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Original Article

Antioxidants prevent indomethacin-induced oxidative damage in tongue tissues of rats

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ABSTRACT

Background and Aims: Indomethacin (Indo) is an important nonsteroidal anti-inflammatory drug that has beneficial effects as a pain killer, but also has side effects. In this study, the protective effects of vitamins, selenium and ranitidine (Ran) against the side effects of Indo on tongue tissue were investigated.

Methods: Rats were divided into six groups: the control group as group I; the group given a vitamin-selenium (Vitamin C, 100 mg/kg; Vitamin E, 100 mg/kg, beta carotene, 15 mg/kg, selenium, 0.2 mg/kg) combination as group II; the group treated with Indo as group III; the group administered vitamins-selenium and Indo (25 mg/kg, single dose) as group IV; the group given Ran (150 mg/kg) as group V; and the group given Ran and Indo as group VI. The experiments were done for 3 days. Animals received vitamins-selenium and Ran for 3 days, 2 h before the Indo administration (in same dose and time). Tongue tissues were taken and homogenized.

Results: In the supernatants, glutathione levels, superoxide dismutase, catalase and glutathione-dependent enzyme activities were reduced. Protein carbonyl, lipid peroxidation levels, lactate dehydrogenase and myeloperoxidase activities were increased in the group that was given Indo. Administration of vitamins-selenium and Ran reversed the levels and activities of the tested parameter in the Indo group.

Conclusion: We suggest that vitamins, selenium and Ran have powerful protective effects on Indo-induced tongue injury in rats.

Keywords: Beta carotene, Vitamin C, Vitamin E, Indomethacin, Tongue tissue, Selenium

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most preferred drug classes as a result of their multiple drug actions, which include analgesic, anti-inflammatory and antipyretic effects. They generally inhibit cyclo-oxygenase (COX) enzymes which convert arachidonic acid to eicosanoids like thromboxane and prostaglandins (Bacchi, Palumbo, Sponta, & Coppolino, 2012; Baker & Perazella, 2020), whose elevated expressions are associated with increased cardiovascular diseases (Radi & Khan, 2019).

Indomethacin (Indo) is one of the most commonly used NSAIDs due to its unique pain killer effect, and is an indole-acetic acid derivative (Bindu, Mazumder, & Bandyopadhyay, 2020). Despite their effectiveness and useful aspects, both Indo and other NSAIDs have been reported to cause serious gastrointestinal system and kidney injury (Varghese, Faith, & Jacob, 2009; Turkyilmaz, Coskun, Bolkent, & Yanardag, 2019a, Eraslan, Tanyeli, Güler, Kurt, & Yetim, 2020). Indo has been reported to increase free radical production by different mechanisms (Turkyilmaz, Arda Pirincci, Bolkent, & Yanardag, 2019b). The drug results in radical production either via tumour necrosis factor alpha (TNF-a) stimulation in gastric ulcer and cancer risk (Moustafa, Khoder, El-Awady,

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& Zaitone, 2013), or by facilitating the breakdown of Indo on mitochondrial functioning and oxygen uptake (Basivireddy, Jacob, & Balasubramanian, 2005). This situation has challenged scientists to come up with solutions for preventing the deteriorative effect of Indo.

Vitamin C (Vit C) and vitamin E (Vit E) are the two most important vitamins that work synergistically. Their protective effects either alone or in combination can be termed a second line of defence. They support the endogenous antioxidant defence system through the regeneration of important molecules like reduced glutathione (GSH), preventing lipid peroxidation of membrane structure, facilitating the synthesis of different hormones and neurotransmitters, improving immune systems, etc. (Wagner, Buettner, & Burns, 1996; Shaik-Dasthagirisaheb et al., 2013; Michalczyk, Czuba, Zydek, Zając, & Langfort, 2016). In addition, beta carotene, a precursor of vitamin A (Vit A), also has vital roles such as free radical neutralization, prevention of peroxidative process progression and regulation of inflammatory systems (Dragnev, Rigas, & Dmitrovsky, 2000; Valko et al., 2007; Rocha et al., 2018). Vit A has been reported to stabilize thiol groups of membranes (Rutkowski & Grzegorczyk, 2012). Selenium (Se) is an essential micronutrient whose necessity carries great importance for regulation of inflammation (Hariharan & Dharmaraj, 2020), its existence in selenoproteins like glutathione peroxidase (Cai, Zhang, & Li, 2019), and participation in redox signalling and homeostasis (Guillin, Vindry, Ohlmann, & Chavatte, 2019). Ranitidine (Ran), a histamine H_2-receptor antagonist, is effective in reducing excess gastric acid production and secretion (Ryan, Barker, & Hawcutt, 2020). Its protective effect on Indo-induced small intestinal injury was proven by Turkyilmaz et al., (2019b).

The tongue can be negatively affected for many reasons, including age, alcohol consumption, cigarette use and NSAID utilization (Baek et al., 2017; Kalogirou & Tosios, 2019; Bayrak, Arda-Pirincci, Bolkent, & Yanardag, 2021). For this reason, we directed our attention to preventing tongue damage induced by Indo via the proven protective effects of antioxidants and Ran.

MATERIALS AND METHODS

Animals

The procedure of this study was carried out according to the Local Ethics Committee on Animal Research at Istanbul University (Approval No: 2008/21). Male Sprague Dawley rats, aged 2.5-3.0 months, were chosen. All the animals had access to standard pellet chow and tap water.

Experimental design

The current study was intended to investigate the protective effects of an antioxidant combination and Ran against Indo-induced tongue injury in rats. Rats were randomly split into six groups of eight animals per group. Group I was control animals. Group II was control animals that received Vit C (100 mg/kg per day), Vit E (100 mg/kg per day), beta carotene (15 mg/kg per day) and sodium selenate (Se) (0.2 mg/kg per day) for 3 days by gavage technique. Group III was animals that received Indo at 25 mg/kg at a single dose by gavage technique (Koc, Imik, & Odabasoglu, 2008). Group IV was animals given Vit C, Vit E, beta carotene and Se over 3 days, with the final dose of this combination given 2 h before Indo administration (in the same dose and time). Group V was animals given Ran at a dose of 150 mg/kg per day for 3 days by gavage technique. Group VI was animals who received Ran in the same dose and time, and its final dose was applied 2h before Indo administration. After 6h of Indo administration, all animals were sacrificed under anaesthesia. Vit C, Se and Ran were dissolved in distilled water. Vit E was (in its acetate form) dissolved in distilled water just like Vit C. Beta carotene was prepared in sunflower oil. Indo was prepared by dissolving Tween-80 and 0.9% NaCl (1mL:9mL, respectively).

Biochemical experiments

After all animals were sacrificed, tongue tissues were taken and kept in physiological saline at -80 °C. Tongue tissues were homogenized in cold physiological saline to make up 10% homogenates (weight/volume). The homogenates were centrifuged at 20000 x g for 10 minutes. After the centrifugation procedure, collected supernatants were used to evaluate reduced glutathione (GSH; Beutler, 1975), lipid peroxidation (LPO; Ledwozyw, Michalak, Stepień, & Kadziołka, 1986) and protein carbonyl (PC; Levine et al., 1990) levels. For determining enzyme activities, the following parameters were chosen, respectively: catalase (CAT; Aebi, 1984), superoxide dismutase (SOD; Mylroie, Collins, Umbles, & Kyle, 1986), glutathione-S-transferase (GST; Habig & Jakoby, 1981), glutathione peroxidase (GPx; Wendel, 1981), lactate dehydrogenase (LDH; Bais & Philcox, 1994) and myeloperoxidase (MPO; Wei & Frenkel, 1991). The protein levels of tongue tissue homogenates were evaluated by referencing the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

Statistical analyses

Values are expressed as the "*mean* ± *standard deviation* (SD)" and analysed statistically using GraphPad Prism (version 6.0, GraphPad Software, San Diego, California, USA). An unpaired t-test, analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed to determine the significance of difference at $P < 0.05$.

RESULTS

The tongue GSH, LPO and PC levels of all groups are shown in Figure 1. Administration of antioxidants caused a significant decrease in GSH levels ($P < 0.01$) and insignificant elevations in LPO and PC levels of the control group. Indo reduced GSH levels but increased LPO and PC levels significantly compared to the control group ($P < 0.001$, $P < 0.01$, respectively). Antioxidants reversed these abnormal levels in the Indo group in a significant manner ($P < 0.001$, $P < 0.05$, respectively). It was determined that Ran decreased GSH and LPO levels insignificantly while its administration significantly increased PC levels as compared to the control group ($P < 0.05$). Treatment with Ran in the Indo group reversed GSH levels significantly ($P < 0.001$), while its effect on LPO and PC levels was insignificant (Figure 1).

In Figure 2, CAT, SOD, GST and GPx activities of all groups are given. According to the results, antioxidant administration increased CAT activity and decreased SOD activity insignificantly, while no differentiation was detected in GST activity when compared to the control group. In addition, a significant diminishment was deter-

mined at GPx activity of the antioxidant group as compared to the control group ($P < 0.0001$). It can be said that Indo decreased SOD, GST and GPx activities significantly compared to the control group ($P < 0.01$, $P < 0.05$, $P < 0.0001$, respectively). However, CAT activities were insignificantly affected in the Indo group as compared to the control group. The administration of antioxidants to the Indo group increased CAT, SOD and GST activities significantly ($P < 0.01$, $P < 0.001$, respectively), while GPx activities were insignificant. Ran caused a remarkable decrease in GPx activity ($P < 0.0001$), although the changesets of CAT, SOD and GST activities were insignificant when compared to the control group. Ran ameliorated all enzyme activities significantly in the Indo group ($P < 0.001$, $P < 0.05$, respectively) (Figure 2).

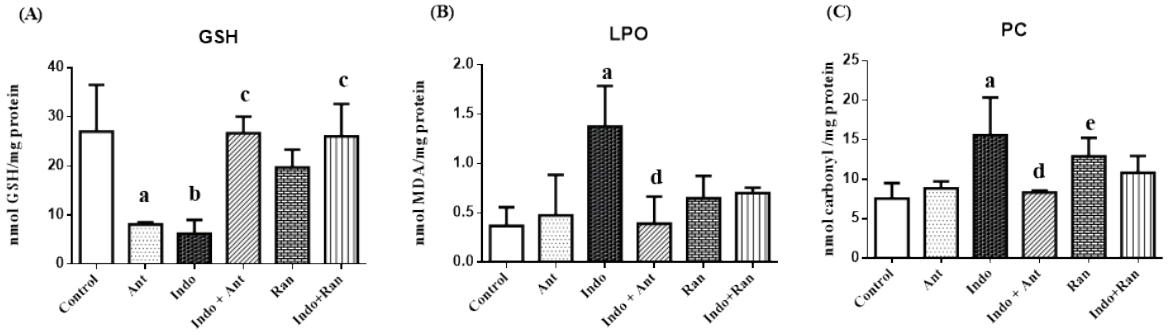

Figure 1. The tongue tissue GSH (A), LPO (B) and PC (C) levels of control and experimental groups. Values are expressed as mean ± SD. [a]$P<0.01$ vs control group, [b]$P<0.001$ vs control group, [c]$P<0.001$ vs Indo group, [d]$P<0.05$ vs Indo group, [e]$P<0.05$ vs control group. Ant: antioxidants (vitamins-selenium); GSH: reduced glutathione; Indo: indomethacin; LPO: lipid peroxidation; PC: protein carbonyl; Ran: ranitidine; SD: standard deviation.

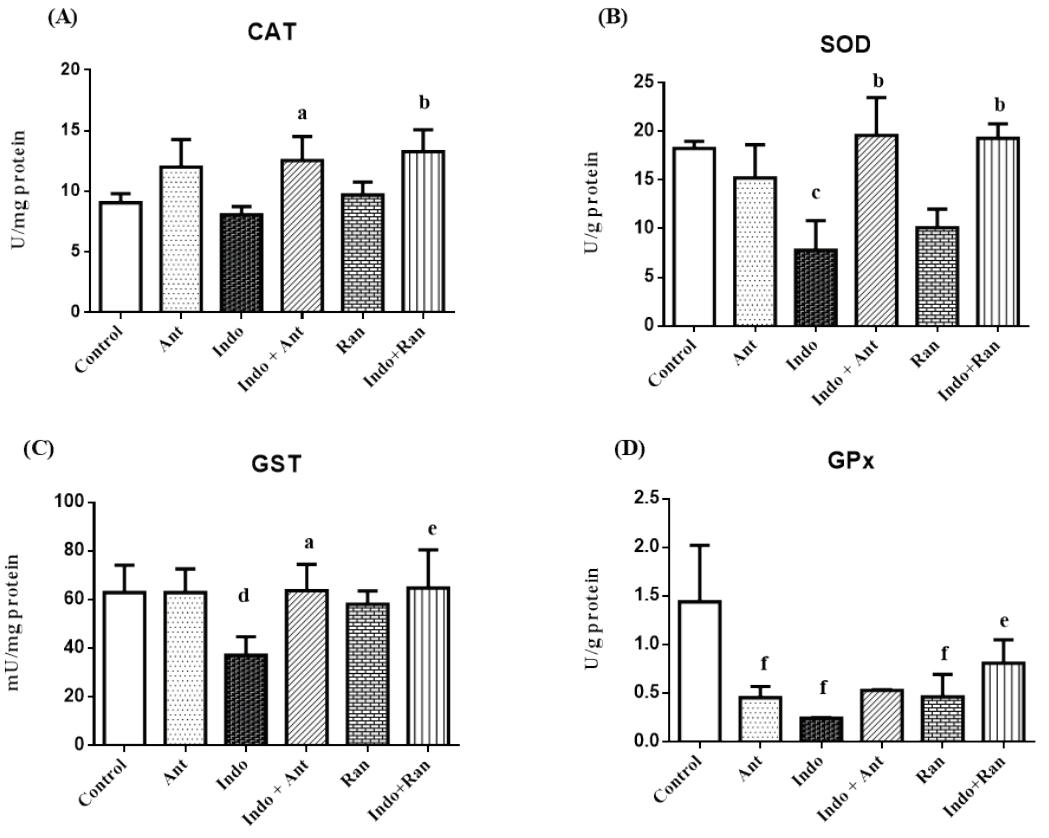

Figure 2. The tongue tissue CAT (A), SOD (B), GST (C) and GPx (D) activities of control and experimental groups. Values are expressed as mean ± SD. [a]$P<0.01$ vs Indo group, [b]$P<0.001$ vs Indo group, [c]$P<0.01$ vs control group, [d]$P<0.05$ vs control group, [e]$P<0.05$ vs Indo group, [f]$P<0.0001$ vs control group. Ant: antioxidants (vitamins-selenium); CAT: catalase; GPx: glutathione peroxidase; GST: glutathione-S-transferase; Indo: indomethacin; Ran: ranitidine; SOD: superoxide dismutase; SD: standard deviation.

The LDH and MPO activities of control and experimental rats are depicted in Figure 3. Antioxidant administration increased LDH activity and decreased MPO activity insignificantly as compared to the control group. Indo administration caused significant increases of both enzyme activities when compared to the control group ($P < 0.001$, $P < 0.0001$, respectively). Ran caused an insignificant increase in LDH activity and didn't change MPO activities of the control group. The antioxidants and Ran significantly decreased LDH and MPO activities in the Indo group ($P < 0.05$; $P < 0.0001$, respectively) (Figure 3).

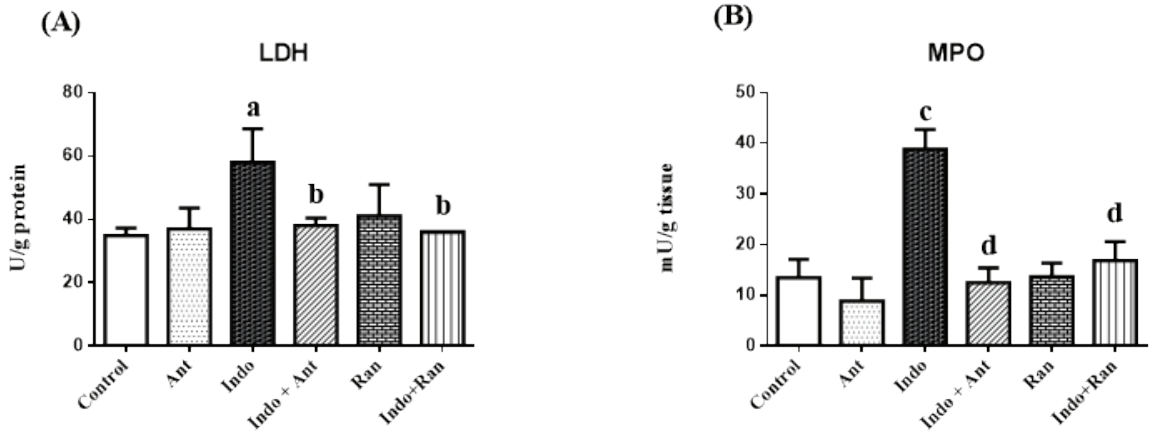

Figure 3. The tongue tissue LDH (A) and MPO (B) activities of control and experimental groups. Values are expressed as mean ± SD. [a]P<0.001 vs control group, [b]P<0.05 vs Indo group, [c]P<0.0001 vs control group, [d]P<0.0001 vs Indo group, Ant: antioxidants (vitamins-selenium); Indo: indomethacin; LDH: lactate dehydrogenase; MPO: myeloperoxidase; Ran: ranitidine; SD: standard deviation.

DISCUSSION

The tongue encounters most of the blood supply in the course of the feeding process, when considering its skeletal muscle structure, taste sensing functions and connections with nerve activity (Toda, Ayajiki, & Okamura, 2012). Unfortunately, it is inevitable that the tongue tissue will encounter free radical circulation when various agents like ethanol or some NSAIDs are used. These agents have recently been implicated in causing the elevation of free radicals, which ultimately harm the tongue tissue (Baek et al., 2019; Bayrak et al., 2021).

Indo has been shown to be a potent pro-oxidant initiator (Nagano et al., 2005). In addition, the drug affects mitochondrial function like all NSAIDs (Bindu et al., 2020). In different tissues, Indo's many free radical production mechanisms exist. One of the mechanisms that affects this function is the inhibitor effect of Indo on gastric peroxidase (Chattopadhyay, Bandyopadhyay, Biswas, Maity, & Banerjee, 2006). This drug causes aconitase inhibition in mitochondria. This leads to the release of free iron following inhibition and decomposition of the aconitase structure, which is stabilized by iron-sulphur interaction. On account of this free iron, hydroxyl radical generation starts (Maity et al., 2009). In addition, studies by Turkyilmaz et al. (2019a and 2019b) on the effect of Indo on gastric and intestinal damage indicates that Indo inhibits intestinal epithelial cells by dramatically decreasing cell proliferation, and also interferes with caspase 3, 8 and 9 expressions in gastric media. The elevation of caspase 8 expression, as well as the diminishment of caspase 3 and 9 expressions, was observed. This diminishment results in the inhibition of COX-2, a related enzyme in apoptosis and apoptosis-related caspase 3 and 9 levels. When all the possible mechanisms are taken into consideration, the elevation of reactive oxygen species (ROS) levels is inevitable. The goal of the present study was to scavenge the deleterious effect of Indo on tongue tissue like gastric and intestinal tissues.

Despite low concentrations of GSH in the biological system, it serves as one of the most effective antioxidants in many organs and tissues. It also serves as a substrate in enzymatic reactions (Jomova & Valko, 2011). GSH is a unique barrier for scavenging of hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$) and hydroxyl (HO) species (Misak et al., 2018). Once GSH levels are decreased, lipid structure can be affected via the initiation of LPO. In this current study, administration of Indo caused GSH diminishment and in turn LPO elevation. These alterations can be explained with the free radical formation potential of Indo on different parts of the gastrointestinal system. One of these parts that is affected by Indo is the stomach. Ugan & Un (2020) reported the same alterations in GSH and LPO levels in their study, which emphasises the deleterious effects of Indo in this tissue. Our results for LPO are also in line with previous reports (Varghese, Faith, & Jacob, 2009; Moustafa et al., 2013). PC can be considered products of the peroxidation process, which harms both the lipid and protein structure of membranes (Sivalingam et al., 2007). Indo caused an elevation of PC levels of Indo-treated tongues, perhaps the same way as with the alteration of GSH and LPO levels. This elevation is also evidence for increased oxidative stress caused by Indo. The present results are in accordance with reports by Banerjee, Maity, Nag, Bandyopadhyay, & Chattopadhyay (2008). The administration of the antioxidant combination reversed the levels of these parameters in the Indo group. Vit C protects GSH from further oxidation, and helps in the stabilization of the GSH/GSSG ratio. Vit E aids the regeneration of Vit C, and also protects the membrane structure from

unwanted LPO (Ali, Ahsan, Zia, Siddiqui, & Khan, 2020). Beta carotene also supports lipid protection (Ali et al., 2020), while Se as selenoprotein can diminish ROS production (Cai et al., 2019). Ching, Haenen & Bast (1993) proved that H_2-blockers like Ran and cimetidine had powerful radical scavenging activity. Based on this approach, we can assume that Ran might have increased GSH levels, and as well decreased LPO and PC levels due to its proven antioxidant effect.

Oxygen is necessary for the progression of life processes. Mitochondria utilise oxygen with a high ratio during aerobic reactions, however, a small percent of the oxygen is transformed to $O_2^{\cdot-}$ (Assi, 2017). Although this function is normal for cells, elevation of this situation leads to the excessive formation of radical molecules including $O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$ (Kajarabille & Latunde-Dada, 2019). In tongue tissues of Indo treated rats, lessened activities of GPx, SOD, CAT and GST (enzymatic defence system against ROS) were observed. Similarly, decreased antioxidant enzyme activities were reported in Indo-induced organ damage and ethanol-induced tongue injury by Abdallah, (2010), Koriem, Gad, & Nasiry (2015), Atalay et al., (2016) and Bayrak et al. (2021), respectively. The diminishments of GSH levels may be as a result of its excessive depletion, which thereafter also affects GSH-dependent enzymes like GPx and GST. GPx is also a selenoprotein, capable of directly scavenging H_2O_2 (Guillin et al., 2019). GST employs GSH as a co-substrate for detoxifying harmful substances. SOD transforms $O_2^{\cdot-}$ to H_2O_2, a substrate for both CAT and GPx (Ali et al., 2020). The vitamin combination, including Se and Ran, increased the activities of the aforementioned enzymes in the Indo group. This ameliorating effect can be associated with the existence of Se for stabilizing GPx activity. In addition, Vit E is also capable of scavenging a large scale of radical molecules (from peroxides and superoxides derived by xanthine), while Vit C helps in its regeneration (Miyazawa, Burdeos, Itaya, Nakagawa, & Miyazawa, 2019). Beta carotene and Ran may have also decreased radical substances by stabilizing GSH levels and ameliorating antioxidant enzyme activities in the tongue.

LDH is an important enzyme for carbohydrate metabolism. Its elevated activities can be considered proof of possible oxidative damage. Indo has been reported to cause changes in energy metabolism. It affects mitochondrial functions by altering energy flux from aerobic to anaerobic glycolysis (Khan, Yusufi, & Yusufi, 2019). By considering this theory and the formation of the tongue structure as resembling skeletal muscle, the present results suggest that LDH elevation in the Indo treated group is connected with oxidative damage. Moreover, Pawar, Anap, Ghodasara & Kuchekar (2011) reported elevated serum LDH activities in Indo-induced enterocolitis via emphasising the inhibition of COX and prostaglandins by Indo. Ben Amara et al. (2011) reported that the ameliorating effect of Vit E and Se on LDH enzyme activities might be related to the antiradical/antioxidant activities of the vitamin. H_2-receptor antagonists like cimetidine and Ran were also reported to have an inhibitory effect on LDH, although Ran was less inhibitory than cimetidine (Gill, Sanyal, & Sareen, 1991). The outcome of the present finding is in agreement with the aforementioned reports. Hence, the combination of vitamins, Se, and Ran successfully decreased LDH activity in Indo-induced tongue injury.

MPO (an important constituent of neutrophils) is released when the infiltration of leukocytes occurs. Through this approach, Indo has been proven to increase MPO activities in gastric tissues (de Araújo et al., 2018), and as well cause inflammatory reactions and epithelial losses in small intestinal tissues (Yamamoto, Itoh, Nasu, & Nishida 2014). Besides, Indo has been declared as having an elevator effect on MPO-associated neutrophil infiltration in Indo-induced ulcer models (Abdel-Raheem, 2010). In addition, some NSAIDs have been reported to be targets of mitochondrial electron transport system (ETC) complex I, thereby increasing mitochondrial oxidative stress (Bindu et al., 2020). Naturally, this is an indicator for increased H_2O_2 levels as parallel to elevated oxidative stress. In this study, tongue MPO activities of the group to which Indo was administered were found to be increased. Vitamin combination with Se may have decreased this activity in the group via radical scavenging effect. Ran was also proven to decrease excess MPO activities by reducing neutrophil infiltration in gastric tissue. The present findings for Ran are in accordance with reports by de Araújo et al. (2018).

CONCLUSION

According to the results, Indo caused an elevation in oxidative stress in the tongue tissue of rats. We may conclude that the protective effects of vitamin-Se treatments and Ran administrations on this injury either using their antioxidative properties or their positive effects on the regulation of energy metabolism.

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