The effect of ginger (*Zingiber officinale*) essential oil on catalase in rat kidney tissue

ABSTRACT

We were aimed to investigate the effect of ginger (Zingiber officinale) essential oil on catalase release in rat kidney tissue by histopathological and immunohistochemically method. This study, 21 male Wistar albino rats were used. Rats were divided into three groups: control, 100 mg/kg ginger essential oil (G100), and 500 mg/kg ginger essential oil (G500). Hematoxylin-eosin staining method was used for histopathological evaluations. Immunohistochemically localization of catalase in kidney tissue was determined by streptavidin-biotin peroxidase method. As a result of histopathological evaluations, an increase in glomerulus diameter was observed in kidney tissues of G100 and G500 groups. In addition, vacuolar degeneration was observed in the proximal and distal tubule epithelial cells in the renal cortex of the G100 group. The immunoreactivity of catalase in the renal cortex region; In the control group, it is strong in the proximal tubules and very weak in the collecting ducts. In the G100 group, catalase immunoreactivity was weak in the proximal tubules and distal tubules and strong in the collecting ducts. In G500 group, weak catalase immunoreactivity was observed in only proximal tubules. Strong catalase immunoreactivity was detected in the proximal tubules of the kidney medulla regions of the rats in all groups. Furthermore, there was strong catalase immunoreactivity in collecting ducts in the medullary region of the G100 group. We think that ginger essential oil can be used in appropriate doses and durations to reduce kidney damage.

Keywords: Catalase, ginger essential oil, kidney

INTRODUCTION

Ginger (*Zingiber officinale*) belonging to the *Zingiberaceae* family is a medicinal plant whose root or rhizome has been used as a spice or herbal medicine for many years (Karna et al., 2012). Ginger root is used to relieve or treat some common ailments such as headaches, colds, nausea, and vomiting. Ginger contains 1-3.3% essential oil (Karna et al., 2012). Ginger essential oil and its components have been studied mostly for their flavor and fragrance. However, in recent years, it has attracted a lot of attention around the world due to its multi-purpose functional uses.

Having numerous therapeutic effects, ginger is widely used in public medicine for its many health benefits in various diseases including diabetes (Al Hroob et al., 2018), cancer (Chen et al., 2018), ulcers (Liu et al., 2015), obesity (Suk et al., 2017), chronic diseases such as Alzheimer's (Cuya et al., 2018), cardiovascular (Liu et al., 2013) diseases and depression (Kukula-Koch et al., 2018).

Oxidative stress occurs due to the imbalance between production and elimination of reactive oxygen species (ROS), one of the free radicals, such as superoxide anions (O_2-) and hydroxyl radicals (OH-). Overproduction of ROS causes oxidation of cellular compounds and cell

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Research Article

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death (Alonso-Alconada et al., 2012; Nita and Grzybowski, 2016). Excessive oxidative stress is responsible for the initiation and progression of cell damage/death in organs (Cayir et al., 2011; Galle, 2001; Zhu et al., 2011). There are endogenous antioxidant defense mechanisms, including antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase, in protecting or reducing oxidative stress (Akbulut et al., 2014; Cayir et al., 2011; Forbes et al., 2008).

Catalase is a tetrameric enzyme consisting of 60 kDa subunits containing a heme group and a NADPH molecule (Scibior and Czeczot, 2006). It plays a role in preventing or delaying oxidative damage by catalyzing hydrogen peroxide (H_2O_2) and converting it to H_2O and O_2 (Yu et al., 2007).

Glomerular diameter is an indicator of glomerular hypertrophy. Focal segmental glomerulosclerosis (FSGS) is linked to a variety of illnesses, including unilateral renal agenesis, diabetes, eclampsia, and a high protein diet (Fogo, 2000).

In this study, it was aimed to investigate the protective effect of ginger essential oil on kidney tissue.

MATERIALS AND METHODS

Animals and experimental design

We used 200-250 g male *Wistar albino* rats housed in a sterile environment at 22 ± 3°C and 60–65% humidity in a room under a 12-hour light-dark cycle. Animals were permitted access to pellet feed and tap water *ad libitum*. Groups was formed from randomly selected rats as 7 rats in each group as follows.

Control group (n = 7): No application was made to the rats in this group. 100 mg/kg/day ginger essential oil (G100 Group) (n = 7): The rats in this group were administered 100 mg/kg/day ginger essential oil (Hekimhan Herbal-Antalya, Türkiye) by oral gavage for 10 days (Jeena et al., 2011). 500 mg/kg/day ginger essential oil (G500 Group) (n = 7): The rats in this group were administered 500 mg/kg/day ginger essential oil by oral gavage for 10 days.

The experiment was finished by following the ethics committee rules throughout the study. At the end of the experiment, rats in all groups were anesthetized with 15 mg/kg xylazine (Rompun; Bayer, İstanbul, Türkiye) and 75 mg/kg ketamine (Ketalar; Pfizer, İstanbul, Türkiye) before sacrifice by cervical dislocation. The kidney tissue samples were removed and placed in 10% formalin.

Histopathological procedure

After the kidney tissue samples were fixed in 10% formaldehyde solution, they were blocked in paraffin after routine histological procedures. Hematoxylin-Eosin (H&E) staining technique was applied to the 5 µm sections taken from the blocks to examine the general structure of the tissue.

Glomerulus diameters were measured using Image J (v1.50i) software from kidney tissue samples. A total of 975 glomerulus diameters were measured, including 325 glomerulus diameters from each group.

Immunohistochemical procedure

The streptavidin-biotin-peroxidase technique, one of the indirect methods, was used to the sections (5 µm) taken to the lams coated by chrome aluminum gelatin (Hsu et al., 1981). The sections were then incubated in 3% H_2O_2 prepared in methanol for 15 min to prevent the endogenous peroxidase activity. After the deparaffinization and rehydration processes. They were then applied heat at the maximum temperature in a microwave oven for 10 min (800 watt) in the citrate buffer solution (Ph 6.0) to bring antigens into the open after washing with the PBS. The blocking solution A was dripped to prevent the nonspecific binding (Histostain-Plus IHC Kit, HRP, broad-spectrum Ref.) after washed by PBS. The anti-catalase (Santa Cruz sc271358, it was diluted at the rate of 1/500) were applied on the sections in a humid

environment at the ambient temperature for 1 h. The Broad-Spectrum Antibody was dripped on the sections since it was against the type produced by the primary antibody. The HRP streptavidin was incubated at the ambient temperature for 15 min after washing with PBS. The 3,3'-Diaminobenzidine tetrahydrochloride (DAB) substrate solution (0.5 mg DAB/ml; Dako Corporation, Carpinteria, USA) was added for the chromogen practice and then, Gil III hematoxylin was used for the background staining. The slides were examined in a research microscope and their photos were taken (Leica DM4000B, Germany). The immunohistochemically evaluation was made by considering staining characteristic and staining density of the target cells. The evaluation was made by two independent observers by giving values from 0 to 4 in accordance with the characteristics including no staining (-), very weak staining (+), weak staining (++), moderate staining (+++), and strong staining (++++) (Aras et al., 2023).

Statistical analyses

SPSS 18 (IBM Corp., New York, USA) package program was used to evaluate the data obtained in the study. The Kolmogorov-Smirnov test was utilized to assess the normality of the group data. Data determined to be normally distributed were tested with one way analysis of variance (ANOVA) followed by post-hoc Duncan test. P values below 0.05 ($P<0.05$) were considered statistically significant.

RESULTS

Histopathological results

In the kidney tissue samples taken from all groups, 325 glomerulus diameters were measured in randomly selected areas from each group. When the control and experimental groups were compared, there was no significant difference between the control and G500 groups, and between the G100 and G500 groups. But the difference was significant between the control and G100 groups ($p<0.05$, Table 1, Figure 1).

Table 1. Statistical evaluation of glomerulus diameter levels of rats according to groups (um)

Groups	n	Average ± S.D.
Control	325	90,29±13,57
G100	325	95,12±14,09*
G500	325	92,67±15,69

*: $p<0.05$ relative to control was accepted.

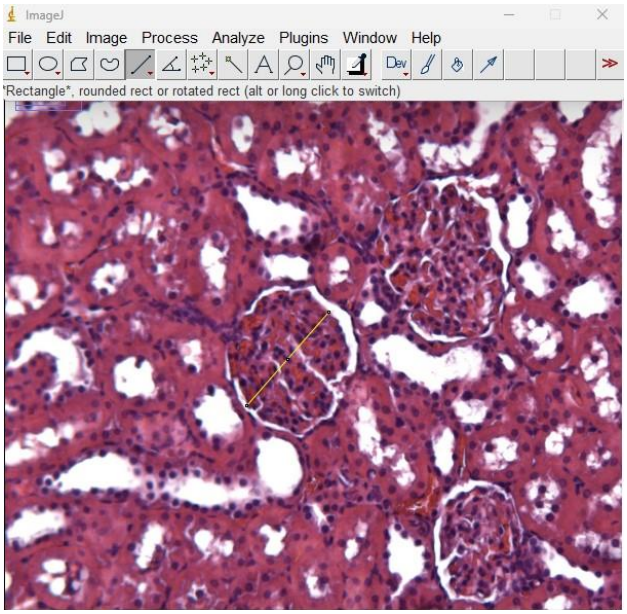

Figure 1. Sample glomerulus measurement with Image J (v1.50i) application. Yellow line is glomerulus diameter.

Renal corpuscles containing glomerulus in the cortex region of the control group kidney tissue and both distal and proximal tubules around it were in normal structure. When compared to the kidney tissues of the control group, an increase in Bowman's space was observed in the kidney tissue of the G100 group. And vacuolar degeneration was observed in the proximal and distal tubule epithelial cells in the cortical region. In the G500 group kidney tissue, an enlargement of the Bowman space of some glomerulus was observed, while a narrowing of the Bowman space was observed in some regions. In addition, casts were showed in some cortical proximal tubule lumens in the kidney tissue of the G500 group (Table 2, Figure 2).

Table 2. Comparison of catalase immunoreactivity between groups.

Cortex and Medulla Cortex	Control	G100	G500
Proximal tubules	++++	++	++
Distal tubules	-	++	-
Mesangial cells	-	-	-
Collecting ducts	+	++++	-
Medulla			
Proximal tubules	++++	++++	++++
Distal tubules	-	-	-
Collecting ducts	-	++++	-
Vascular endothelium	-	-	-

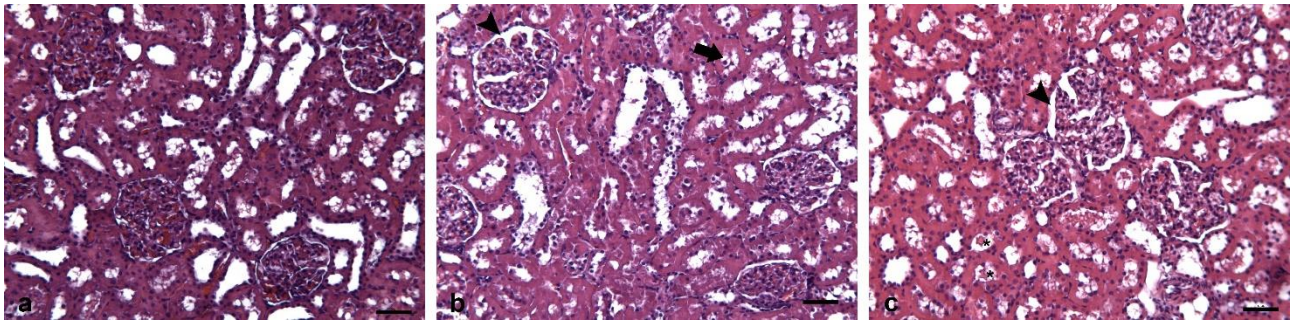

Figure 2. Rat kidney tissues. a) Control group, b) G100 group, c) G500 group. Arrowhead: Bowman spaces, Black arrow: Vacuole degeneration, p: Proximal tubules, d: Distal tubules, *: Casts. Hematoxylin & Eosin Staining (H&E). Bar 50 µm.

Immunohistochemical results

Catalase immunoreactivity was examined separately in cortex and medulla regions of rat kidney tissues in all groups. In the cortex region; In the control group, there was strong (++++) catalase immunoreactivity in proximal tubules and very weak (+) catalase immunoreactivity in collecting ducts, but no catalase immunoreactivity in distal tubules and mesangial cells. In the G100 group, there was no catalase immunoreactivity in mesangial cells, but there was weak (++) catalase immunoreactivity in proximal and distal tubules, as well as strong (++++) catalase immunoreactivity in collecting ducts. In the G500 group, weak (++) catalase immunoreactivity was observed in the proximal tubules. No catalase immunoreactivity was observed in distal tubules, mesangial cells and collecting ducts (Figure 3).

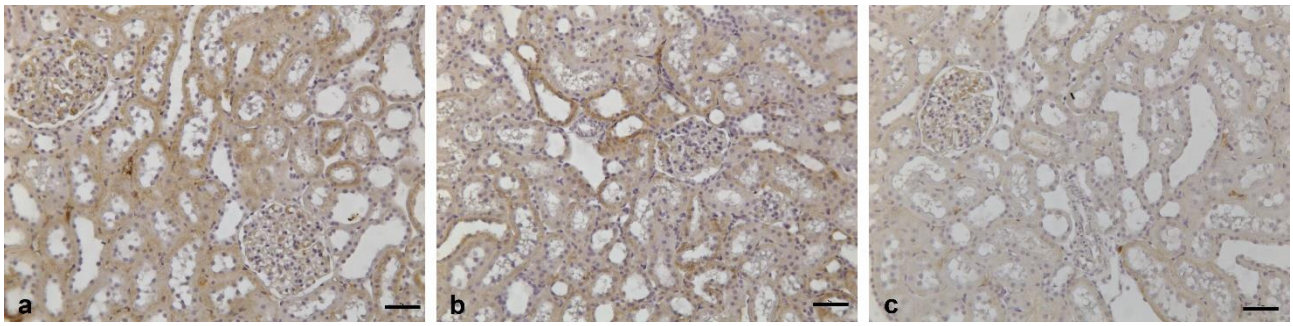

Figure 3. Rat kidney (cortex) tissue. a) Control group, b) G100 group, c) G500 group. Arrow (m): Mesangial cell, c: Collecting ducts, p: Proximal tubules, d: Distal tubules. Catalase immunoreactivity. Bar: 50 µm.

When the medulla region of the kidney tissue of all groups was examined. In the control and G500 groups, there was strong (++++) catalase immunoreactivity in the proximal tubules, but no catalase immunoreactivity was observed in the distal tubules and collecting ducts. In the G100 group, there was strong (++++) catalase immunoreactivity in the proximal tubules and collecting ducts. There was no catalase immunoreactivity in distal tubules. No catalase immunoreactivity was observed in the vascular endothelium in the kidney tissues of all examined groups (Figure 4).

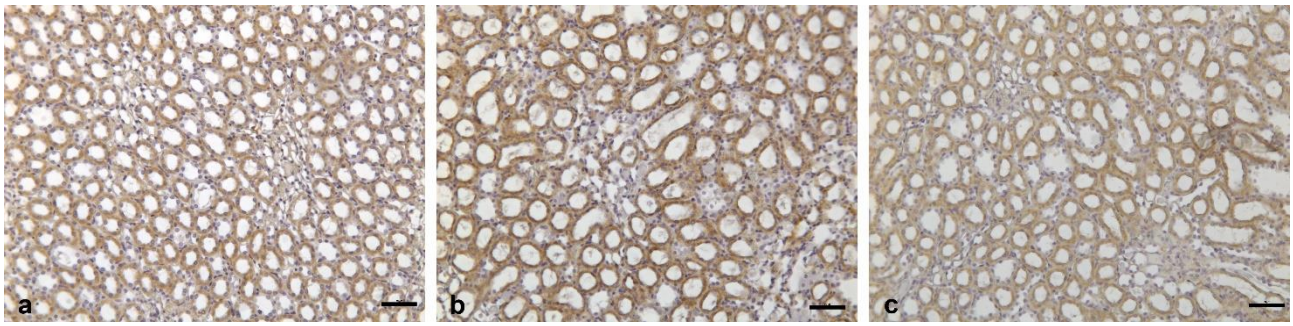

Figure 4. Rat kidney (medulla) tissue. a) Control group, b) G100 group, c) G500 group. c: Collecting ducts, p: Proximal tubules, d: Distal tubules Catalase immunoreactivity. Bar: 50 µm.

DISCUSSION

The use of plants or components obtained from plants is increasing day by day due to the natural antioxidants they contain. Along with this increase, the incidence of some side effects increases due to errors caused by use (for example, misdiagnosing the plant, high doses, long-term use). As a result of such adverse reactions, serious side effects have been reported in important organs such as the liver, kidney, heart and brain (Shaw, 2010; Shaw et al., 2012).

Products from plants may protect against kidney damage by increasing endogenous antioxidants (Palipoch, 2013). In the study conducted with *Oenanthe javanica* extract, almost no kidney damage was found in glomeruli and kidney tubules in all groups (Tae et al., 2014). In addition, it has been reported that administration of 600, 750 and 900 mg/kg *Moringa stenopetala* extract did not show significant histopathological changes in mouse kidney compared to the control group (Ghebreselassie et al., 2011). Many herbs can cause damage to kidney tissue depending on the increase in the applied dose (Parveen et al., 2010; Paul and Didia, 2012). For example, it has been shown that significant cytoplasmic vacuolation occurs in the renal tubular cells of rats administered 50 mg/kg *Teucrium polium* (Khleifat et al., 2021). It has been reported that *Moringa oleifera* methanolic extract, 3.5mg/kg extract in Guinea pig kidney tissue has a normal histological structure in the kidney tissue, and 4.6 mg/kg extract application causes deterioration in the distal convoluted tubules and glomerulus in the kidney sections, and occlusion of the interlobular vein. In addition, it has been reported that the application of 7.0 mg/kg extract caused interstitium infiltration with inflammatory cells in the kidney sections, disruption in the proximal convoluted tubule and glomerulus, and formation of tubular lumina containing amorphous eosinophilic material (Paul and Didia, 2012).

It has been reported that the ginger plant is protective against kidney damage caused by lead, iron doxorubicin, gentamycin, metalaxyl. In addition, ginger rhizome extract has been shown to play a protective role against kidney damage caused by diabetes by improving oxidative stress, inflammation, and apoptosis (Ademiluyi et al., 2012; Ajith et al., 2008; Al

Hroob et al., 2018; Gholampour et al., 2017; Reddy et al., 2014; Sakr et al., 2011).

Measurement of glomerulus diameter in kidneys is important in the histopathological evaluation of various diseases (Kotyk et al., 2016). Therefore, kidney glomerulus diameters of the study groups were evaluated. It has been reported that ethephon, which is used to develop nephrotoxicity experimentally, causes a decrease in the glomerulus diameter. In addition, it has been shown that the application of fresh garlic extract together with the ethephon substance increases the decreasing glomerulus diameter (Albrakati, 2021). In our study, there was an increase in glomerulus diameters in the experimental groups. And this increase is thought to be due to the antioxidant properties of ginger.

In our study, histopathological changes in kidney tissue of two different doses of ginger essential oil (100 and 500 mg/kg/day) were investigated on healthy rats without any chemical or disease formation. In the control group kidney tissue, renal corpuscles containing glomerulus in the cortex region and tubules with distal and proximal folds around them were found to be in normal structure. An increase in Bowman's space was observed in the kidney tissue of the G100 group, and vacuolar degeneration was observed in the proximal and distal tubule epithelial cells in the cortical region. In the kidney tissue of the G500 group, an increase in Bowman's space was observed in some regions and narrowing in some regions. In addition, adhesions were observed between the parietal and visceral leaves of Bowman's capsule in some glomeruli. In addition, casts were seen in some cortical proximal tubule lumens in the G500 group.

In previous studies, it has been reported that catalase immunoreactivity is seen in tubular cells, especially in the distal tubules in control group (Lee et al., 2019). Tae et al., (2014) reported that catalase immunoreactivity is weak in the distal tubules of the kidney in control group. Bakir et al., (2017), while catalase immunoreactivity was not observed in the distal tubules, a strong catalase immunoreactivity was observed in the proximal tubules in control group. In our study, catalase immunoreactivity in the kidney tissues of the control group was examined in the cortex and medulla regions. Consistent with the study of Bakir et al., (2017) catalase immunoreactivity was strong in the proximal tubules in the cortex region of the control group. When the medulla regions were examined, strong catalase immunoreactivity was observed in the proximal tubules in all groups.

It has been reported that administration of *Populus tomentiglandulosa* extract significantly increases the immunoreactivity intensity of catalase in the kidney (Lee et al., 2019). It has been suggested that application of *Ocimum basilicum* leaf extract increases the immunoreactivity concentration of catalase in the kidney in acetaminophen-induced kidney damage in mice (Karaali et al., 2018). It has been shown that the intensity of catalase immunoreactivity in kidney tissue of *Oenanthe javanica* extract increased approximately 2-fold compared to the control group (Tae et al., 2014). In another study, it was reported that the immunoreactivity of catalase in the kidney medulla of mice administered *Onosma nigricaule* was quite weak. It has also been reported that catalase immunoreactivity was not seen in the distal tubules. It has been shown that catalase immunoreactivity is quite intense in the renal cortex and proximal tubules (Bakir et al., 2017).

In our study, weak catalase immunoreactivity was observed in the cortex region, proximal tubules and distal tubules, and strong catalase immunoreactivity was observed in the collecting ducts in the G100 group, while weak catalase immunoreactivity was observed only in the proximal tubules in the G500 group. When the medulla regions of the kidney tissues of the rats in all groups were examined, strong catalase immunoreactivity was observed in the proximal

tubules in all groups, while strong catalase immunoreactivity was observed in the collecting ducts only in the G100 group. No catalase immunoreactivity was observed in the vascular endothelium and distal tubules in the kidney tissues of all studied groups.

CONCLUSION

As a result, in this study, it was observed that the essential oil obtained from the ginger plant, which has been used as a spice for many years, causes some damage to the kidney tissue, and at the same time, it reduces the release of catalase, which is one of the endogenous enzymatic antioxidants, with the increase in the dose.

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Conflict of interest: The authors have no conflicts of interest to report.

Ethical statement or informed consent: This study was carried out at Kafkas University Research Animals Application Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Kafkas University (KAU-HADYEK, Ref No:2021/035 Date: 25.03.2021).

Author Contributions: YYA, MM and MÖ contributed to the project idea, design and execution of the study. ŞYA, HA and AG contributed to the acquisition of data. MM and HA analyzed the data. YYA and TŞ drafted and wrote the manuscript. HA and MÖ reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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