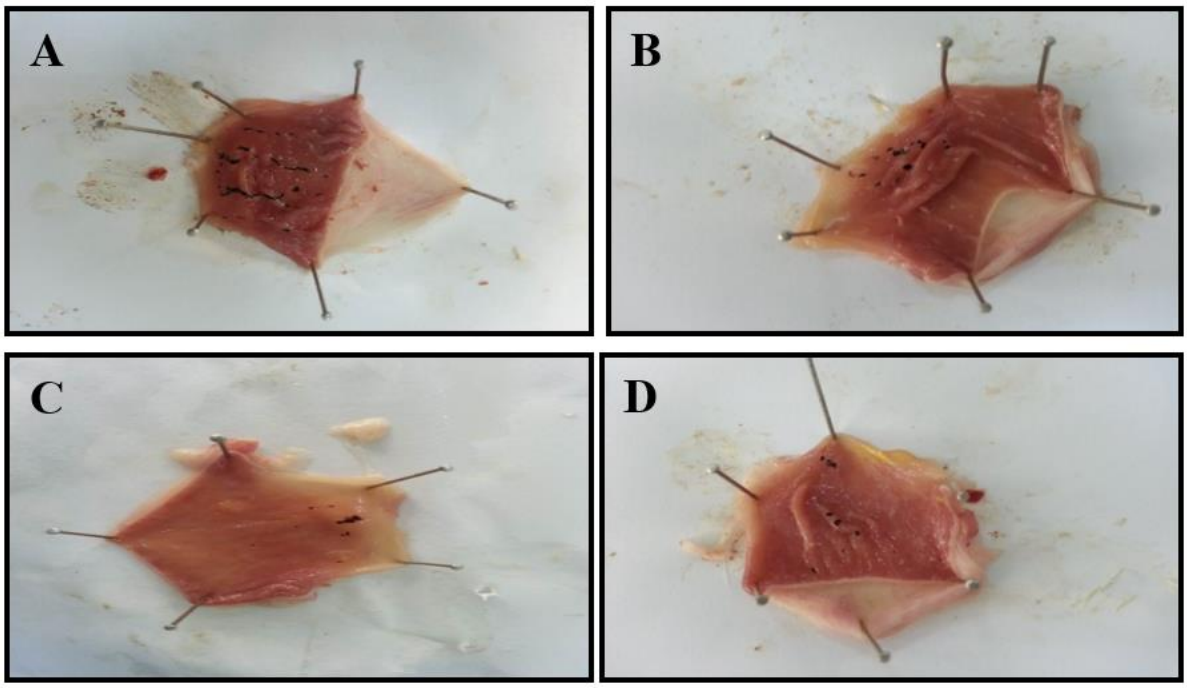

Figure 1. Ulcerous areas in the gastric tissues of indomethacin (IND)-induced rat by orally administrated (A). Sections of the gastric tissues after IND-administration were obtained from some experimental groups. The B, C and B sections show some ulcerative areas: B, the cinnamon group (400 mg/kg body wt.); C, Cumin group (400 mg/kg body wt.) and D; sumac group (400 mg/kg body wt.)

Comparison of enzymes' activities in rats' stomach tissues

The enzyme activities were measured to demonstrate the function of the antioxidant defense system in the prevention of ulcer formation and ulcers in rat gastric tissues. The results are presented in figures and tables shows that IND administration increased the LPO level compared to healthy rat tissues. In contrast to IND, all doses of species and positive control drug, famotidine, reduced the LPO level in rat stomach tissues. These results showed that species has a reducing effect on LPO in tissues Similarly, MPx and CAT enzyme activities were increased in the IND administration rats (Fig. 4,5,6).

Figure 4. Effects of different doses of species extracts and single dose of famotidine (FAM) on the catalase (CAT) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

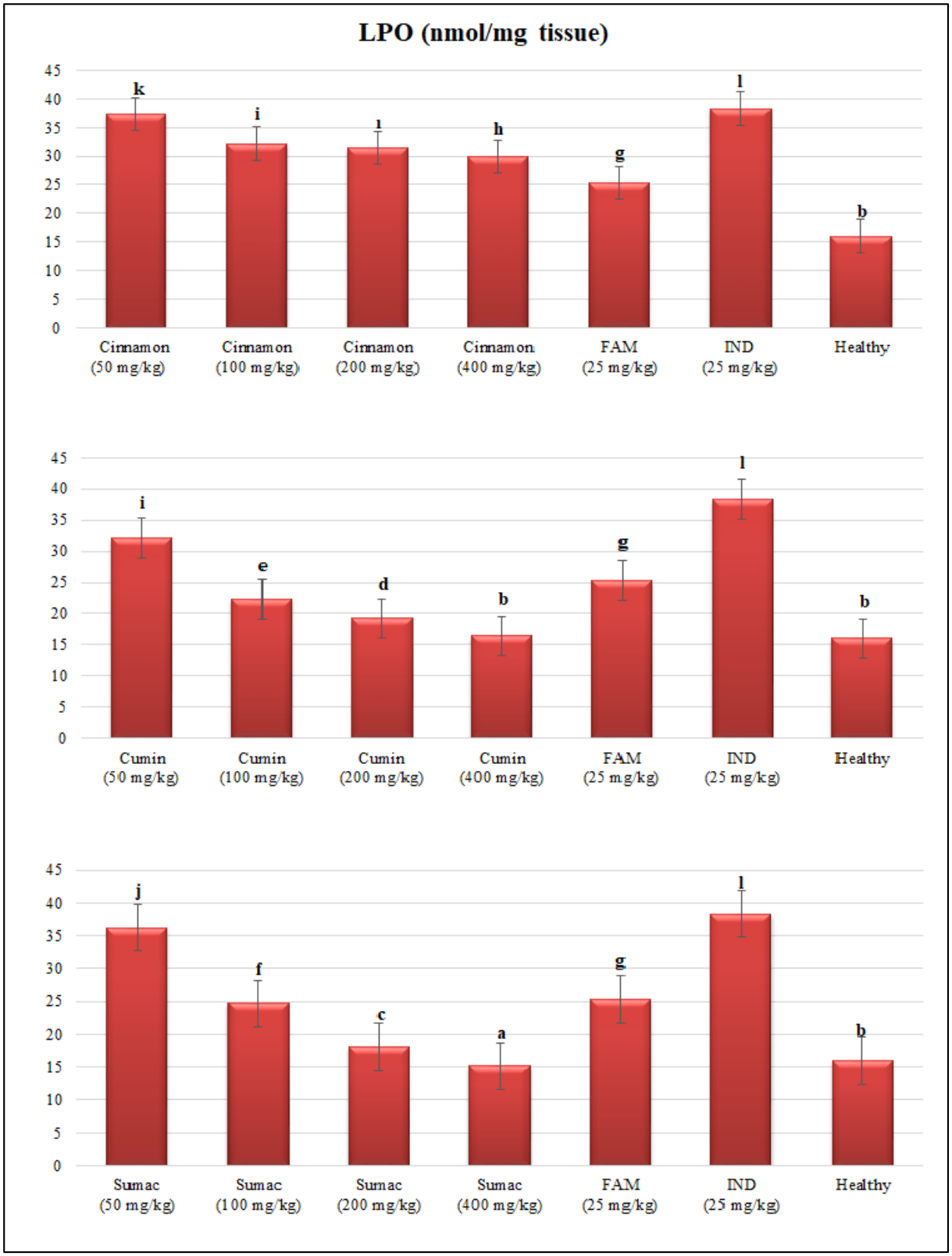

Figure 5. Effects of different doses of species extracts and single dose of famotidine (FAM) on the amount of lipid peroxidation (LPO) in rat's indomethacin (IND)-induced gastric tissue.Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

Figure 6. Effects of different doses of species extracts and single dose of famotidine (FAM) on the myeloperoxidase (MPx) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

This increase was reduced to the healthy group level owing to the spice samples applied at different doses and famotidine (Fig. 4 and Fig. 6). On the other hand, indomethacin increased SOD enzyme activity and glutathione levels in contrast to healthy tissues ($p<0.05$) (Fig. 2 and 3). This increase, caused by indomethacin, brought the applied spice extracts close to nearly healthy tissue. These results prove to be as protective as famotidine, a positive drug.

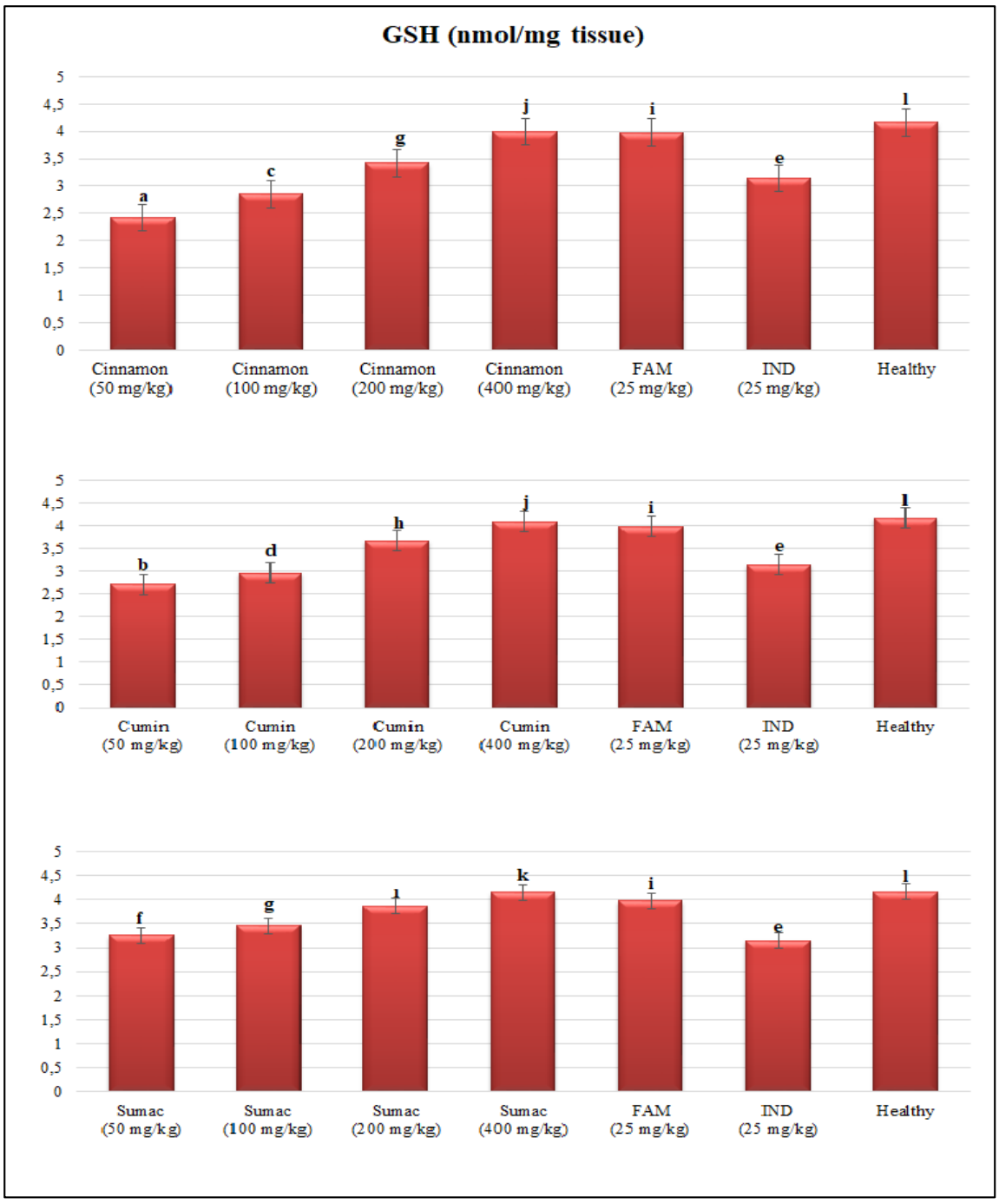

Figure 2. Effects of different doses of species extracts and single dose of famotidine (FAM) on the amount of glutathione (GSH) in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

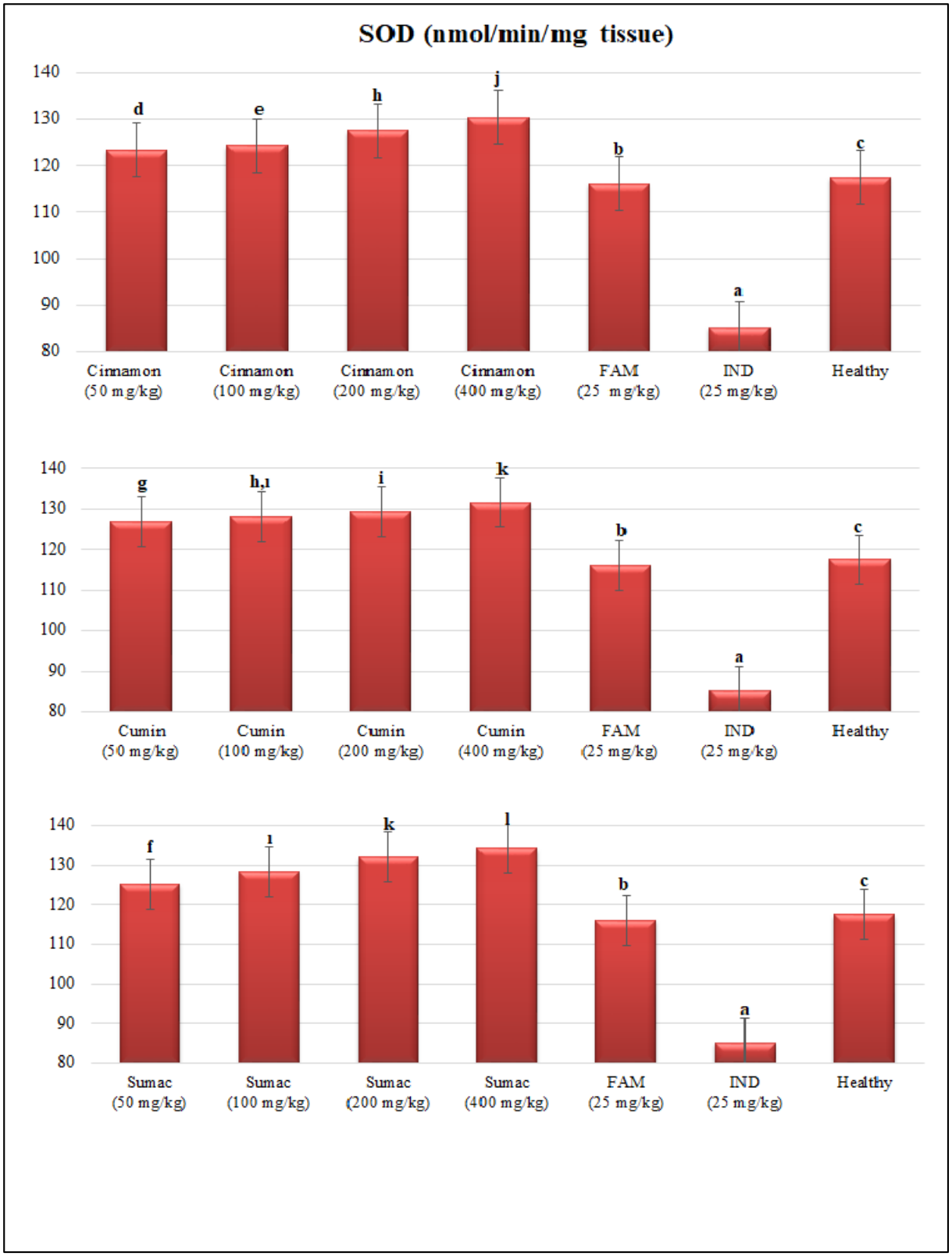

Figure 3. Effects of different doses of species extracts and single dose of famotidine (FAM) on the superoxide dismutase (SOD) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

DISCUSSION

The organism gives a physiological response to the immunological mechanisms in arousal, infection or trauma. This response demonstrates that the level of arachidonic acid is highly active. Thus, enzymes such as cyclo-oxygenase, 5-lipoxygenase, cytochrome P450 hydroxylase and epoxygenase provide for the formation of prostaglandins. (Odabasoglu et al., 2011). NSAIDs such as indomethacin, which is frequently used in daily life, interfere with the synthesis of prostaglandin and inhibit its release. It also increases the reabsorption of the hydrogen ions, thereby increasing the acid release. (Whittle, 1981).

The pathogenesis of NSAID-induced peptic ulcers is quite complex and many factors are connected. Recent advances in cellular and molecular biology have highlighted the importance of various prostaglandin-independent mechanisms. So, pharmacogenetic studies will be much more useful in elucidating damage.

In addition to adding flavor to the food, spices can be used as antimicrobial (garlic, mustard, thyme, red currant, cinnamon, clove), antioxidative (rosemary, sage, thyme, sumac, clove), blood pressure lowering (garlic) It is also used as donor (cement), aphrodisiac (vanilla), painkiller (clove) and soothing (sage) (Yıldız and Kılınc, 2010; Gurib, 2006). There are very few studies on the anti-ulcerogenic effects of cinnamon, cumin and sumac on the spices used widely in our country and in the world in the literature reviews made. There is little information about the mechanism of action in these studies. The current work is planned to determine whether the spices used so widely are effective on the digestive system, the organ system of which food is first, and will also shed light on the future use of these plants. So, the present study examined the gastroprotective effects of four doses of species (cinnamon, cumin and sumac) (50, 100, 200 and 400 mg/kg body wt.) on IND-induced gastric damage in rats (Table 1). Experiments were carried out using four different dosages of extracts from cinnamon, cumin and sumac spices. As a control group, rats given tap water were used. Naturally, there was no damage in the stomach of rats used as control group. Ulcerative hemorrhages were significantly detected in the IND rats used as a negative control group (Table 1) (41.1 / mm^2); the damage to the rat stomach was very low (7.1 / mm^2) in the FAM group given as positive control. For the extracts supplied with IND, it was determined that the damages were inhibited at different levels and that the damaged areas were reduced by doses. It was also found that gastric damage was reduced more effectively when given at a dose of 400 mg / kg for all three spice extracts. The current results show that all three spice varieties inhibit gastric damage, indicating that the most effective gastroprotective spice in these spices is also caraway (Table 1 and Figure 1). Orally administered IND (25 mg/kg body wt. dose) has caused gastric damage. This damage is caused by the inhibition of prostaglandins synthesized by COX-1 and COX-2. But it is much less active against COX-2 than against COX-1 (Whitle, 1981; Dengiz et al., 2007; Karakus et al., 2009; Tanas et al., 2010; Odabasoglu et al., 2011). However, in most of the studies have shown that ROS also play an important role in the mucosal damage caused by IND. (Elliot and Wallace, 1998; Miura et al., 2002; Karakus et al.,2009).

The IND carries out mucosal damage with the pro-oxidant property. It produces LPO by initiating ROS and interferes with endogenous antioxidant systems. (Miura et al., 2002; Tanas et al., 2010). Recent studies have shown that pro-oxidants expeditiously block cells' antioxidant systems, which causes ROS formation. As a result of this process, oxidative damage occurs (Elliot and Wallace, 1998; Miura et al., 2002; Dengiz et al., 2007).

As indicated by this mechanism, the level of LPO is shown to be greatly increased in tissues where IND is applied. But this increase in the treatment groups, the spice extracts and the FAM group, was found to be reduced to a level close to healthy tissue ($P<0.05$) (Sandip et al., 2000; Odabasoglu et al., 2006).

Living organisms have both enzymatic and nonenzymatic antioxidant defense systems to neutralize oxidative stress. The most important enzymatic defense mechanisms include SOD, CAT and glutathione metabolism enzymes (GPx, GST and GR). GSH, tocopherols (E-vitamin), ascorbic acid (C vitamine), A-vitamine and phenolic substances are non-enzymatic antioxidant defense system components (Atalay et al., 2016; Odabasoglu et al., 2006; Halici et al., 2011).

A number of studies have reported that many NSAIDs, such as IND, reduce SOD activity in the stomach (El-Missiry et al., 2001; Mizoguchi et al., 2001; .de la Lsatra et al., 2002; Tanas et al., 2010). SOD is the most important enzyme that disables superoxides. The CAT enzyme is another enzyme that converts H_2O_2 into water. GSH is an antioxidant molecule found in all tissues that electron transfers different free radicals. It has been shown that GSH protects cell membranes from oxidative damage in gastric tissues (Kaplan et al., 2012; Atalay et al., 2016; Odabasoglu et al., 2006; Halici et al., 2011).

In addition to neutralizing GSH, H_2O_2 and its oxygen radicals, it also plays a role in the stimulation of prostaglandin synthesis in gastric tissues. As the radical oxygen molecules in organisms multiply, the level of GSH and other endogenous antioxidants decreases. At the level of these, the hypothalamus weakens the gastric mucosa against oxidative damage. This means; The decrease in the amount of SOD and GSH is indicative of an excessive increase in the amount of oxygen radicals such as LPO level. This judgment has been recorded in the literature with a number of studies (Sakurai and Yamasaki, 1994; Yoshikawa et al., 1997; Hiraishi et al., 1994; Atalay et al., 2016; Odabasoglu et al., 2006; Tanas et al., 2010).

SOD plays an important role in eliminating gastric damage by partially preventing oxidative damage. SOD destroys the highly reactive radical O_2 ($O_2^{-\cdot}$) by converting it into the less reactive H_2O_2 that can be destroyed by the CAT reaction. In Figure 3 we seemed that IND reduced SOD. This means that superoxide radicals could not convert to H_2O_2 by SOD. But CAT activity was increased in IND administrated tissues. The increase in CAT activity means that there is an increase in the amounts of H_2O_2, but how? It is reported that superoxide radicals spontaneously convert to H_2O_2 and peroxil (HO_2^-) radicals in acidic media (Mahadik and Scheffer, 1996). This spontaneous dismutation is fastest in pH 4.8. In addition, superoxide and perhydroxil radicals react with each other which causes and oxidation and a reduction. As a result of this dismutation, H_2O_2 and O_2 occurs (Weiss and Lobuglio, 1982). Thus, CAT activity in IND-administrated tissues might be increased because of this spontaneously dismutation (Figure 4).

CAT is a highly reactive enzyme that reacts with H_2O_2 to form water and molecular oxygen, and can also form methanol, ethanol, formic acid or phenols by donating hydrogen. (Sedlak and Lindsay, 1968; Elliot and Wallace, 1998). In the present study we established that all doses of species extract and FAM decreased CAT activity (Figure 4), which was increased by IND in rat gastric tissues. Our study found that SOD activity was reduced by IND ($P<0.05$), and that all doses of species extract and FAM increased SOD activity to near control group levels ($P<0.05$) (Figure 3). If species increases the activity of SOD, why CAT activity decreases in the same tissues?

In the presence of the Fe and Cu metals, H_2O_2 reacts with superoxides which results with the most reactive and damager free oxygen radical, hydroxyl radical (Weiss and Lobuglio, 1982). This reaction is called as Haber – Weiss reaction. Haber – Weiss reaction occurs in the presence of catalyst or without catalyst. The reaction without catalyst is very slow. The second way catalyzed by Fe^{3+} is very fast. Hydrogen peroxide could be changed to hydroxyl radicals via Fenton reaction in the presence of this iron. The mechanisms of these reactions were demonstrated by Figure 3 (Freeman and Crapo, 1982; Szabo, 1987).

On the other hand, Chen et al. (1998) suggested that CAT stimulates the expression of mRNA and the protein for COX-2 in rats' aortic smooth muscle cells, despite not affecting the expression of either mRNA or the protein for COX-1. That is, CAT exerted a biphasic effect on prostaglandin synthesis and enhanced prostaglandin production at low concentrations. This suggests that, at low concentrations, increased CAT activity may cause inflammation as reflected by increased COX-2 activity. One of the factors causing the IND-induced gastric ulceration process is possibly an augmentation of CAT activity, which was ascertained in the results of the present experiment (Figure 4).

The MPO assay has had widespread use as an index of neutrophil infiltration in various gastric injuries (Karakus et al., 2009; Tanas et al., 2010). As shown in Figure 6, the MPO activity in IND-administrated rat stomach tissues increases in comparison with that occurring in the tissues of healthy rats ($P<0.05$). The increase in this enzyme activity level may be associated with increases in the levels of neutrophil infiltration and H_2O_2 in those gastric damaged tissues administered with IND. The activity level of this enzyme was alleviated by each dose of species, acting counter to the IND. Similarly, FAM also decreased MPO activity. It has been reported that the release of MPO from gastric cells is another indication of the degree of ulceration, with NSAIDs such as IND also exerting their effects via inhibition of MPO pathways (Karakus et al., 2009; Tanas et al., 2010). Tissue MPO activity is a sensitive and specific marker of acute inflammation and reflects polymorphonuclear cell infiltration into the parenchyma. The effect of species on decreasing MPO activity may be related to its gastroprotective ability.

In conclusion, this experiment showed that IND successfully induced ulcers in rat stomachs. The species extract reduced ulcers at a greater magnitude when compared to FAM. The levels of MPO, anti-oxidant system enzymes (SOD and CAT), LPO and GSH were adversely affected by ulcer induction. Administrated drugs (FAM and species extract) alleviated the adverse effects of ulceration on these parameters. The gastroprotective properties of species extract could be related to its positive effects on the antioxidant system and MPO activity in IND-induced gastric ulcers in rats.

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DISCLOSURE STATEMENT

All authors declare that there are no conflicts of interest.

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