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Antioxidative role of propolis on LPS induced renal damage

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Abstract

Sepsis is a systemic infectious disease that leads to shock, organ failure, and death and requires urgent treatment. Animal model studies of sepsis and endotoxemia have revealed that antioxidant compounds prevent the progression of multi-system organ failure and reduce death rate. In the present study aimed to establish the effect of propolis, which has been proven to have antibacterial, anti-inflammatory and antioxidant activities in recent years, on lipopolysaccharide (LPS)-induced renal damage. 40 *Sprague dawley* rats were randomly divided into five equal groups (n = 8): Control (0.9% NaCl), LPS (30 mg/kg), propolis (250 mg/kg), propolis + LPS, and LPS + propolis. After completion of the experimental protocol, Malondialdehyde (MDA) levels were measured using blood serum samples obtained from the rats. The kidney samples of the rats were examined histopathologically. As a result, it was determined that LPS increased MDA levels in the blood serum samples and it caused histological changes in the kidney tissue such as tubular damage, mild ischemic injury, ischemic damage in the form of vacuolization, tubular epithelial vacuolization, vascular congestion, and glomerular atrophy. Contrary to these results, MDA levels of serum decreased in the propolis + LPS, and LPS + propolis groups, and also histological findings improved. These results showed that protective effect of propolis against kidney damage caused by LPS.

Keywords: Sepsis; Propolis; LPS-induced; MDA Levels; Rat

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Introduction

LPS (a bacterial endotoxin) is a glycolipid with an amphiphilic character that is regularly used in experimental models of septic shock. LPS is released whenever gram (-) bacteria die or split (1) and lead to sepsis by stimulating the inflammatory response of the immune system. Sepsis is one of the most prevalent reason of death in intensive care units (2). Septic shock may result in severe hypotension, metabolic acidosis, organ damage, multiple organ failure, acute lung injury, and death. While respiratory failure is one of the early organ dysfunctions in sepsis, the kidney, liver, coagulation system, central nervous system, and gastrointestinal system disorders are common and are among the other problems that increase death rate. However, the oxygen-free radicals resulting from sepsis lead to tissue injury by inducing DNA damage, proteins denaturation, and peroxidation of membrane lipids (3,4). There are studies in the literature showing that antioxidant compounds applied to neutralize the effects of oxygen-free radicals have beneficial effects (5).

Propolis, a resin mixture collected by honeybees from various herbal sources, contains many useful biological compounds. There are many studies showings that propolis has antibacterial, antioxidant and anti-inflammatory activities. Most previous studies have investigated the hepatoprotective effect of propolis on LPS-induced endotoxemia. Doganyigit et al. (6) found that propolis had a hepatoprotective effect on experimental endotoxemia. In another study, it was reported that propolis and caffeic acid obstruct NO

production in macrophages without that causing cytotoxicity, suppressed LPS-induced signaling pathways and did not cause hepatotoxicity by powerful anti-inflammatory activity (7). Basnet et al. (8) showed that di-caffeoylquinic acid derivatives from the propolis water extract had a stronger hepatoprotective effect than chlorogenic acid and caffeic acid. In a study investigating ethanolic extract of propolis and its active ingredients, it was reported that propolis and CAPE strongly inhibited cyclooxygenase activity in lung homogenates of LPS-treated rats, while caffeic acid, ferulic acid, pinocembrin, and chlorogenic acid and cinnamic acid did not inhibit this activity (9).

The kidney is the other organ affected by endotoxins due to the production and release of inflammatory mediators. Increased cytokine expression to protect the host from infection after acute ingestion of LPS results in the disruption of cellular redox balance (10). The overabundant ROS accumulation in renal tissues give rise to cellular damage which begins with damage to the critical super molecules like DNA, protein, and lipid (11,12). Morover, lipid peroxidation leads to various toxic effects such as declined membrane fluidity, deterioration of mitochondrial functions and impaired antioxidant enzyme functions (13). Many studies have revealed that propolis has a nephroprotective effect (14,15). Therefore, we explored the effect of propolis on nephrotoxicity excited by LPS administration.

Materials and Methods

Origin and chemical analysis of propolis

In the present study was used to popular type propolis collected from the vicinity of the Kayseri province in central Anatolia (Turkey). The chemical content of the propolis used was determined according to the study of Silici and Kutluca (2005) (16). The chemical content of propolis used in the study is shown in **Table 1**.

Experimental protocol

In this study, a total of 40 Sprague Dawley female rats (200-300 g; Erciyes University, Kayseri, Turkey) was used. The study was carried out with the approval of Erciyes University ethics committee (Protocol no: 10/8). The water and nutrient requirements of the rats housed in their cages were provided at 21°C and in a light / dark environment lasting 12 hours in normal day conditions.

The rats were randomly divided into five groups and treated LPS to induce endotoxemia (*Escherichia coli* 0111: B4; Sigma-Aldrich Chemie, Deisenhofen, Germany). The groups received the following treatment:

1. Group (Control): 0.1 ml of saline (0.9 % NaCl), i.p.
2. Group (LPS): 30 mg/kg LPS, i.p.
3. Group (Propolis only): 250 mg/kg of propolis (oral gavage)
4. Group (Propolis + LPS): 250 mg/kg propolis (oral gavage), 60 minutes before LPS
5. Group (LPS + Propolis): 250 mg/kg propolis (oral gavage) 60 minutes after LPS

Following the completion of the test protocol, rats were sacrificed under anesthesia and kidney tissue samples were collected. Kidneys were fixed in 10% neutral buffered formalin and embedded in paraffin for histopathological investigation (17).

Table 1. Chemical content of propolis used in this study (GC-MS)

Compounds	%	Compounds	%
Flavonoids	41,2	**Aromatic acids and their esters**	27,13
4,5 dimethoxy-(2-propenyl) 2-phenol		Caffeic acid	
Pinocembrin		Ferulic acid	
Chrysin		Benzoic acid	
Galangin		Caffeic acid phenetyhl ester	
Organic and fatty acids	12,63	**Alcohol, ketone and terpenes**	19,04
Decanoic acid		2-propen-1-ol	
4-pentenoic acid		5-3,3-dimethyl-cyclohexanone	
Cinnamic acid		2-Nonadecanone	
3-hydroxy-4-methoxycinnamic acid		Gamma-eudesmol	
2-propenoic acid		Beta-eudesmol	
3,4-dimethoxycinnamic acid		Alpha-eudesmol	
Coumaric acid		Alpha-bisabolol	
9-Octadecanoic acid		2-propen-1-one	
Octadecanoic acid			

Histological analysis

Kidney tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of 5 µm thickness were processed for routine Periodic Acid Schiff (PAS) staining. (18). Slides examined under Olympus BX51 microscope to see general histological structure.

Biochemical analysis

Serum MDA levels of animals were analyzed according to the protocol described by Yoshioka et al. (19). This method is based on the measurement of the intensity of the pink-colored complex formed by thiobarbituric acid, a lipid peroxidation end product at 535 nm (20).

Statistical analysis

Data of serum MDA levels from experimental groups were analyzed by GraphPad Prism 6.0 (Graphpad Software Inc., San Diego, California) and presented as mean ± SD. Data were analyzed using one-way ANOVA and Tukey's post-hoc test for multiple comparisons. P<0.05 was considered statistically significant.

Results

Compared with the control and propolis group, the MDA levels increased in the group administered LPS (P<0.05). In the propolis +LPS group one hour before LPS ingestion, the MDA level was higher than the control group but lower than the LPS group (p<0.05). Similarly, the propolis + LPS group had a higher MDA level than the control group but lower than the LPS group (**Fig. 1**). Although MDA levels in the propolis +LPS group were lower than the LPS + propolis group, the difference between the groups was not statistically significant (P>0.05).

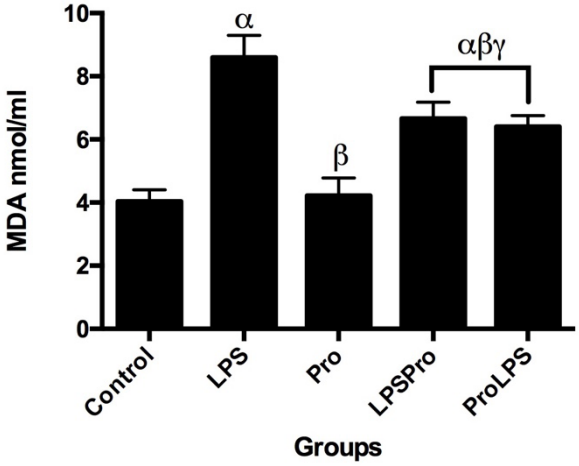

Figure 1. Serum MDA levels of groups. The histogram bar graph data are expressed as mean ± SD, and compared by compared by one-way ANOVA and Tukey's post hoc test for multiple comparisons test (α P<0.05 versus control group; β P<0.05 vs. LPS group; γ P<0.05 vs. Propolis group; ψ P<0.05 vs. LPS + Propolis group).

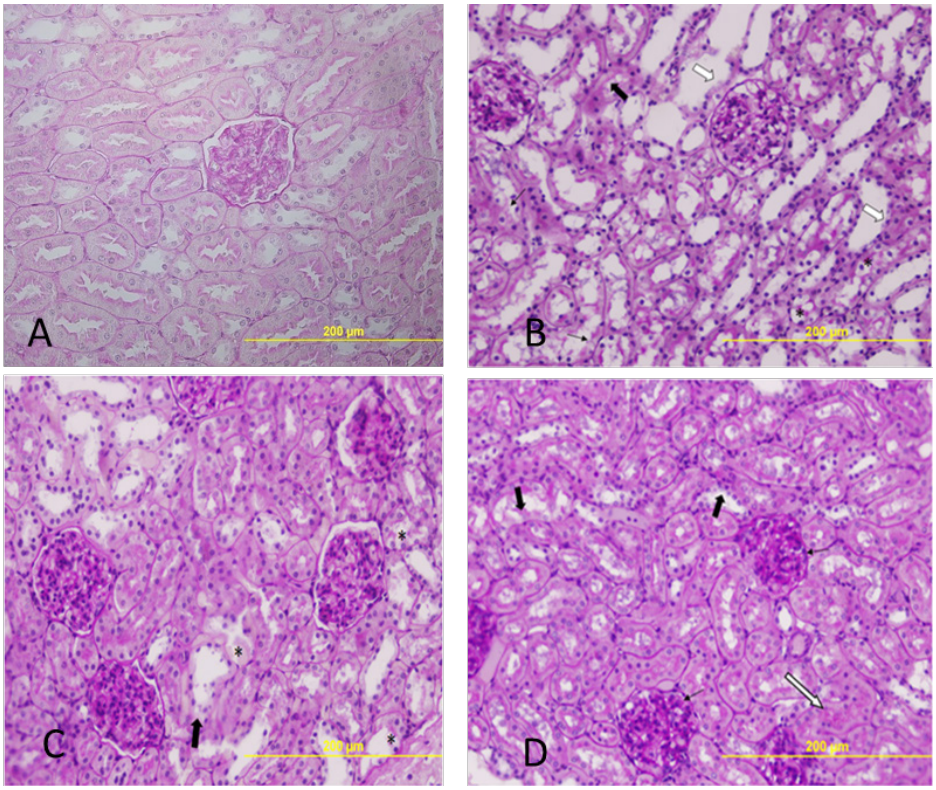

Figure 2. A: Group 1 (control animals) revealed normal kidney structure (x40, PAS). **B:** Group 2 (LPS) kidney sections of rats demonstrated slight tubular damage (black arrow), ischemic damage in the form of vacuolization (thin black arrow), tubular dilatation (thick white arrow) and tubule epithelial vacuolization (*) (x40, PAS). **C:** Group 4 (Propolis+LPS) kidney sections of rats demonstrated ischemic damage (thick black arrow), tubule epithelial degeneration (*), (x40, PAS). **D:** Group 5 (LPS+Propolis) kidney sections demonstrated ischemic damage (thick black arrow), vascular congestion (thin white arrow) and reduced Bowman's space (thin black arrow), (x40, PAS).

As a result of histopathological evaluation of kidney sections stained with Periodic Acid-Schiff (PAS) stain, it was observed that the normal histological structure was preserved in the control and propolis-treated groups (**Fig. 2A**). Tubular damage, mild ischemic damage, ischemic damage in the form of vacuolization, tubular epithelial vacuolization, vascular occlusion and glomerular atrophy were observed, especially in the kidney sections of rats treated with LPS only. (**Fig. 2B**). While in propolis + LPS group kidney sections of rats treated with propolis before LPS challenge showed mild ischemic injury (**Fig. 2C**), in group LPS + propolis sections of rats treated with propolis after LPS challenge showed mild ischemic injury, tubule epithelial vacuolization, vascular congestion and glomerular atrophy (**Fig. 2D**).

Discussions

Hydroxyl radicals resulting from oxidative stress form double bonds with lipids in cell and organelle membranes. A series of reactions occur with the resulting free radical-lipid interaction. Thus, many lipid peroxidation products such as MDA are formed (21). MDA is an end product of lipid peroxidation and a marker of oxidative damage. MDA can easily diffuse from its site of formation and is cross-linked to lipids and proteins in the membrane structure, causing deterioration of membrane integrity and permeability. In the current study, the increase the levels of MDA in serum in the group that was applied LPS display lipid peroxidation to have developed. Similarly, MDA levels are elevated in studies performed by creating an empirical endotoxemia model with LPS on many different organ systems (22,23).

Studies in recent years demonstrated that antioxidant agents improve renal damage when used immediately after bacterial infusion. For example, Garoui et al. (24), evaluated the biochemical changes in cobalt-exposed rats and investigated the potential role of Tunisian propolis versus the cobalt-exposed kidney damage. A statistically meaningful increase in plasma urea and creatinine levels was observed in treated female rats and their pups. In addition, the administration of propolis (along with cobalt-exposure) caused low levels of malondialdehyde, high antioxidant activity and abnormal histopathological changes at low severity. Similarly, there are a larger number of studies demonstrating that propolis has antioxidant activity and reduces MDA levels (20). In our study, it was observed that MDA levels decreased in the propolis+LPS, and LPS+propolis groups compared to the LPS group. This result reveals that propolis reduces oxidative stress caused by LPS.

According to light microscopic examinations exhibited normal renal corpuscles and tubules in the control and propolis groups. Tubular damage, mild ischemic damage, ischemic damage in the form of vacuolization, tubular epithelial vacuolization, vascular occlusion and glomerular atrophy were observed, especially in the kidney sections of rats treated with LPS only. The kidneys of animals given propolis prior to LPS application only had mild ischemic damage and low levels of tubular epithelium degeneration. Propolis has been shown to reduce the histopathological damage caused by LPS in the kidneys, and these results are also compatible with previous studies (6, 15).

The chemical composition of propolis used in the current study includes flavonoids (such as pinocembrin, pinobanksin, chrysin and galangin), aromatic acids (such as benzoic, caffeic and ferulic acids), aromatic acid esters (such as caffeic acid phenethyl ester), aromatic aldehydes (such as benzyl p-coumarate, benzyl ferulate, phenylethyl caffeate and cinnamyl cinnamate), and aromatic alcohols, ketones and terpenes. Among them, especially flavonoids and phenolic compounds found in the structure of propolis have been reported to have many useful biological activities. It has been reported that chrysin has potent anti-inflammatory, anti-cancer, and antioxidant properties (25). The flavanone pinocembrin has strong antifeedant, anti-inflammatory, and antioxidant activities (26).

Results from in recent year studies show that galangin, with its antioxidant and radical scavenging activities, is capable of modulating enzyme activities and push down the genotoxicity of chemicals (27). A study shows that caffeic acid phenethyl ester (CAPE), a bioactive component of propolis extract, may protect against cisplatin-induced nephrotoxicity (28) and acute treated of CAPE suppressed ischemia-reperfusion induced renal lipid peroxidation and tissue damage in rats (29, 30). Gurel et al. (31), showed the effect of CAPE and α-tocopherol on nitric oxide manufacture and antioxidant enzyme activities upon renal ischemia-reperfusion damage. The most significant results were encountered at myeloperoxidase activities, and pretreatment with CAPE importantly depressed the tissue myeloperoxidase activity showing the inibition of the neutrophil sequestration inside the kidney (31). In another study, Ogeturk et al. (32), investigated the protective effect of CAPE on carbon tetrachloride (CCl4)-induced kidney injury. CCl4 application was found to induced significant histopathological damage at the kidney. Glomerular and tubular degeneration, interstitial cell infiltration, fibrosis and vascular congestion in the peritubular blood vessels were showed in the renal cortex. It was observed that this histopathological damage was improved in rats treated with CCl4 + CAPE (32). Rossi et al. (9) investigated the effect of propolis on cyclooxygenase activity in lung samples of LPS-administration rats. They observed that propolis and CAPE strongly inhibited COX activity in lung samples of LPS-administration rats, while caffeic, ferulic, cinnamic and chlorogenic acids and pinocembrin did not inhibit this activity. In another study, it was found that quercetin (one of the propolis components) reduced LPS-induced NO production and returned ROS levels to normal (33).

Conclusions

In the current study, kidney sections of rats treated with LPS demonstrated tubular damage, ischemic injury, ischemic damage in the form of vacuolization, tubule epithelial vacuolization, vascular congestion and glomerular atrophy. In comparison with the kidney sections of rats treated with LPS, the administration of propolis with LPS showed a mild effect against LPS-induced kidney injury. In this study, propolis was administered at a dose of 250 mg/kg 1 hour before and after LPS administration. Both biochemical analyses (MDA level) of serum samples and histo-

logical analyses of kidney tissues show that propolis improves oxidation damage in kidneys induced by LPS.

Conflict of interest statement

There is no conflict of interest between authors.

Ethical Review and Approval

Ethical approval was obtained from the Erciyes University Veterinary Faculty Ethics Committee for the study (Protocol no: 10/8)

References

1. Rietschel ET, Brade H, Holst O, Brade L, Müller-Loennies S, Mamat U, Zahringer U, Schumann RR. Bacterial endotoxin: chemical constitution, biological recognition, host response, and immunological detoxification. In: Rietschel ET, Wagner H, ed. Pathology of Septic Shock. Current Topics in Microbiology and Immunology, vol 216.Springer, Berlin, Heidelberg; 1996: 39-81.
2. Iskit AB. Sepsiste Deneysel Modeller. Hacettepe University Faculty of Medicine, Department of Medical Pharmacology. Turkish Journal of Intensive Care 2005; 5(2): 133-136.
3. Otero-Anton E, Gonzalez-Quintela A, Lopez-Soto A, Lopez-Soto A, Lopez-Ben S, Llovo J and Perez LF. Cecal ligation and puncture as a model of sepsis in the rat: influence of the puncture size on mortality, bacteremia, endotoxemia and tumor necrosis factor alpha levels. European Surgical Research 2001; 33(2): 77-79.
4. Bone RC. Gram-negative sepsis. Background, clinical features, and intervention. Chest 1991;100(3): 802-8.
5. Powell RJ, Machiedo GW, Rush BF, Jr & Dikdan GS. Effect of oxygen-free radical scavengers on survival in sepsis. American Surgery 1991; 57: 86-88.
6. Doganyigit Z, Kup FO, Silici S, Deniz K, Yakan B and Atayoglu T. Protective effects of propolis on female rats' histopathological, biochemical and genotoxic changes during LPS induced endotoxemia. Phytomedicine 2013; 20(7): 632– 639.
7. Búfalo MC, Ferreira I, Costa G, Francisco V, Liberal J, Cruz MT, Lopes MC and Sforcin JM. Propolis and its constituent caffeic acid suppress LPS-stimulated pro-inflammatory response by blocking NF-κB and MAPK activation in macrophages. Journal of Ethnopharmacology 2013; 149 (1): 84-92.
8. Basnet P, Matsushige K, Hase K, Kadota S and Namba T. Four di-O-caffeoyl quinic acid derivatives from propolis. Potent hepatoprotective activity in experimental liver injury models. Biological and Pharmaceutical Bulletin 1996; 19(11): 1479-84.
9. Rossi A, Longo R, Russo A, Borrelli F and Sautebin L. The role of the phenethyl ester of caffeic acid (CAPE) in the inhibition of rat lung cyclooxygenase activity by propolis. Fitoterapia 2002; 73: 30-37.
10. Finkel T and Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408 (6809): 239-247.
11. Randerath K, Randerath E, Smith CV and Chang J. Structural origins of bulky oxidative DNA adducts (type II I-compounds) as deduced by oxidation of oligonucleotides of known sequence. Chemical Research in Toxicology 1996; 9(1): 247-254.
12. Mallis RJ, Buss JE and Thomas JA. Oxidative modification of Hras: S-thiolation and S-nitrosylation of reactive cysteines. Biochemical Journal 2001; 355: 145-153.
13. Sugino K, Dhi K, Yamada K and Kawasaki T. The role of lipid peroxidation in endotoxin-induced hepatic damage and the protective effect of antioxidants. Surgery 1987; 101(6): 746752.
14. Baykara M, Silici S, Ozcelik M, Guler O, Erdogan N and Bilgen M. In vivo nephroprotective efficcay of propolis against contrast-induced nephropathy. Diagnostic and Interventional Radiology 2015;21(4): 317-21.
15. Ulusoy HB, Ozturk I and Sonmez MF. Protective effect of propolis on methotrexate-induced kidney injury in the rat. Renal Failure 2016; 38(5): 744-750.
16. Silici S and Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of Ethnopharmacology 2005; 99 (1): 69-73.
17. Bancroft JD, Stevens A and Turner DR. Theory and Practice of Histological Techniques. 3rd ed. Churchill Livingstone, Philadelphia; 1990, 167: 43-49.
18. Nangaku M, Alpers CE, Pippin J, Shankland SJ, Adler S, Kurokawa K, Couser WG and Johnson RJ. A new model of renal microvascular endothelial injury. Kidney International 1997; 52 (1): 182-94.
19. Yoshioka T, Kawada K, Shimada T and Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. American Journal of Obstetrics & Gynecology 1979;135(3): 372-376.
20. Eraslan G, Kanbur M, Silici S, Altınordulu S and Karabacak M. Effects of cypermetrin on some biochemical changes in rats: the protective role of propolis. Experimental Animals 2008; 57(5): 453-460.
21. Niki E, Yamamoto Y, Komuro E and Sato K. Membrane damage due to lipid oxidation. American Jounal of Clinical Nutrition 1991; 53: 201-205.
22. Wu QJ, Wang YQ and Qi YX. The protective effect of procyanidin against LPS-induced acute gut injury by the regulations of oxidative state. Springerplus 2016; 5(1): 1645.
23. Qi T, Li H and Shuai L. Indirubin improves antioxidant and anti-infalmmatory functions in lipopolysaccharide-challenged mice. Oncotarget 2017; 8(22): 36658-36662.
24. Garoui El M, Hamadi F, Soudani N, Boudawara T and Zeghal N. Propolis attenuates cobalt induced nephrotoxicity in adult rats and their progeny. Experimental and Toxicological Pathology 2012; 64 (7-8): 837-846.
25. Keun Ha S, Moon E and Kim YS. Chrysin suppresses LPS-stimulated proinflammatory responses by blocking NF-$_{κ}$B and JNK activations in microglia cells. Neuroscience Letters 2010; 485 (3): 143–147.
26. Napal DNG, Carpinella CM and Palacios MS. Antifeedant activity of ethanolic extract from Flourensia oolepis and isolation of pinocembrin as its active principle compound. Bioresource Technology 2009; 100(14): 3669–3673.
27. Heo YM, Sohn JS and Au WW. Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. Mutation Research 2001;488(2): 135–150.
28. Ozen S, Akyol O, Iraz M, Sogut S, Ozugurlu F, Ozyurt H, Odaci E and Yildirim Z. Role of caffeic acid phenethyl ester, an active component of propolis, against cisplatin-induced nephrotoxicity in rats. Journal of Applied Toxicology 2004; 24(1): 27–35.
29. Irmak MK, Koltuksuz U, Kutlu NO, Yagmurca M, Ozyurt H, Karaman A and Akyol O. The effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in comparison with α-tocopherol in rat kidneys. Urological Research 2001; 29(3): 190–193.
30. Ozyurt H, Irmak MK, Akyol O, Sogut S. Caffeic acid phenethyl ester changes the indices of oxidative stress in serum of rats with renal ischaemia-reperfusion injury. Cell Biochemistry and Function 2001; 19: 259–263.
31. Gurel A, Armutcu F, Sahin S, Sogut S, Ozyurt H, Gulec M, Kutlu NO and Akyol O. Protective role of a-tocopherol and caffeic acid phenethyl ester on ischemia–reperfusion injury via nitric oxide and myeloperoxidase in rat kidneys. Clinica Chimica Acta 2004; 339 (1-2): 33–41.
32. Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N and Sarsılmaz M. Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. Journal of Ethnopharmacology 2005; 97(2): 273–280.
33. Zhang C, Walker LM and Mayeux PR. Role of nitric oxide in lipopolysaccharide-induced oxidant stress in the rat kidney. Biochemical Pharmacology 2000; 59: 203–209.