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N-Acetyl Cysteine Has Both Gastro-Protective and Anti-Inflammatory Effects in Experimental Rat Models: Its Gastro-Protective Effect Is Related to Its In Vivo and In Vitro Antioxidant Properties

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ABSTRACT

N-acetyl cysteine (NAC), a metabolite of sulphur-containing amino acid cysteine, is used as an antioxidant and a mucolytic agent. Therefore, we aimed to investigate anti-inflammatory and anti-ulcerative effects of NAC. We also intended to determine the relation between antiulcer effect of NAC and its antioxidant properties by biochemical evaluation. In this study a total of 15 rat groups (n = 6 per group) were used for inflammation and ulcer experiments. Anti-inflammatory effects of NAC have been investigated on six rat groups with carrageenan (CAR)-induced paw oedema model. Antiulcer effects of NAC have been investigated on 24 h fasted nine rat groups with IND-induced ulcer model in the presence of positive (LAN, RAN, FAM, and OMEP), negative (untreated IND group) and intact control groups. In biochemical analyses of stomach tissues; glutathione S-transferase (GST), catalase (CAT), myeloperoxidase (MPO), and superoxide dismutase (SOD) enzyme activities and lipid peroxidation (LPO) and the glutathione (GSH) levels were determined. All doses of NAC exerted significant anti-inflammatory effect; even the effect of 900 mg/kg NAC was similar with that of DIC and IND. In gastric tissues NAC administration decreased the level of LPO and activity of CAT, which were increased by IND. Furthermore, NAC increased the GSH level and SOD and GST activities, which decreased in ulcerous stomach tissues. Only MPO activity increased in both IND and NAC groups when compared to healthy rat group. We determined that NAC has both anti-inflammatory and anti-ulcerative effects. J. Cell. Biochem. 117: 308–319, 2016.

KEY WORDS: N-ACETYL CYSTEINE; INDOMETHACIN; GASTROPROTECTIVE EFFECT; MYELOPEROXIDASE; PAW OEDEMA; ANTIOXIDANT ENZYME

Inflammatory diseases such as rheumatoid arthritis, asthma, and hepatitis are major causes of morbidity in humans. Today, it is well known that chronic inflammation also leads to the development of cancer, cardiovascular diseases, and neurodegenerative diseases [Moncada et al., 1973; Odabasoglu et al., 2007; Albayrak et al., 2011; Oral et al., 2011; Uzkeser et al., 2012]. Two cycloocxygenase enxymnes, Cox-1 and COX-2 converst arachidonic acid into prostanoids in definite ratios. Nonsteroidal anti-inflammatory drugs (NSAIDs) which used to reduced fever, pain and inflammation inhibits COX enzymes [FitzGerald and Patrono, 2001; Odabasoglu et al., 2007, 2008, 2012] resulting in inhibited prostaglandin synthesis. Therefore gastric lesions revealed as an important adverse effect by inhibiting prostaglandin biosynthesis [Koc et al., 2008; Karakus et al., 2009]. Among NSAIDs, Indomethacin has been widely used to reduce inflammation, pain and fever in humans. It also causes an injury on the gastric mucosa due to the inhibition of COX enzymes and suppression of prostaglandins. [Elliot and Wallace, 1998]. Hydroxyl radicals ($OH^{\bullet}$) or superoxide anions ($O_{2}^{-\bullet}$) and hydrogen peroxide (H_2O_2), which are reactive oxygen species, cause lipid peroxidation in tissues [Elliot and Wallace, 1998; Karaca et al., 2009; Odabasoglu et al., 2012]. Oxidative stress also contributes in the development of many different pathologies such as local or systemic inflammatory disorders, respiratory disorders, rheumatoid arthritis,

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atherosclerosis, and cancer [Kumtepe et al., 2010; Albayrak et al., 2010, 2011; Odabasoglu et al., 2012; Cimini et al., 2015]. Aerobic organisms contributes in enzymatic and non-enzymatic antioxidant defence systems against reactive oxygen species. These antioxidants can be listed as; catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPO), reduced glutathione (GSH), glutathione reductase (GR), glutathione *S*-transferase (GST), vitamins and flavonoids [Odabasoglu et al., 2006, 2012]. Furthermore, antioxidative properties of the various anti-secretory drugs such as histamine H_2 receptor antagonists and proton pump inhibitors are also shown [Farzin and Attarzadeh, 2000].

N-acetyl cysteine (NAC) is a metabolite of the sulphur-containing amino acid cysteine. It has the molecular formula of $HSCH_2CH(NHCOCH_3)CO_2H$ and formula weight of 163.19 (Fig. 1). In humans it can be administered orally or by intravenous infusion and can also be inhaled using a nebulizer. Currently it is used as an antioxidant and a mucolytic agent. The therapeutic potential of NAC has been examined and is currently being further investigated across a range of illnesses as an antidote for specific toxins, as a bio-protective agent against oxidative stress and ischemic injury, and as a treatment for certain mental and physical illnesses. In addition, athletes also occasionally use it as a dietary supplement, in particular. Its widespread use is partly a function of its ready availability. NAC preparations for oral use are available without prescription from pharmacies and health food stores worldwide. It has a regulatory authority approval for intravenous use for the treatment of paracetamol (acetaminophen) overdose, and as a second line agent for the treatment of acrylonitrile and methacrylonitrile poisonings [Dodd et al., 2008]. Moreover, NAC has been purported to have anti-inflammatory properties [Farshid et al., 2010]. Induction of the pro-inflammatory transcription factors activator protein1 (AP-1) and NF-κB is inhibited by NAC. These transcription factors have been found to be induced in response to oxidative stress, supporting the argument that the anti-inflammatory properties of NAC are due to its mechanism of action as an antioxidant [Dickinson and Forman, 2002; Farshid et al., 2010].

This study aimed to (a) evaluate antioxidant potency (in vitro) of n-acetyl cysteine; (b) determine the effects of n-acetyl cysteine (Fig. 2) on carrageenan-induced acute inflammation; (c) investigate the protective effect of n-acetyl cysteine against indomethacin ulcer; and (d) determine the effects of n-acetyl cysteine on the myeloperoxidase enzyme and antioxidant defence systems which are important markers in the development of gastric mucosal lesions and protection from gastric damage.

MATERIALS AND METHODS

CHEMICALS

All chemicals obtained to Sigma Chemical (Germany). Other substances; n-acetyl cysteine (Mukonex eff. Tabl., 600 mg) from TriPharma-Turkey, diclophenac (Miyadren retard Tabl., 100 m) from Fako-Turkey, lansoprazole (Lansor capsule, 30 mg) from Sanovel-Turkey, famotidine (Neotab tabl., 40 mg), omeprazole (Demeprazol tabl., 20 mg), ranitidine (Ranitab tabl., 40 mg), and indomethacin (Endol capsule, 25 mg) from Deva-Turkey, were provided.

ANIMALS

Two different experiment was performed. In first experiment, we used 54 rats and studied IND induced gastric damage on these rats. In

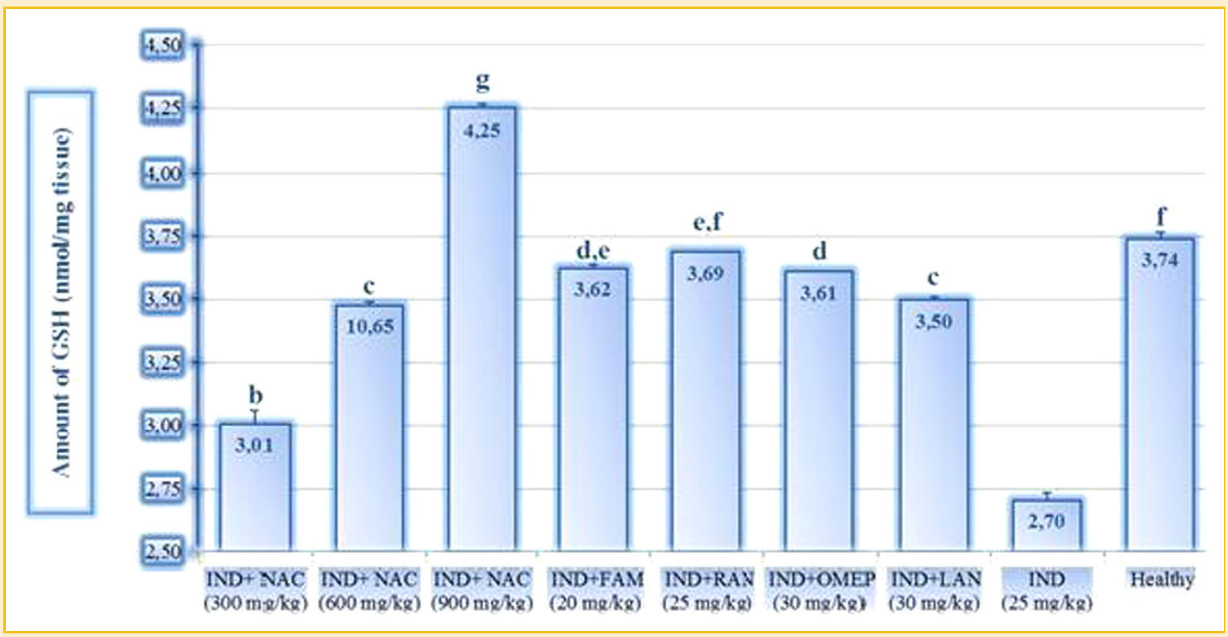

Fig. 1. Effects of different doses of N-acetyl cysteine (NAC) and single dose of ranitidine (RAN), famotidine (FAM), lansoprazole (LAN) and omeprazole (OMEP) on the amount of glutathione (GSH) in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($P < 0.05$). Results are means ± SE of three measurements.

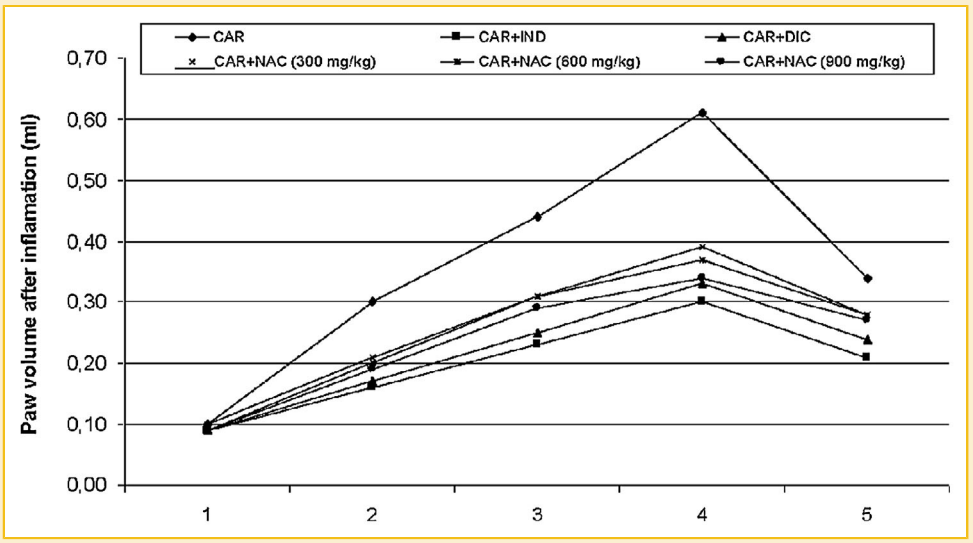

Fig. 2. Effects of three doses of NAC, one dose of IND or DIC on CAR-induced rats paw volumes in time (hour) post carrageenan.

second experiment, 36 rats were used in order to observe CAR induced paw oedema. Wistar rats in 180–190 g of weight were obtained in experimental research laboratory. Before the application, the rats were grouped and hold under the same conditions [Bowd, 1998]. CAR was injected into each animal into the group (0.1 mL of 1% per animal) and in addition n-acetyl cysteine (300, 600, and 900 mg/kg), indomethacin (25 mg/kg) and diclofenac (25 mg/kg) were given indicated doses.

TOTAL ANTIOXIDANT ACTIVITY ASSAY

Antioxidant activity of the n-acetyl cysteine (NAC) was determined using the thiocyanate method [Halici et al., 2005]. Briefly, doses of n-acetyl cysteine (0.5, 1, 2.5, 5, and 10 mg/kg doses) (1 mg) in 1 ml distilled water was confused and then was added 5 ml of phosphate buffer (0.2 M, pH 7.0) and 5 ml linoleic acid emulsion (0.02M, pH 7.0). 0.5608 g of linoleic acid, 0.5608 g of Tween 20 and 100 ml of phosphate buffer was mixtured. The mixture was homogenized and was incubated at 37°C.

The degree of oxidation was measured according to the thiocyanate method 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 (0.02M, in 3.5% HCl). The mixture stood for 3 min and the peroxide value was then determined by reading the absorbance at 500 nm using a UV-visible spectrophotometer (Thermo-Spectronic-HEλIOS β). A control was performed with linoleic acid but without the extract. Trolox, alpha tocopherol, and ascorbic acid solutions, prepared under the same conditions as described above, were used as positive controls. Inhibition % (I) was calculated using the following equation:

$$I = (1 - \text{absorbance of sample at } 500\,\text{nm}/\text{absorbance of control at } 500\,\text{nm}) \times 100.$$

CARRAGEENAN-INDUCED PAW OEDEMA IN RATS

The effect of NAC and diclofenac on carrageenan-induced paw oedema was researched in accordance with proposed method in this study. [Moncada et al., 1973; Tanas et al., 2010]. Briefly, three different doses of n-acetyl cysteine as 300, 600, and 900 mg/kg were orally applied to the experimental group. Plethysmometric measurements were done in order to calculate the paw volumes of the rats and 0.1 mL of 1% carrageenan injection was administered into the hind paw of each rat by waiting around 1 h from the last injection. The change in paw volumes was reported by six replicate measurements which performed at 60 min intervals by plethysmometry. Animals who receive equal volumes of diclofenac (25 mg/kg) and carrageenan (control) were compared in terms of the anti-inflammatory potency of n-acetyl cysteine.

INDOMETHACIN-INDUCED GASTRIC DAMAGE

In proposed method, the effects of several drugs used in ulcer treatments which are n-acetyl cysteine, lansoprazole, famotidine, omeprazole, and ranitidine on were applied onto indomethacin-induced gastric damage [Halici et al., 2005]. Protective effects of n-acetyl cysteine were compared with not only H_2-receptor blockers, ranitidine and famotidine, but also the proton pump inhibitors lansoprazole and omeprazole. Animals were separated to nine different groups and each of them consist of six rats. Food is not allowed to all groups during 24 h whereas groups have free access to water. At the 24 h, N-acetyl cysteine (300, 600, and 900 mg/kg body weight doses, prepared by suspending in water), lansoprazole (30 mg/kg body weight), famotidine (20mg/kg body weight), ranitidine (25 mg/kg), and omeprazole (30 mg/kg body weight) were orally injected to assigned groups of rats. Five minutes after the drug administrations, indomethacin (25 mg/kg body weight) were given to all rats by oral gavage. One group was determined to be the control group, which received only distilled-water. Six hours after the indomethacin administration, each groups were sacrificed with sodium thiopental (50 mg/kg). Stomachs of rats were collected and taken into a greater curvature. Then, they were washed with serum physiological solution (0.9% NaCl). There were no macroscopically visible lesions that was observed in order to calculate the gastric damage score. Therefore, the ulcerous stomach region was ingrained on a planar surface with small pins. The width of ulcer area was measured by using

millimeter paper. The score of ulcer index was based upon the area of the ulcers present in the glandular portion of the stomach (mm^2/rat). The indomethacin group was compared with the healthy group. The protective effect of NAC was evaluated with respect to the results of the indomethacin, lansoprazole, famotidine, omeprazole, and ranitidine groups. % inhibition of ulcer areas in accordance with the indomethacin group was calculated by the following formula:

$$\%\text{Inhibition} = 1 - [\text{Ulcer area treatment}/\text{Ulcer area control(indomethacin)} \times 100].$$

BIOCHEMICAL INVESTIGATION OF STOMACH TISSUES

After calculating the ulcer area, the enzymes activities such as superoxide dismutase (SOD), glutathione s-transferase (GST), catalase (CAT), myeloperoxidase (MPO), and the levels of lipid peroxidase (LPO) and total glutathione (GSH) were determined. Stomach tissues were ground by liquid nitrogen to prepare the tissue homogenates. Then, 0.5 g each of these tissues were treated with 4.5 ml of appropriate buffer. This tissue mixtures were homogenized by an ultra-turrax homogenizer for 15 min. Homogenates were centrifuged by using a refrigerated centrifuge at 4°C. Then, these supernatants were used for the determination of the biochemical enzymatic activities.

SOD ACTIVITY

SOD activity was defined to Sun et al. [1988]. The generation of superoxide radicals produced by xanthine and xanthine oxidase reacts with nitro blue tetrazolium (NTB) to form formazan dye. The degree of inhibition of this reaction was measured at 560 nm and SOD activity is determined as millimole per minute per milligram tissue (mmol/min/mg tissue).

CAT ACTIVITY

The CAT enzyme activity was determined as the amount of enzyme required to decompose one nanomole of H_2O_2 per minute, at 25°C and pH 7.8 and was followed at 240 nm [Aebi, 1984]. The results of CAT activity are expressed to be mmol/min/mg tissue.

GST ACTIVITY

The GST activity was determined in accordance with the purposed method [Habig and Jacoby, 1981]. Spectrophotometric measurements was done at 340 nm in a 4 ml cuvette that containing tissue homogenate, 0.1 M PBS (pH 6.5), 30 mM glutathione and 30 mM 1-chloro-2,6-dinitrobenzene. The GST enzyme activity was determined as nanomole per minute per milligram protein (nmol/min/mg tissue).

MPO ACTIVITY

The modified Bradley method was applied in order to determine MPO activity [Bradley et al., 1982]. Frozen and thawed (three times cycle) samples were centrifuged at 1500 × g for 10 min at constant temperature (4°C). Hundred milliliter of supernatant is added to 1.9 mL of 10 mM phosphate for (pH 6.0), and 1 ml of 1.5 mM o-dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide in order to measure MPO activity. The changes in absorbance of each sample were determined by UV–vis spectrophotometer at 450 nm wavelength. MPO activity of gastric tissues was expressed to be μmol/min/mg tissue.

TOTAL GSH DETERMINATION

The method of Sedlak and Lindsay was obeyed to determine the amount of of GSH in the gastric mucosa [Sedlak and Lindsay, 1968]. The mucosal surface of the stomach was collected and weighed. After that samples were homogenized in 2 ml of 50 mM Tris–HCl buffer which contained 20 mM EDTA and 0.2 M sucrose at pH 7.5. Precipitation was achieved by 0.1 ml of 25% trichloroacetic acid and the precipitate was discarded after the centrifugation at 4200 rpm for 40 min at 4°C. The supernatant of the homogenate was taken to determine GSH by using 5,5′-dithiobis(2-nitrobenzoic acid). Absorbance was recorded at 412 nm wavelength. The results of the GSH level in the gastric mucosa were expressed to be nmol/mg tissue.

DETERMINATION OF LIPID PEROXIDATION LEVEL

The levels of LPO were measured by estimating malondialdehyde (MDA) using the thiobarbituric acid test [Ohkawa et al., 1979; Halici et al., 2005]. At first, the rat′s stomach tissue were removed and rinsed cold saline. The bristles or any blood adhering on the epidermis were carefully removed to minimize the possibility of interference of haemoglobin with free radicals. The stomach tissues was collected and weight and then homogenized in 10 ml of 100 g/l KCl. The homogenate (0.5 ml) was added a solution which contain 0.2 ml of 80 g/l sodium laurylsulfate, 1.5 ml of 200 g/l acetic acid, and 1.5 ml of 8 g/l 2-thiobarbiturate, and 0.3 ml distilled water. These tissue mixtures were incubated at 98°C for 1 h. After cooling, 5 ml of n-butanol/pyridine (15:1) was added and was vortexed for 1 min and then the mixture centrifuged for 30 min at 4000 rpm. The supernatant absorbance was measured at 532 nm. A standard curve was generated using 1,1,3,3-tetramethoxypropane. All samples were measured in triplicate. The results were expressed as nmol MDA per gram wet tissue (nmol/g tissue).

STATISTICAL ANALYSES

The enzyme activities, the levels of GSH and LPO, differences between the paw volumes and ulcer areas was determined in accordance with the according with one-way analysis of variance (ANOVA) with the positive and negative control groups by using SPSS 11.0 software. Differences among the groups were reached using the Duncan option, and significance was declared at $P < 0.05$.

RESULTS

IN VITRO ANTIOXIDANT ACTIVITY OF N-ACETYL CYSTEINE (NAC)

The total antioxidant capacity of NAC was demonstrated by thiocyanate methods in our study. The results compared with the positive controls, trolox, alpha tocopherol, and ascorbic acid and after 96 h incubation with linoleic acid emulsion, the different doses of NAC was summarized as % inhibition in Table I. The all doses of NAC and the positive control groups reduced peroxide production when compared to control groups.

TABLE I. Anti-Oxidant Activity of Five Doses of NAC and One Dose of Positive Controls (Alpha Tocopherol, Trolox, and Ascorbic Acid) on the Linoleic Acid Oxidation, In Vitro

	Antioxidant activity (in vitro)	
Samples	Means of Absorbance (96. hours, 500 nm)[a]	% Inhibition
NAC (0.5 mg/ml)	0.50 ± 0.001b,c	69
NAC (1 mg/ml)	0.23 ± 0.000a	86
NAC (2.5 mg/ml)	0.09 ± 0.001e	94
NAC (5 mg/ml)	0.05 ± 0.001b,c	97
NAC (10 mg/ml)	0.04 ± 0.001b	98
Alpha-tocopherol (1 mg/ml)	0.041 ± 0.001b	97
Trolox (1 mg/ml)	0.06 ± 0.001c	96
Ascorbic acid (1 mg/ml)	0.7 ± 0.000d	56
Control	1.60 ± 0.010f	–

Means in the same column by the same letter are not significantly different to the Duncan test ($P<0.05$).
[a]The average values of three calculations are presented as mean ± SE.

CARRAGEENAN-INDUCED PAW OEDEMA

Carrageenan injection in rats led to a time-dependent increase in paw volume (Table II and Fig. 2). The increase in paw volume was observed starting from 1 h and it reached the maximum level at the 4 h after injection. Carrageenan-induced paw oedema was significantly reduced in a dose-dependent manner by NAC at 1st, 2nd, 3rd, 4th and 5 h after injection of carrageenan (Fig. 2). 300, 600 and 900 mg/kg doses of NAC significantly ($P < 0.01$) reduced the paw oedema by 36, 39 and 44%, respectively, at the 4 h (Table 2). Diclofenac (25 mg/kg) and IND (25 mg/kg) also significantly reduced the oedema by 46 and 51%, respectively. These results demonstrated that DIC, IND and all doses of NAC (Fig. 2) had a significant anti-inflammatory effect. The anti-inflammatory effect of IND, was stronger than that of diclofenac and all doses NAC.

GASTROPROTECTIVE EFFECT OF N-ACETYL CYSTEINE (NAC) ON INDOMETHACIN INDUCED GASTRIC DAMAGE

The gastroprotective effect of 300, 600, and 900 mg/kg doses of NAC on IND-induced gastric damage were macroscopically determined in rats (Table III and Fig. 3). There were remarkable hyperaemias in the stomachs of indomethacin group. In the NAC, lansoprazole, famotidine, omeprazole, and ranitidine given groups, hyperaemias were very slight compared to indomethacin-given rats. The ulcer index in rats receiving 300, 600, and 900 mg/kg NAC was 8.3 ± 0.7, 5.5 ± 0.6, and 1.7 ± 0.7, respectively. In the indomethacin, lansoprazole, famotidine, omeprazole and ranitidine groups, ulcer area were detected as 52.8 ± 1.3f, 1.8 ± 0.5, 3.2 ± 0.5, 6.7 ± 0.7, and 12.7 ± 0.7, respectively. Lansoprazole, famotidine, omeprazole, ranitidine, and 300, 600, and 900 mg/kg doses of NAC reduced the ulcer areas at a rate of 96.6%, 94%, 87.3%, 76%, and 84.3%, 90%, and 97%, respectively, compared to the indomethacin group (Table III). These results showed that lansoprazole, famotidine, omeprazole, ranitidine, and all doses of NAC had significant protective effect against the indomethacin induced gastric damage.

COMPARISON OF ENZYME ACTIVITIES IN RATS' STOMACH TISSUES

To evaluate the role of oxidative and antioxidative systems in the ulceration process, the levels of antioxidant enzymes (SOD, CAT, and GST), GSH, LPO, and MPO were measured in the stomach tissues of rats. The results are presented in Figures 4–8. Figure 4 shows that IND administration increased the LPO level compared to healthy rat tissues. In contrast to IND, all doses of NAC and other standard drugs reduced the LPO level in rat stomach tissues. These results showed that NAC has a reducing effect on LPO in tissues. Nevertheless, SOD and GST enzyme activities and GSH levels were found to be low in the tissue of rats given IND compared to healthy rat tissues (Figs. 5, 8 and 9). However, the activities of these enzymes and the level of GSH were increased by the administration of all doses of NAC and other standard anti-ulcer drugs compared to the stomach tissues of rats given IND. On the other hand, as can be seen from Figure 6, IND increased the CAT activity in comparison to healthy stomach tissues. In contrast to the tissue of rats given IND, all doses of NAC, LAN, FAM, OMEP, and RAN reduced the activity of this enzyme ($P < 0.05$).

We also measured the MPO activity in gastric tissues, which demonstrates neutrophil infiltration into tissues (Fig. 7). The administration of IND increased MPO activity compared to healthy group. All doses of NAC also significantly increased the MPO activity ($P < 0.05$), whereas the administration of LAN, FAM, OMEP, and RAN significantly decreased it.

DISCUSSION

Antioxidants contribute in many processes including the prevention of chain initiation, decomposition of peroxides, binding of transition metal ion catalysts, prevention of continued hydrogen abstraction

TABLE II. Effects of N-Acetyl Cysteine (NAC), Indomethacin (IND) and Diclofenac (DIC) on Carrageenan (CAR)-Induced Paw Edema (4 st h) in rats

Treatments	N	Dose (mg/kg body wt.)	Paw volume before inflammation (ml)	Difference between paw volumes (ml) at 4 hours[a]	Inhibition (%)[b]
CAR+NAC	6	300	1.11	0.39 ± 0.01c	36
	6	600	1.07	0.37 ± 0.02b,c	39
	6	900	1.04	0.34 ± 0.01b	44
CAR+DIC	6	25	1.03	0.33 ± 0.01a,b	46
CAR+IND	6	25	1.19	0.30 ± 0.01a	51
CAR (control)	6	–	0.98	0.61 ± 0.03d	–

Means in the same column by the same letter are not significantly different to the Duncan test ($P<0.05$).
[a]Mean edema volume (ml) ±SEM of six animals in each group.
[b]Percentage inhibition in paw volumes in relation to carrageenan group. Three doses of NAC, IND, and DIC treated groups were compared with CAR group. N, number of animals.

TABLE III. Effects of Different Doses of N-Acetyl Cysteine (NAC) and Single Dose of Ranitidine (RAN), Famotidine (FAM), Lansoprazole (LAN), and Omeprazole (OMEP) on Indomethacin (IND)-Induced Gastric Damage in Rats

Treatment	N	Dose mg/kg body wt.	Ulcer index (mm^2/rat)[a]	% Inhibition[b]
IND+NAC	6	300	8.3 ± 0.7d	84.3
IND+NAC	6	600	5.5 ± 0.6c	89.6
IND+NAC	6	900	1.7 ± 0.7a,b	96.8
IND+FAM	6	20	3.2 ± 0.5b	93.9
IND+RAN	6	25	12.7 ± 0.7e	75.9
IND+OMEP	6	30	6.7 ± 0.7c,d	87.3
IND+LAN	6	30	1.8 ± 0.5a,b	96.6
IND	6	25	52.8 ± 1.3f	–
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]Percentage inhibition in ulcer index in relation to indomethacin group.
[c]Nothing administrated. N, the number of rats.

and radical scavenging [Aksakal et al., 2011; Albayrak et al., 2011; Odabasoglu et al., 2012]. In this study, we performed an in vitro antioxidant activity assay to confirm in vivo antioxidant effects of NAC. For this purpose, we used the thiocyanate method in the presence of positive controls, trolox, alpha tocopherol and ascorbic acid. NAC (0.5, 1, 2.5, 5, and 10 mg/ml), ascorbic acid (1 mg/ml), α-tocopherol (1 mg/ml) and trolox (1mg/ml) after 44 h incubation with linoleic acid emulsion are also summarized as inhibition (%) in Table I. NAC, ascorbic acid, α-tocopherol, and trolox inhibited linoleic acid oxidation, when compared to the control. All doses of NAC and trolox, except for ascorbic acid exerted significant antioxidant activities when compared to the control ($P < 0.05$). NAC exhibited potent antioxidant activities with 69. 0–97.5% inhibition of linoleic acid peroxidation, respectively. Thus, it is suggested that the antioxidant properties of NAC might contribute in both gastroprotective and anti-inflammatory processes.

Inflammation is a physiological response of the organism against different stimulants such as trauma and infections. During inflammation, the arachidonic acid cascade is highly activated by cyclooxygenase and 5-lipoxygenase enzymes and formation of eicosanoids occurs. Many different inflammatory mediators such as prostaglandins, thromboxanes, and leukotrienes released during inflammatory reactions. Although drugs with a central mechanism of anti-pyretic and anti-inflammatory actions are known to down-regulate fever and inflammation, the role of antioxidant mechanism pathway in mediating the action of such agents is still unknown.

CAR induced paw inflammation, is an accepted method for evaluating anti-inflammatory agents, provokes a local, acute inflammatory reaction [Salvemini et al., 1996; Odabasoglu et al., 2008; Tanas et al., 2010]. CAR-induced paw edema occurs with contribution of inflammation mediators such as NO, PG, cytokines and free radicals, such as H_2O_2, superoxide, and hydroxyl radical etc. [Salvemini et al., 1996; Odabasoglu et al., 2008; Karaca et al., 2009; Tanas et al., 2010]. The levels of antioxidant parameters were evaluated in paw tissues to determine the effects of antioxidant defences on the acute inflammation process.

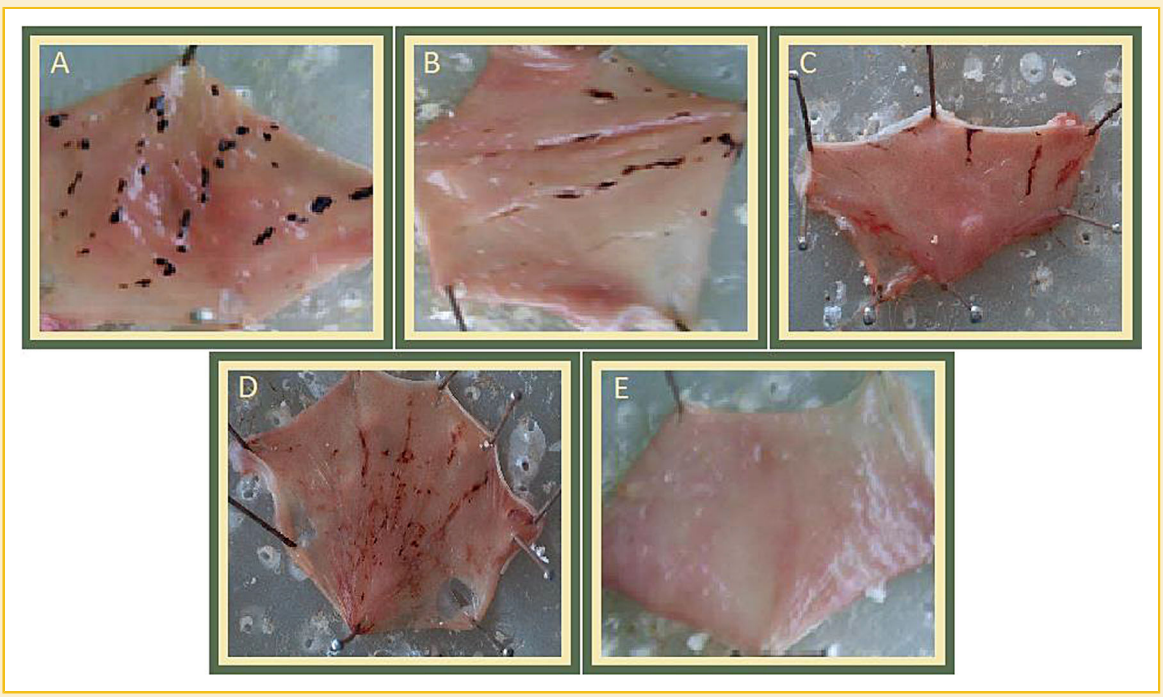

Fig. 3. Ulcerous areas in the gastric tissues of indomethacin (IND)-induced rat by orally administrated two doses of N-acetyl cysteine (NAC) and single dose of ranitidine (RAN). Sections of the gastric tissues after IND-administration were obtained from some experimental groups. The A–E sections show some ulcerative areas: (A) the control group (IND, 25 mg/kg body wt.); (B) IND-administrated plus NAC group I (600 mg/kg body wt.); (C) IND-administrated plus NAC group II (900 mg/kg body wt.); (D) IND-administrated plus RAN group (25 mg/kg body wt.); and (E) untreated group (healthy).

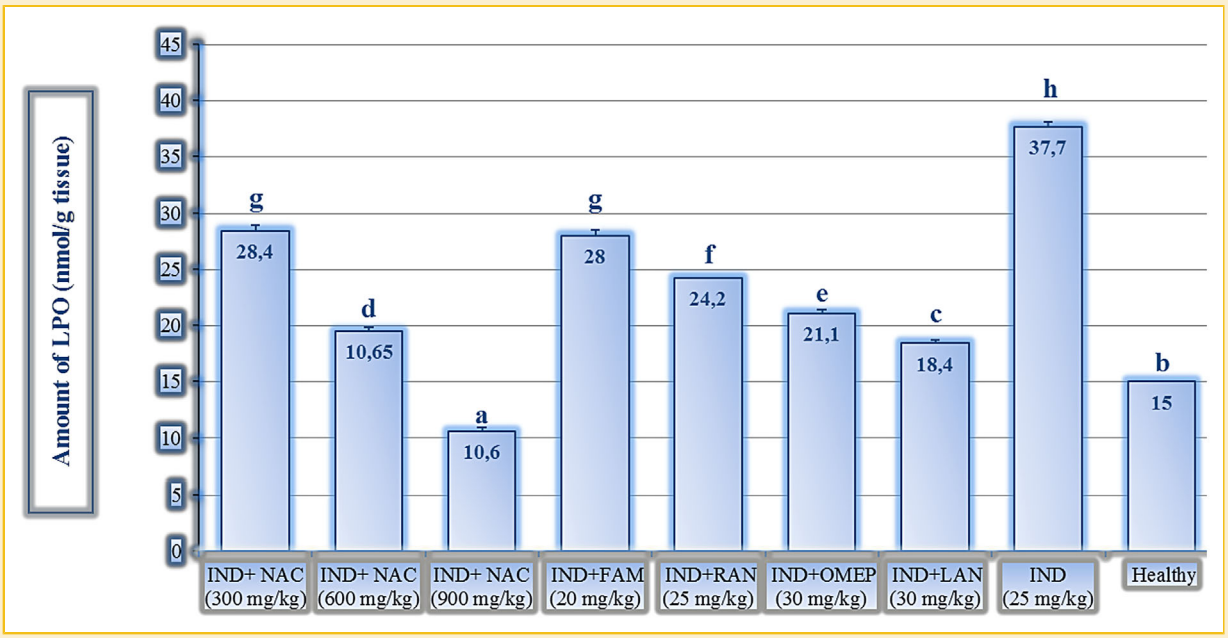

Fig. 4. Effects of different doses of N-acetyl cysteine (NAC) and single dose of ranitidine (RAN), famotidine (FAM), lansoprazole (LAN), and omeprazole (OMEP) on the amount of lipid peroxidation (LPO) in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($P<0.05$). Results are means ± SE of three measurements.

This study investigated the anti-inflammatory effects of the NAC, a potent antioxidant agent. Anti-inflammatory effects of NAC was observed in the CAR-induced paw oedema test, and its effects were compared to that of DIC and IND. In our experiment, the mean paw volume of the control group reached peak at the fourth hour. In all groups inflammation started to decrease after the fourth hour. 300, 600 and 900 mg/kg doses of NAC significantly ($P < 0.01$) reduced the CAR-induced paw oedema by 36, 39, and 44%, respectively, at the

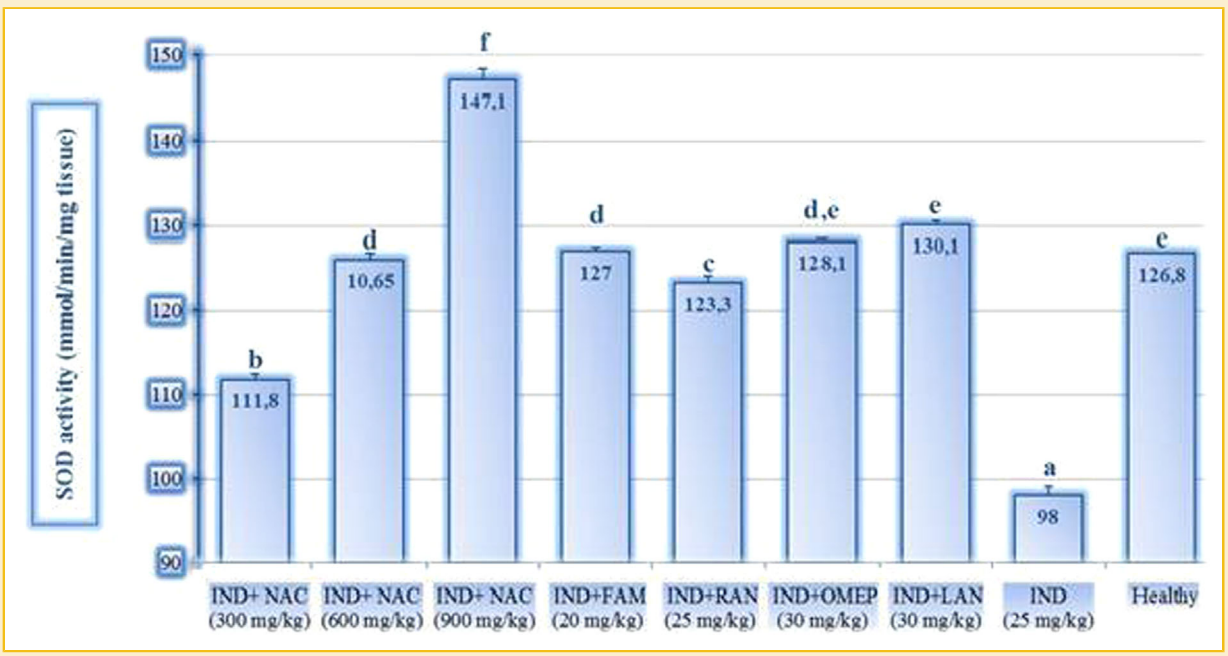

Fig. 5. Effects of different doses of N-acetyl cysteine (NAC) and single dose of ranitidine (RAN), famotidine (FAM), lansoprazole (LAN) and omeprazole (OMEP) on the activity of superoxide dismutase (SOD) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($P < 0.05$). Results are means ± SE of three measurements.

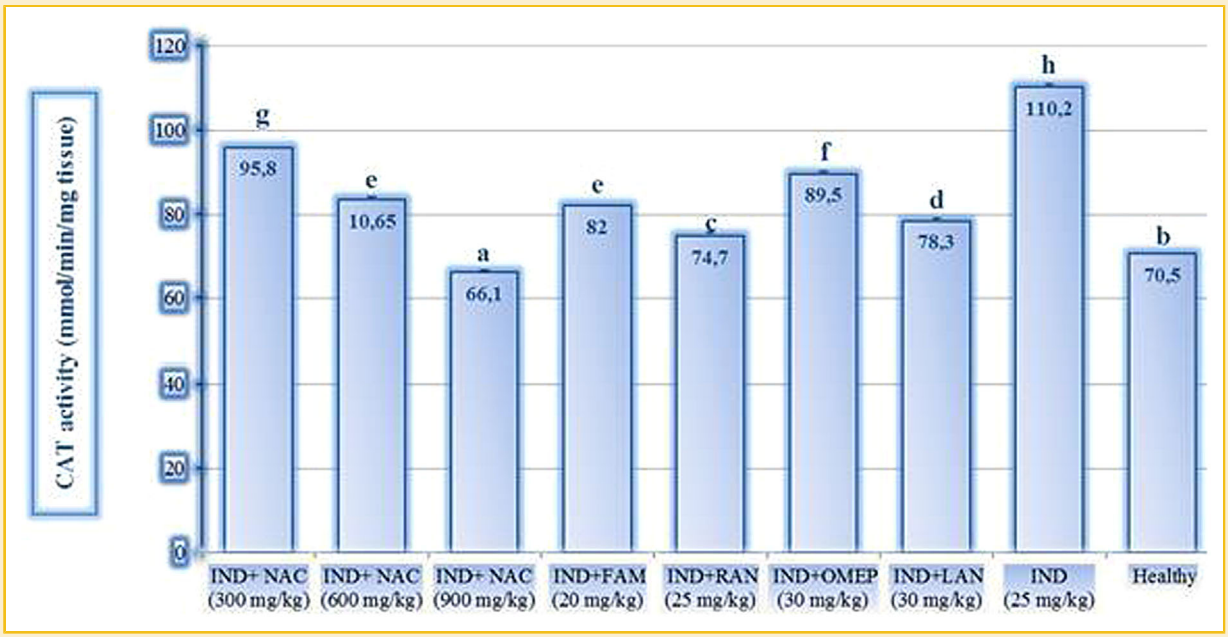

Fig. 6. Effects of different doses of N-acetyl cysteine (NAC) and single dose of ranitidine (RAN), famotidine (FAM), lansoprazole (LAN) and omeprazole (OMEP) on the activity of catalase (CAT) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($P<0.05$). Results are means ± SE of three measurements.

fourth hour (Table II and Fig. 2). DIC and IND showed the highest anti-inflammatory effect in this experiment. In the present study, we also determined that NAC has anti-inflammatory properties. Similar results have also been recorded in the literature. It has been reported that the effects of NAC in various inflammatory diseases might be related to the inhibition of NFκB, iNOS, and MPO activations, through decreased oxidative stress and also reduction in proinflammatory chemical mediators [Allen and Garner, 1980; Farshid et al., 2010].

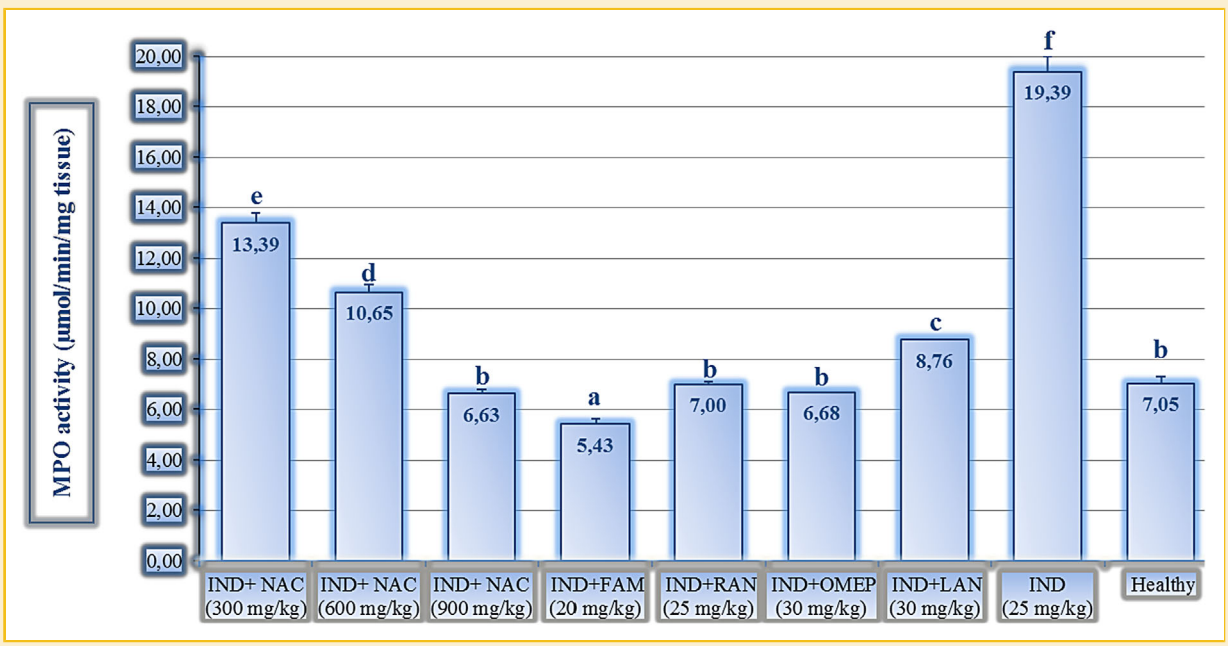

Fig. 7. Effects of different doses of N-acetyl cysteine (NAC) and single dose of ranitidine (RAN), famotidine (FAM), lansoprazole (LAN), and omeprazole (OMEP) on the activity of myeloperoxidase (MPO) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($P < 0.05$). Results are means ± SE of three measurements.

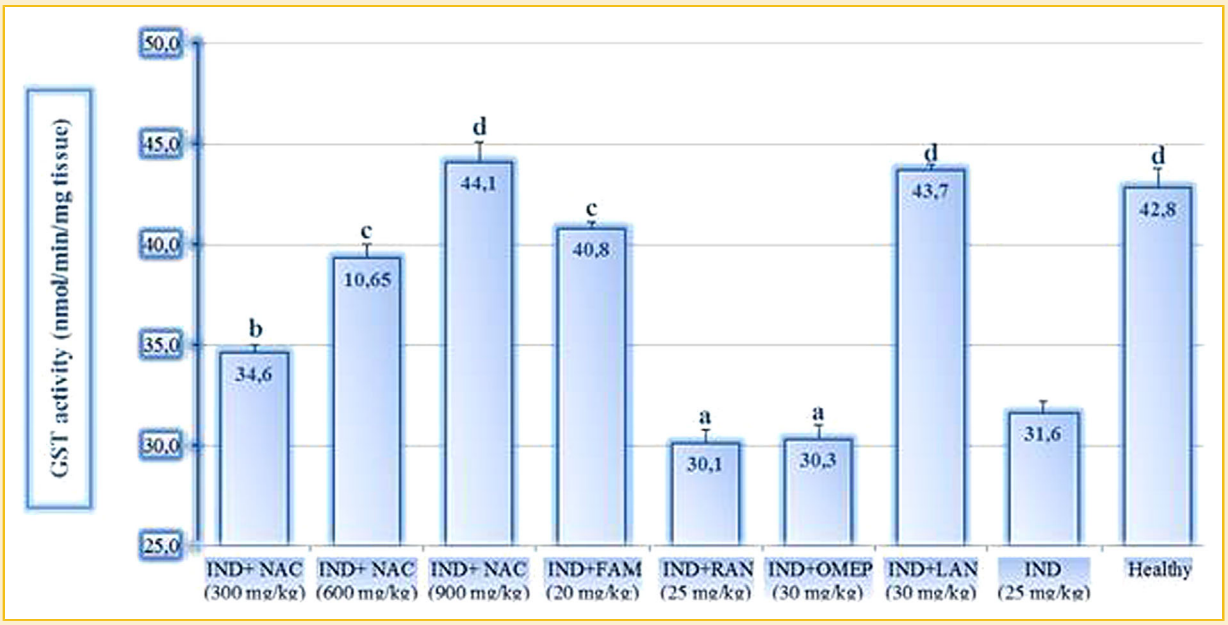

Fig. 8. Effects of different doses of N-acetyl cysteine (NAC) and single dose of ranitidine (RAN), famotidine (FAM), lansoprazole (LAN) and omeprazole (OMEP) on the activity of glutathione s-transferase (GST) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($P < 0.05$). Results are means ± SE of three measurements.

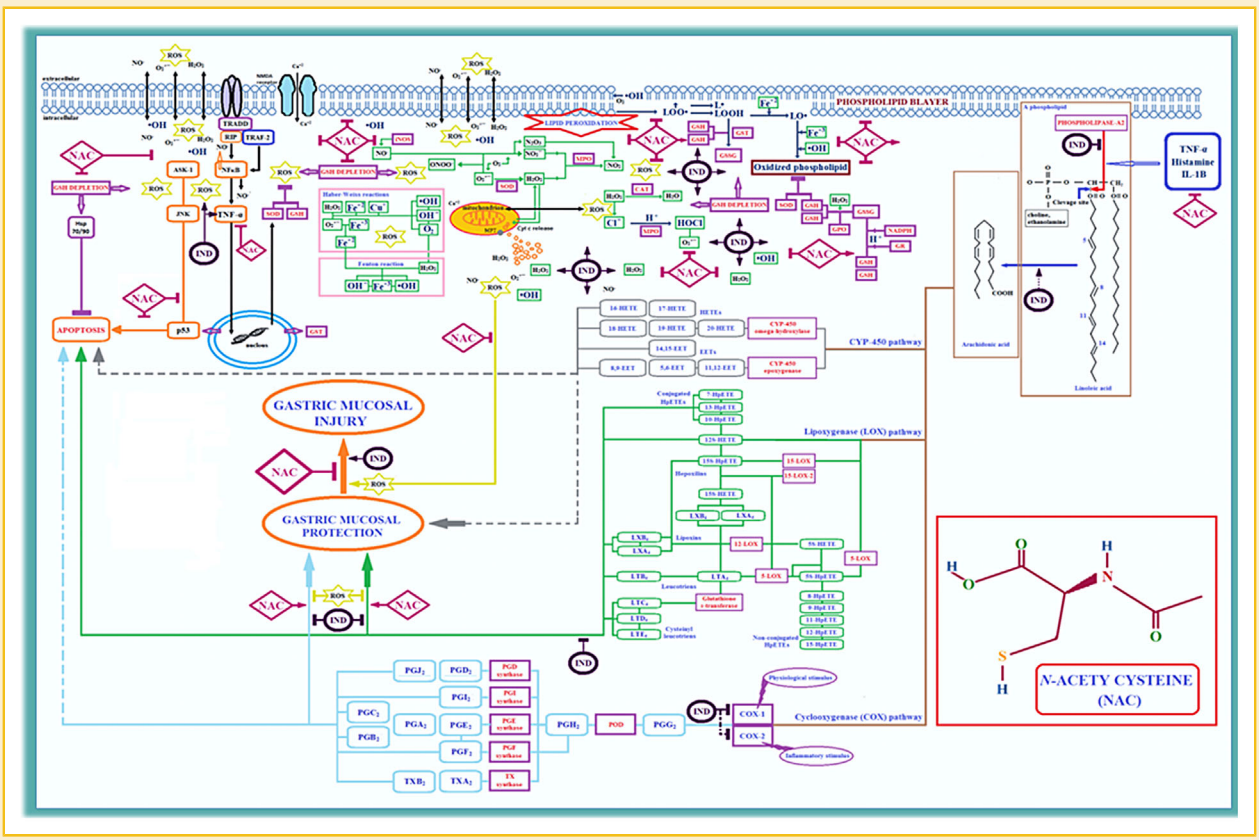

Fig. 9. The effect mechanism appearances of N-acetyl cysteine (NAC) in organization scheme of the gastric mucosal injury.

In this study we also evaluated the antiulcerogenic effects of three doses (300, 600, and 900 mg/kg) of NAC on indomethacin-induced gastric damage in rats. We compared antiulcer effect of NAC with that of four commercially available controls [lansoprazole (30 mg/kg body weight), omeprazole (30 mg/kg body weight), famotidine (20 mg/kg body weight) and ranitidine (25 mg/kg body weight)]. 25 mg/kg indomethacin induced significant gastric damage in the rats (Table III). On the other hand, also gastroprotective effects of NAC have been reported. While previous literature reported that NAC′s gastroprotective effects on different experimental models like stress-, aspirin-, different antirheumatic drugs-, and ethanol-induced gastric mucosal injuries, the gastroprotective effect mechanism of NAC is still not clear [Allen and Garner, 1980; Szabo et al., 1981; Carvalho et al., 2007; Yadav et al., 2013].

IND cause gastric ulcer via inhibiting COX-1 and COX-2 enzymes in the stomach tissue resulting in decreased synthesis of cytoprotective prostaglandins [Whittle, 1981]. However, recent studies have also demonstrated that ROS play important roles in the pathogenesis of mucosal damage caused by IND, ethanol, and other agents over the inhibition of COX enzymes [Yoshikawa et al., 1993; Carvalho et al., 2007; Halici et al., 2011]. IND causes oxidative stress and up-regulates LPO by generating ROS, thereby interfering with the mucosal cells' endogenous antioxidant systems [Yoshikawa et al., 1993; Halici et al., 2005; Muthuraman and Sood, 2010]. In addition, Yadav et al. [2013] showed that IND induced oxidative stress, triggering mucosal TNF-α that activated NF-κB and JNK MAPK signalling in mice. Similarly, our results showed that IND caused a significant increase in the LPO levels ($P < 0.05$) in the stomach tissues of rats (Fig. 4). In contrast to IND, the administration of NAC, LAN, FAM, OMEP, and RAN significantly ($P < 0.05$) decreased the LPO level in stomach tissues.

Enzymatic and non-enzymatic defence mechanisms such as SOD, GST, GR, GPO, and CAT are present in living organisms against the toxicity and tissue damage of ROS. Decreased SOD levels have been reported (Fig. 5) in rat stomach tissues after NSAID administration [Karakus et al., 2009; Halici et al., 2011; Polat et al., 2011; Kaplan et al., 2012; Suleyman et al., 2012]. Our results agree with these findings. As can be seen from Figure 5, IND administration caused a decrease in SOD activity in stomach tissues. SOD is an antioxidant enzyme that plays an important role in eliminating gastric damage. However, SOD activity was found to be high in the tissues of rats given NAC and anti-ulcer drugs (Fig. 5). SOD converts the highly reactive radical O_2 ($O_2-\bullet$) into the less reactive $H_2O_2\bullet$ CAT also destroys H_2O_2 to water and molecular oxygen. We found that IND reduced SOD activity. This means that SOD could not convert superoxide radicals to H_2O_2.

However, CAT activity was increased by IND administration. Increased CAT activity is an indicator of increased amounts of H_2O_2, but this seems in contrary with our SOD results. Superoxide radicals reported to convert in H_2O_2 and perhydroxyl ($HO_2\bullet$) radicals without enzyme activity in acidic media and this is fastest in pH 4.8 [Mahadik and Scheffer, 1996]. In addition, superoxide and perhydroxyl radicals react with each other, which cause oxidation and a reduction, resulting in high levels of H_2O_2 and O_2 [Weiss and Lobuglio, 1982]. Thus, CAT activity in the tissue of rats given IND might increase because of this spontaneous dismutation. The increased CAT activity in our study suggested that the exposure to oxidative stress resulted in the ability to detoxify the H_2O_2 via the catalase enzyme and accumulation of H_2O_2. CAT is a highly reactive enzyme that converts H_2O_2 to water and molecular oxygen, and can also produce methanol, ethanol, formic acid or phenols by donating hydrogen [Bradley et al., 1982; Elliott and Wallace, 1998].

In this study, we established that all doses of NAC, LAN, FAM, OMEP, and RAN decreased CAT activity (Fig. 6), which was increased by IND in rat stomachs. The current results also showed that IND decreased SOD activity while NAC, LAN, FAM, OMEP, and RAN increased the activity of this enzyme. Because NAC increases the activity of SOD, it is interesting to see a decrease in CAT activity in the same tissues. According to Haber-Weiss reaction H_2O_2 reacts with superoxides, which results in the most reactive and damaging free oxygen radical, the hydroxyl radical, in the presence of Fe and Cu metals [Weiss and Lobuglio, 1982]. The Haber–Weiss reaction occurs with or without contribution of a catalyst. The reaction without a catalyst is very slow while the second way catalysed by Fe^{3+} is very fast. Hydrogen peroxide also could be metabolized into hydroxyl radicals via the Fenton reaction in the presence of this iron [Koc et al., 2008]. Nevertheless, Chen et al. (1998) suggested that CAT stimulates COX-2 enzyme in the aortic smooth muscle cells of rats, but not COX-1. Namely, 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.

We also determined that GST activity, which is a multigene family of isoenzymes responsible for the detoxification of xenobiotics in aerobic organisms, was elevated by NAC in IND-induced gastric tissues in which IND was significantly alleviated (Fig. 9). Although GST activity was decreased by IND, 600 and 900 mg/kg doses of NAC increased the activity of this enzyme. GST substrates include the toxic products of tissue damage, the hydroperoxide products of oxidative damage and aromatic xenobiotics (Fig. 9). GST is a catalyser of reactions in which reduced glutathione acts as a nucleophile, conjugating to and facilitating the removal or reduction of the second substrate. In all organisms that exhibit GST activity, multiple forms of enzyme (which may or may not be tissue-specific) has been discovered [Hayes and Pulford, 1995].

Reduced glutathione contributes in the maintenance of cells in a reduced state, serves as an electron donor for certain anti-oxidative enzymes (e.g., glutathione peroxidase) and the formation of conjugates with some harmful endogenous and xenobiotic compounds via the catalysis of GST [Odabasoglu et al., 2008; Pourahmad et al., 2010]. The gastroprotective effects of NAC can be related increased GSH content of rats stomachs (Fig. 8). Namely, all doses of NAC increased GSH levels in the stomach tissues, which were decreased by IND. Previous reports, has also reported that GSH level was decreased by IND [Albayrak et al., 2010; Halici et al., 2011; Polat et al., 2011; Kaplan et al., 2012; Suleyman et al., 2012]. Our results suggested that NAC has a supporting effect on antioxidant defence systems in the gastric tissues of rats. NAC is a precursor of cysteine, the

rate-limiting step in glutathione synthesis also scavenges ROS. Under the conditions of oxidative stress, glutathione becomes depleted and this can be reversed by NAC supplementation [Fitzpatrick et al., 1999].

The MPO enzyme is widely used as an index of neutrophil infiltration in various gastric injuries [Atkuri et al., 2007; Albayrak et al., 2010]. As shown in Figure 7, MPO activity in the stomach tissues of rats given IND was found to be high compared to the tissues of healthy rats ($P < 0.05$). The increase in this enzyme activity level may be associated with the increases in the levels of neutrophil infiltration and H_2O_2 in those damaged gastric tissues containing IND. However, the administration of positive anti-ulcer drugs decreased the MPO activity [Potrich et al., 2010; Yadav et al., 2013]. In view of the present results, it can be concluded that anti-ulcer drugs used in the current study possess a reducing effect on neutrophil infiltration into gastric mucosal tissues. Compatible with our results, it has been found 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 [Mizoguchi et al., 2001; Atkuri et al., 2007; Karaca et al., 2009; Karakus et al., 2009; Yadav et al., 2013]. Nevertheless, similar to IND, NAC strongly increased the MPO activity compared to that of healthy stomach tissues. This result suggests that NAC possess an increasing effect on the MPO activity. In contrast to the present results, it has been found that NAC administration decreased the MPO activity in both ethanol-induced and stress-induced gastric tissues [Potrich et al., 2010].

In conclusion, this study showed that indomethacin induced gastric ulcers in rats succesfully. NAC administration reduced ulcers at a as well as famotidine, lansoprazole, omeprazole and ranitidine. The levels of MPO, anti-oxidant system enzymes (GST, SOD, CAT), LPO, and GSH were adversely affected by ulcer induction. NAC, famotidine, lansoprazole, omeprazole, and ranitidine administration reduced the adverse effects of ulceration on these parameters. Antiulcer effects of NAC can be contributing in its positive effects on the antioxidant system and MPO activity in indomethacin-induced gastric damage in rats. Moreover, NAC administration exerted a significant anti-inflammatory effect. Having both anti-inflammatory and gastroprotective effects may provide a high usage of NAC in both inflammatory and ulcerative conditions.

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