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Original Article

Protective effects of *Cinnamomum zeylanicum* and *Zingiber officinale* extract against CCl4-induced acute kidney injury in rats

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ABSTRACT

Introduction: The toxicity induced by toxic substances and medications is one of the principal reasons for acute kidney injury. The purpose of this study was to investigate the effect of *Cinnamomum zeylanicum* and *Zingiber officinale* extracts on the kidney of the rats intoxicated with carbon tetrachloride (CCl4).

Methods: In this study, thirty-six Wistar rats randomly divided into six groups: I) control, II) cinnamon 25mg/kg + ginger 125mg/kg, III) CCl4, IV) CCl4+ cinnamon 50mg/kg, V) CCl4+ ginger 250mg/kg, VI) CCl4+ cinnamon 25mg/kg and ginger 125mg/kg. *Cinnamomum zeylanicum* and *Zingiber officinale* extracts were injected for 14 days. On the 14th day, the rats in the CCl4 and the pretreatment groups were administered with 1mg/kg of CCl4 and olive oil mixture (1:1 v/v). Forty-eight hours after the injection of CCl4, blood samples were taken to conduct subsequent biochemical tests. Also, the kidney removed and histological alterations as well as oxidative markers were investigated.

Results: The administration of CCl4 increased the levels of urea, uric acid, creatinine and malondialdehyde; while decreased the levels of serum albumin, total protein, total antioxidant capacity and renal tissue antioxidant enzymes. Pretreatment with *Cinnamomum zeylanicum* and *Zingiber officinale* extracts, especially with a combination of them, led to considerable improvement in these values compared to the CCl4 group.

Conclusion: The results suggest that hydroalcoholic extracts of *Cinnamomum zeylanicum* and *Zingiber officinale,* alone or simultaneously, have protective effects against free radicals produced during CCl4 metabolism.

Keywords:
Cinnamomum zeylanicum
Zingiber officinale
Antioxidant
Carbon tetrachloride
Acute kidney injury

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Introduction

Kidneys and the liver are the main targets of toxic substances during the metabolism of poisons and medications (Subasini et al., 2015). Nephrotoxicity has been reported during the metabolism of various substances such as heavy metals, chemicals, fungal toxins and a wide variety of medications (Mahmoud et al., 2012; Tiong et al., 2014; Yousefi et al., 2019). There are two types of renal failure: acute kidney injury (AKI) and chronic kidney disease. The nephrotoxicity induced by toxic substances is the principal cause of AKI and resultant mortality (Shahbazi et al., 2012).

Carbon tetrachloride (CCl4) is an organic solvent and agent in the induction of the AKI model (Yoshioka et al., 2016). The administration of CCl4 changes the antioxidant status and induces severe nephropathy in rats. The free radical of trichloromethyl formed by cytochrome P450 enzyme pending the metabolism of CCl4, quickly reacts with molecular oxygen and produces trichloromethyl peroxyl radical (Mazani et al., 2020). This highly toxic radical attacks the unsaturated fatty acids of phospholipids and results in lipid peroxidation of liver and kidney cell membrane (Hismiogullari et al., 2015).

Natural compounds obtained in plants play an essential role in inhibiting renal toxicity (Preethi and Kuttan, 2009). Many studies have reported the effectiveness of various herbal extracts against the CCl4-induced nephrotoxicity (Chávez-Morales et al., 2017; Yilmaz-Ozden et al., 2016). *Zingiber officinale* (ZO) has been found to have antioxidant, anti-inflammatory, anti-cancer and anti-microbial effects. There are more than 60 volatile and non-volatile active compounds in this plant. The hydrocarbons of monoterpenoid and sesquiterpenoids are examples of volatile compounds. Non-volatile compounds including shogaols, parasols and gingerols are all responsible for the antioxidant activities of the plant. Antioxidant properties of the plant candidates it for protection against oxidative damages and extensively used to prevent various types of diseases (Cifci et al., 2018; Yılmaz et al., 2018). *Cinnamomum zeylanicum* (CZ) is a small, tropical, evergreen tree in southern Asia and indigenous to Sri Lanka. It is used for the treatment of malnutrition, diabetes, acne, digestive diseases, respiratory problems, etc. (Mazani et al., 2020). The main compound of the bark of this tree is cinnamaldehyde. Other compounds like trans-cinnamyl acetate, eugenol, o-methoxy benzaldehyde, benzyl benzoate, beta-caryophyllin, linalool and etc. have been reported from this plant (Sharafeldin and Rizvi, 2015).

Few studies have investigated the protective effects of CZ and ZO extracts against renal injuries induced by medications and toxic substances (Maghsoudi et al., 2011; Shirpoor et al., 2016). Furthermore, no study has explored the synergistic effects of the simultaneous use of these two extracts on kidney injuries. Therefore, this study aimed to investigate the protective effects of cinnamon extract, ginger extract and a combination of these two on the prevention of CCl4-induced AKIs in rats.

Material and methods

Animals

For this study, thirty-six Wistar rats (200-250g) were purchased from the Baquatollah University of Medical Sciences (Tehran, Iran). The rats were acclimatized for one week under standard conditions at room temperature (25±2ºC) with 12h light/dark cycles in the animal unit of Ardabil University of Medical Sciences. This study was approved by the Ethics Committee of Payam Noor University and registered in the Clinical Trials Center of the Islamic Republic of Iran with the code of IR.PNU.REC.1396.4. Animals randomly divided into six groups as follows:

Group I: the rats did not receive any damaging or therapeutic substance and had unlimited access to water and food. They just received 1mg/kg of olive oil (the solvent of CCl4) on the 14th day of the intervention. Group II: the rats in this group received 25mg/kg of CZ+ 125mg/kg of ZO extract for 14 days (Abd-Allah et al., 2016; Eidi et al., 2012). Also, they managed with 1mg/kg of olive oil on the 14th day of the mediation. Group III: in this group, the rats after 14 days received CCl4 (1mg/kg) dissolved in olive oil. Group IV: in this group, after 14 days of pretreatment with the CZ extract (50mg/kg), the rats received CCl4 (1mg/kg) dissolved in olive oil. Group V: in this group, after 14 days of pretreatment with the ZO extract (250mg/kg) the rats received CCl4 (1mg/kg) dissolved in olive oil. Group VI: this group was pretreated with a combination of CZ and ZO extracts (cinnamon [25mg/kg]+ ginger [125mg/kg] extract) for 14 days. They were also received CCl4 (1mg/kg) dissolved in olive oil on the 14th day of the intervention (Mazani et al., 2020).

All rats anesthetized using diethyl ether for 48h after the administration of CCl4 (Mazani et al., 2018). Blood

samples were taken directly from their heart immediately. Then their kidney removed and histological alterations as well as oxidative markers were investigated.

Plants preparation and extraction

The dried form of CZ and ZO were purchased from the traditional medicine market. The genus and species of plants confirmed by an expert in the herbarium of the University of Mohaghegh Ardabili (CZ herbarium No: GUMS-C17 and ZO herbarium No: 1483). The CZ and ZO bark samples were ground into a powder and immersed in a solution consisting of methanol and distilled water with a ratio of 70:30 for five days. Then, extracts filtered and the remaining in the filter paper was returned to the solution of methanol and distilled water once again. After that, they were transferred to glass tubes and kept at room temperature for three days until their solvent evaporated. Finally, to obtain pure extract the extracts were kept at the temperature of 37°C. This process was repeated several times until concentrated extracts were obtained.

Biochemical tests

The superoxide dismutase (SOD) and the glutathione peroxidase (GPx) activities were analyzed using diagnostic *kits* manufactured by Randox Laboratories Ltd (Crumlin, UK). In addition, the measurement of the levels of urea, uric acid, creatinine, albumin and total protein was obtained from Pars Azmoon Company (Tehran, Iran). Thiobarbituric acid, trichloroacetic acid, bovine serum albumin, sodium acetate, 3H2O, CCl4, ferric chloride, and ferrous sulfate were purchased from Merck Company (Germany) and 2,4,6-tri[2-Pyridyl]-s-triasine (TPTZ) was bought from Fluka Company (Sigma, Germany). All the chemicals and other substances used in this study were of analytical grade. The levels of urea, uric acid, creatinine, albumin and serum total protein were determined via colorimetric and spectrophotometric methods (Eppendorf, Ecom-E6125).

Kidney homogenates

Fifty milligram of each of the kidney samples was transferred to 1.5ml of 50mM phosphate buffer and homogenized using Heidolph homogenizer (Silent Crusher). The obtained homogenates were centrifuged at 12000rpm for 20min. Then, the resultant supernatants were removed and used in the measurement of malondialdehyde (MDA), total antioxidant capacity, SOD, GPx and catalase levels.

Measurement of protein concentration

The Bradford protein assay was used to measure the concentration of proteins in the samples based on the binding of Coomassie blue dye to proteins (Bradford, 1976).

Measurement of MDA level

The MDA level, as an indicator of lipid peroxidation, was measured according to the Uchiyama and Mihara (1978) method with minor modifications. In this method, the first 500µl of the homogenized supernatant was mixed with 500µl of trichloroacetic acid. After centrifugation, 400µl of the supernatant was removed and mixed with 2400µl of 1% phosphoric acid. After vortex-mixing, 1ml of 0.67% thiobarbituric acid solution was added to the test tube and after vortex-mixing once again, the tube was placed in a boiling bain-marie for 60min. After cooling, 1600µl of n-butanol was added and the resultant solution was centrifuged at 3000rpm for 10min. The absorbance of the supernatant was measured at 532nm against n-butanol.

Measurement of total antioxidant capacity

Ferric reducing antioxidant power assay was used to measure the total antioxidant capacity based on the reduction of $Fe3^{+}$ to $Fe2^{+}$ in the presence of TPTZ. The reaction of $Fe2^{+}$ with TPTZ leads to the formation of blue color which can be measured at maximum absorption of 593nm (Benzie and Strain, 1996).

Measurement of catalase activity

Catalase activity was determined according to the Aebi (1984) method based on the decomposition of H2O2 in kidney homogenates. To measure catalase activity, 10µl of tissue homogenates were mixed with 5ml of 50mM phosphate buffer (pH=7). Then, 2µl of the resultant solution was mixed with 1ml of 30mM hydrogen peroxide and the changes in absorbance at 240nm against reagent blank were monitored for 1min and the values were recorded as µmol/min/mg protein.

SOD and GPx activity

The evaluation of SOD and GPx activity was conducted according to the instructions provided by the Randox

TABLE 1: Comparison of urea, uric acid, and creatinine in the studied groups.

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
I	61.40±2.30	1.70±0.21	0.40±0.03
II	61.75±3.30	1.70±0.25	0.41±0.04
III	76.40±3.04***	4.06±0.39***	0.53±0.04***
IV	65.00±3.16†††	1.84±0.45†††	0.44±0.01†
V	64.40±1.87†††	1.76±0.43†††	0.44±0.03†
VI	63.40±2.07†††	1.74±0.36†††	0.43±0.02†

Results presented in the table were expressed as the mean±SD for 6 rats in each group. ***shows the significance of the differences relative to the normal control group (P<0.001). † and ††† show significance of the differences relative to the damage control group (P<0.05 and P<0.001 respectively). I: Normal control, II: Control cinnamon 25mg/kg + ginger 125mg/kg, III: CCl4 control, IV: Cinnamon 50mg/kg, V: Ginger 250mg/kg, VI: Cinnamon 25mg/kg + ginger 125mg/kg.

TABLE 2: Comparison of albumin, total protein, and creatinine/albumin ratio in the groups.

Groups	Albumin (g/dl)	Total Protein (g/dl)	Creatinine/Albumin
I	3.80±0.12	6.61±0.29	0.10±0.008
II	4.05±0.35	7.21±0.45	0.10±0.017
III	2.70±0.13***	4.98±0.43***	0.19±0.012
IV	3.57±0.23†	6.14±0.47	0.12±0.009
V	3.58±0.28†	6.21±0.46†	0.12±0.016
VI	3.64±0.29†	6.35±0.41†	0.11±0.005

Results presented in the table were expressed as the mean±SD for 6 rats in each group. ***shows the significance of the differences relative to the normal control group (P<0.001). †shows significance of the differences relative to the damage control group (P<0.05). I: Normal control, II: Control cinnamon 25mg/kg + ginger 125mg/kg, III: CCl4 control, IV: Cinnamon 50mg/kg, V: Ginger 250mg/kg, VI: Cinnamon 25mg/kg + ginger 125mg/kg.

kit (Laboratories Ltd., UK). The basis of SOD reaction is the production of free radicals by xanthine and xanthine oxidase that react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride and produce colored molecules which absorb light at 505nm. SOD inhibits the reaction and the level of inhibition depends on the level of SOD activity. Measurement of GPx was started with the oxidation of the glutathione by glutathione peroxidase. Then, it was regenerated in the presence of glutathione reductase and NADPH. The decrease in the absorbance at 340nm was related to the concentration of GPx (U/mg protein) as the result of the conversion of NADPH to NADP.

Histopathological assessments

To conduct histopathological assessments, a piece of kidney tissue was placed in a 10% solution of formalin. After 72h, it was dehydrated in an ascending ethanol series (50-100). Xylene was used as the clearing agent. Then, the samples were transferred to a paraffin bath and after that, samples were transferred to appropriate molds. The tissue sections with 4-5μm of thickness stained through hematoxylin and eosin staining method. Finally, they were investigated by Microscope Camera Leica ICC50 HD to identify histopathological changes (Shokoohi et al., 2019).

Statistical analysis

All of the results were reported in the form of mean±SD. The statistical analyses conducted using one-way ANOVA. The Kolmogorov–Smirnov test was used to analyze the normal distribution of the data. Moreover, for post hoc analyses, the Tukey test was employed. The significance level was set at $P\leq0.05$. All of the statistical analyses conducted using SPSS v16.

Results

Serum biochemical parameters

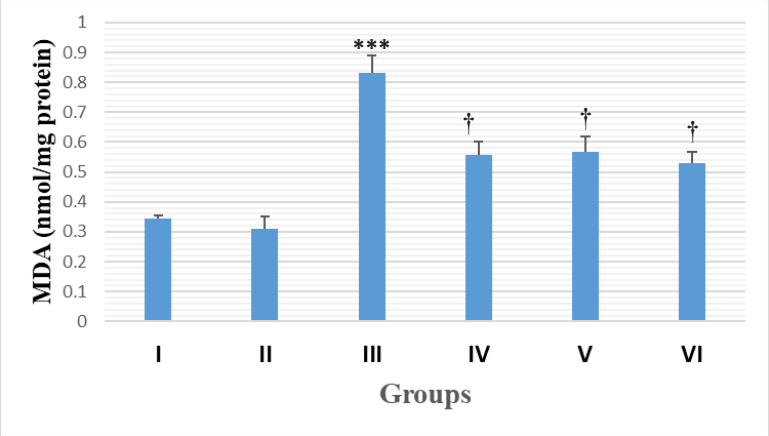

FIGURE 1. The effect of pretreatment with cinnamon and ginger extracts on malondialdehyde (MDA) level in CCl4-damaged rats. The bar signs on top of the columns indicate mean±SD (n=6). ***shows the significance of the differences relative to the normal control group (P<0.001). †shows the significance of the differences relative to the damage control group (P<0.05). I: Normal control, II: Control cinnamon 25mg/kg + ginger 125mg/kg, III: CCl4 control, IV: Cinnamon 50mg/kg, V: Ginger 250mg/kg, VI: Cinnamon 25mg/kg + ginger 125mg/kg.

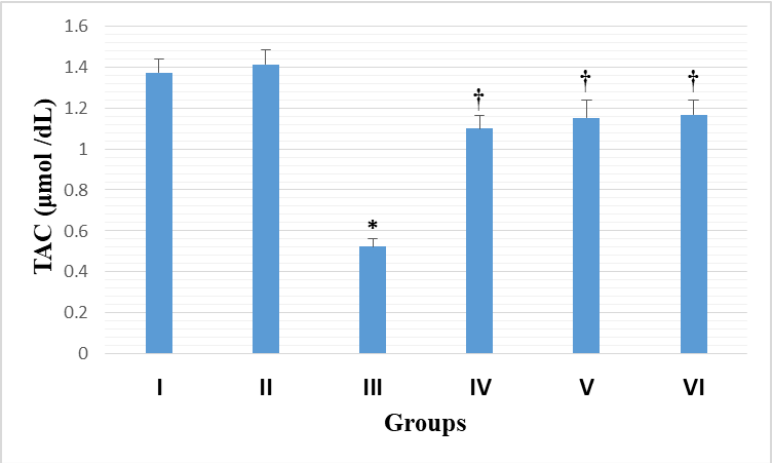

FIGURE 2. The effect of pretreatment with cinnamon and ginger extracts on total antioxidant capacity (TAC) in CCl4-damaged rats. The bar signs on top of the columns indicate mean±SD (n=6). *shows the significance of the differences relative to the normal control group (P<0.05). †shows the significance of the differences relative to the damage control group (P<0.05). I: Normal control, II: Control cinnamon 25mg/kg + ginger 125mg/kg, III: CCl4 control, IV: Cinnamon 50mg/kg, V: Ginger 250mg/kg, VI: Cinnamon 25mg/kg + ginger 125mg/kg.

The effects of CZ and ZO extracts on biochemical factors are presented in Tables 1 and 2. The injection of CCl4 caused a significant increase in the levels of creatinine, urea, uric acid; while decreased levels of serum albumin and total protein compared to the normal control group (P<0.001). Also, it was found that the administration of either CZ or ZO extract, as well as a combination of them, improves biochemical factors in the investigated groups.

Lipid peroxidation

The comparison of MDA level in the rats damaged by CCl4 with that of the healthy ones showed a significant increase after the induction of oxidative stress (P<0.001). Compared to the rats in the injury control group, pretreatment with the extract of CZ, ZO and a combination of these two decreased MDA level by 33.16%, 31.76% and 48.59%, respectively (Figure 1).

Total antioxidant capacity

The injection of CCl4 decreased total antioxidant capacity in kidney tissues by 62.04% compared to the level in the rats of the normal control group (P<0.05). On the other hand, the administration of the CZ extract, ZO extract and a combination of them in the pretreatment groups increased total antioxidant capacity by 50.47%,

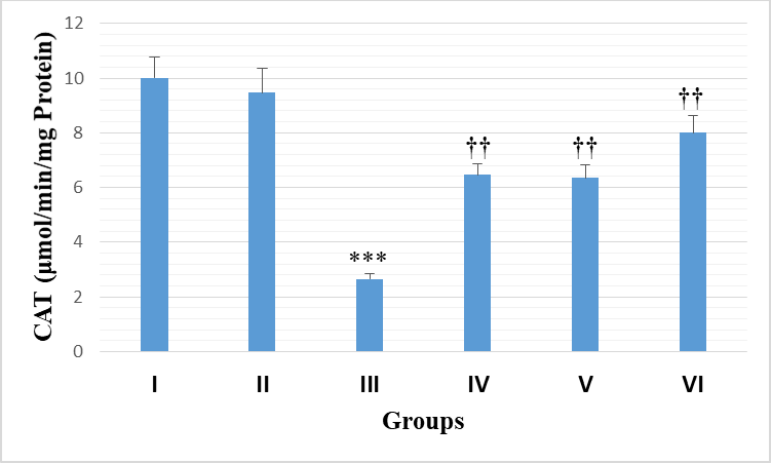

FIGURE 3. The effect of cinnamon and ginger extracts on catalase (CAT) in CCl4-damaged rats. The bar signs on top of the columns indicate mean±SD (n=6). ***shows the significance of the difference relative to the damage control group (P<0.001). ††shows significance of the difference relative to the exposure group (P<0.01). I: Normal control, II: Control cinnamon 25mg/kg + ginger 125mg/kg, III: CCl4 control, IV: Cinnamon 50mg/kg, V: Ginger 250mg/kg, VI: Cinnamon 25mg/kg + ginger 125mg/kg.

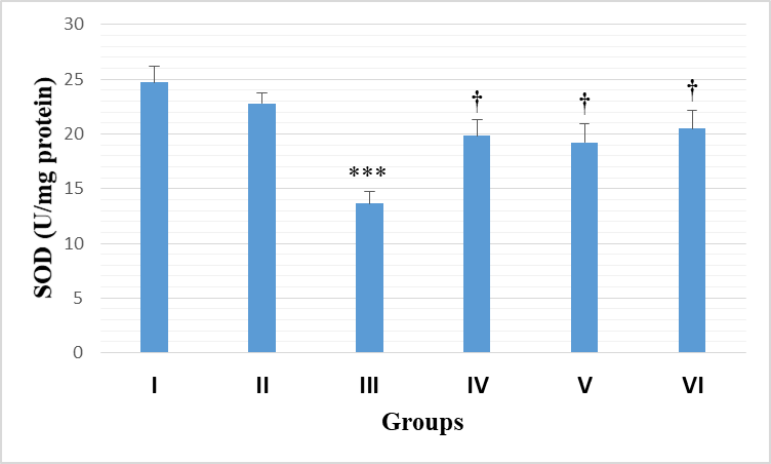

FIGURE 4. The effect of cinnamon and ginger extracts on superoxide dismutase (SOD) in CCl4-damaged rats. The bar signs on top of the columns indicate mean±SD (n=6). ***show the significance of differences relative to the exposure group (P<0.001). †shows significance of the differences relative to the damage control group (P<0.05). I: Normal control, II: Control cinnamon 25mg/kg + ginger 125mg/kg, III: CCl4 control, IV: Cinnamon 50mg/kg, V: Ginger 250mg/kg, VI: Cinnamon 25mg/kg + ginger 125mg/kg.

54.78% and 55.17%, respectively (P<0.05, Figure 2).

Antioxidant enzymes

The CCl4 caused a significant decrease in the activity of GPx (P<0.05), SOD (P<0.001) and CAT (P<0.001) enzymes compared to those in the normal control group. In the pretreatment groups, CZ, ZO and a combination of them led to a significant increase in the activity of CAT and SOD enzymes (P<0.05), and an insignificant increase in the activity of the GPx enzyme (P>0.05) compared to those in the injury control group (Figures. 3-5).

Renal histopathology

Figure 6 shows the histopathological changes of the kidney. Microscopic assessment of kidney tissues in the rats of the normal groups showed a regular morphology of renal parenchyma with well-defined glomeruli and tubules. The injection of CCl4 resulted in marked necrosis in proximal tubules, parenchymatous degeneration, an increase the urinary space diameter and an increase in mononuclear cell infiltration. However, pretreatment with extracts could protect the kidney against these damages to a greater extent.

Discussion

According to epidemiological evidence, AKI is one

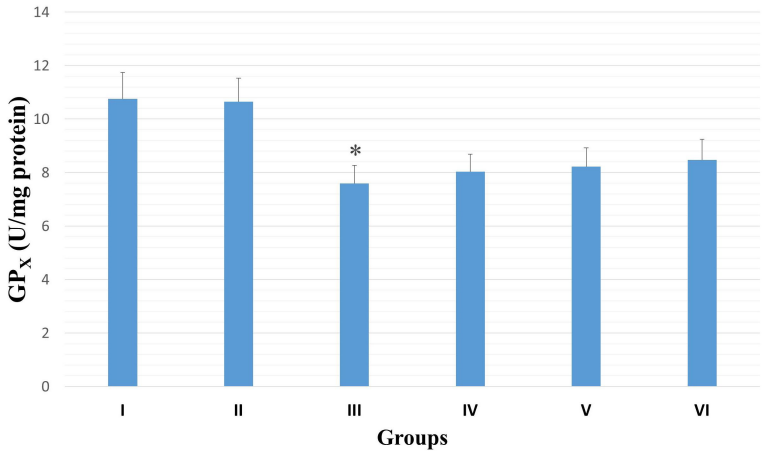

FIGURE 5. The effect of cinnamon and ginger extracts on glutathione peroxidase (GPx) in CCl4-damaged rats. The bar signs on top of the columns indicate mean±SD (n=6). *shows the significance of the differences relative to the control group (P<0.05). I: Normal control, II: Control cinnamon 25mg/kg + ginger 125mg/kg, III: CCl4 control, IV: Cinnamon 50mg/kg, V: Ginger 250mg/kg, VI: Cinnamon 25mg/kg + ginger 125mg/kg.

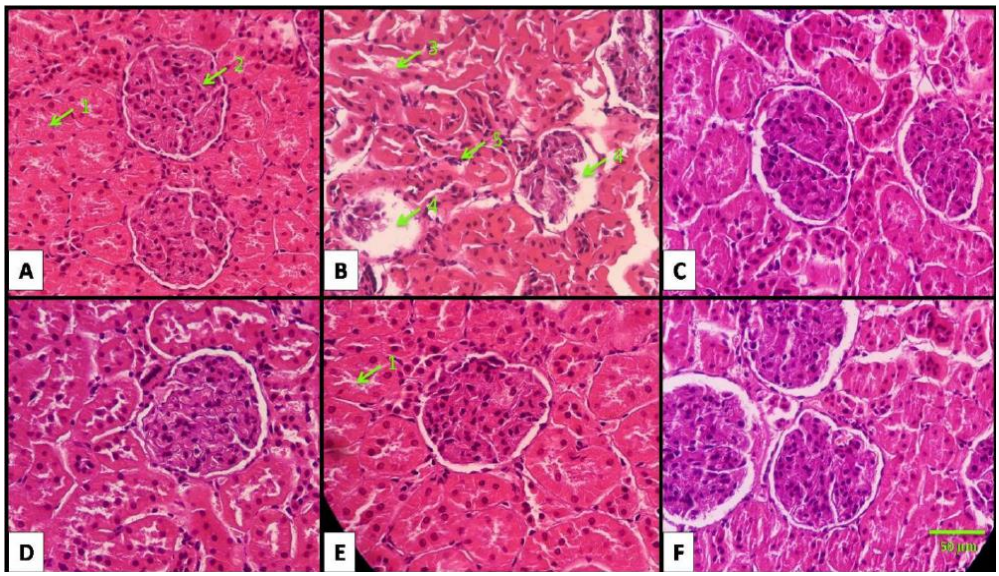

FIGURE 6. TLight microscopy of renal tissue in different groups (400x). Normal renal morphology of proximal tubules (1) and renal corpuscle (2) is seen in the control group (A). Histological changes including proximal tubules (3) and renal corpuscle (4) degeneration and increase the urinary space diameter (4) and mononuclear cell infiltration (5) demonstrated in the CCl4 group (B). Administration of cinnamon and ginger reduces the histological change in treatment groups (C, D and E). C: Cinnamon 50mg/kg, D: Ginger 250mg/kg, E: Cinnamon 25mg/kg + ginger 125mg/kg, F: Control cinnamon 25mg/kg + ginger 125mg/kg.

of the serious problems threatening the health of human beings all over the world. It is increasingly afflicting more people. It can be the result of specific factors as a complication of another disease or medical treatments (Trujillo et al., 2013). Renal function impairments are often accompanied by oxidative stress, which is one of the main causes of kidney diseases (Cachofeiro et al., 2008).

Kidney function was assessed by analyzing the serum level of urea and creatinine. These two parameters are changed after the major damages to the kidney nephrons (Radulović et al., 2015). Moreover, the serum levels of uric acid can be used as an indicator of kidney disease progress (Kang et al., 2002). In the present study, the injection of CCl4 caused an increase in serum urea, uric acid and creatinine, which can be symptomatic of AKI. Serum total protein and serum albumin levels are indicators of liver function and kidney diseases. The decline of serum total protein and serum albumin in the rats damaged by CCl4 might be the consequence of exces-

sive protein leakage caused by glomerular and tubular hypercellularity (Mazani et al., 2018). Pretreatment with CZ and ZO extract and a combination of them led to a significant increase in total protein and albumin levels, and declined the levels of urea, uric acid and creatinine to a considerable extent. It seems that the effective antioxidant compounds of CZ and ZO extracts limited damages caused by CCl4 and thereby prevented kidney injury.

MDA, a reactive aldehyde, produced by peroxidation of unsaturated fatty acids, represents the content of the free radicals resulted from lipid peroxidation and its level shows the extent of lipid peroxidation (Lin et al., 2016). A higher level of MDA is indicative of tissue injury and failure of antioxidant defense mechanisms against free radicals (Kumar et al., 2014; Sudjarwo and Giftania Wardani Sudjarwo, 2017). In our study, the injection of CCl4 caused a significant increase in the MDA level of the damaged rats. This finding was consistent with the findings reported by Ogeturk and colleagues (2005). In their study, the injection of CCl4 led to an increase in the level of lipid peroxidation in kidney tissue. Our findings also revealed that pretreatment with either CZ or ZO extract caused a significant decrease in MDA level. It seems that the compounds present in these extracts can prevent CCl4 induced injuries. The findings reported by Sharma et al. (2018) and Alibakhshi et al. (2018) indicated that cinnamaldehyde and zingerone, the main and the most important components of CZ and ZO, can prevent lipid peroxidation and cell injury.

Cellular homeostasis involves sustaining the balance between antioxidant reactions and reactive oxygen species (ROS) and reactive nitrogen species to maintain reactive substances at an appropriate level and minimize their unwanted and harmful reaction with vital biomolecules (Bartosz, 2003). Evidence suggests that different enzymatic and non-enzymatic antioxidant defense systems in mammals have been developed to deal with ROS. However, pending oxidative stress, their protective capacity against ROS becomes impaired. They may cause a decrease in the activity of CAT, SOD and GPx enzymes and finally lead to a decrease in total antioxidant capacity (Adewole et al., 2007). Our results are consistent with the findings reported in other studies (Ganie et al., 2010; Rajesh and Latha, 2004), in which the injection of CCl4 significantly decreased the activity of the antioxidant enzymes of CAT, SOD, GPx as well as total antioxidant capacity in kidney tissue. Pretreatment with the extracts could partly compensate for the decline in the activity of these enzymes and the resultant decrease in total antioxidant capacity. It seems that the effective antioxidant compounds in these plants can prevent the induction of oxidative stress by CCl4. In other words, these compounds seem to prevent injury to kidney tissue, probably through eliminating free radicals and stimulating antioxidant enzymes.

The renal histopathological findings were related to the amounts of biochemical indices in the studied groups. The injection of CCl4 resulted in glomerular and tubular injuries as well as vasocongestion in the kidney tissues. Further investigations revealed the presence of the primary symptoms associated with acute tubular necrosis, tissue changes in proximal tubules and renal tubular inflammation. These observations were all consistent with the findings reported in Mazani et al. (2018) stud. Pretreatment with the extracts could prevent inflammation and necrosis in tubules and glomeruli to a larger extent. Such an effect on histopathological changes might be due to the presence of antioxidant compounds in cinnamon and ginger. Considering the antioxidant compounds of CZ and ZO which have a phenolic hydroxyl group, it seems that these compounds prevent extensive oxidative damages via eliminating the free radicals produced by cytochrome P450 enzymes from CCl4 (Chericoni et al., 2005; Yanishlieva et al., 2006).

Conclusion

The findings showed that hydroalcoholic extracts of cinnamon and ginger possess antioxidant and protective effects against CCl4-induced damages to kidney tissue. Also, the simultaneous administration of the two extracts can have additive antioxidant and protective effects.

Acknowldgements

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Conflict of interest

The authors declare that they have no conflict of interest.

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