Protective Effects of Geraniol on Long Term Renal Ischemia/Reperfusion Injury in Rats

Senanur Can
https://orcid.org/0000-0001-7248-3812

Mediha Canbek
https://orcid.org/0000-0003-1095-2382

1 Eskişehir Osmangazi University, Faculty Of Arts And Science, Department Of Biology,Eskişehir,Turkey;

Received: 2018.10.09; Accepted: 2019.07.21.

*Correspondence: senanurc@hotmail.com; Tel.: +90-2222393750 (F.L.)

HIGHLIGHTS

- Geraniol (100 mg/kg geraniol) protected the kidneys against oxidative stress.
- 100 mg / kg geraniol showed antioxidant properties and supported the antioxidant defense system.
- SOD, CAT and GPx activities in renal tissue were analyzed through jel electrophoresis.
- Geraniol decreased BUN and CRE levels in kidney parameters in rats.

Abstract: Possible protective effects of geraniol, known as antioxidant properties, were analyzed biochemically and histologically on experimental long-term renal ischemia/reperfusion I/R injury in rats. This study used 3-4 months old male Wistar albino rats and were divided into 4 groups (n = 7) by random selection: Group I (Sham Group), Group II (I/R+SF), Group III (I/R+50 mg/kg geraniol), and Group IV (I/R+100 mg/kg geraniol). A right nephrectomy was performed in all groups under anesthesia. Groups I and II were inoculated with SF (1 ml/kg) and Groups III and IV were inoculated with 50 mg/kg and 100 mg/kg of geraniol, injected intraperitoneally. For Groups II, III, and IV, I/R durations were determined to be 60 mins ischemia and 24 hours reperfusion. At the end of the experiment, Urea (BUN), Creatinine (CRE) activities in the blood serum and the catalase (CAT), glutathione peroxidase (GPx) and Superoxide dismutases (SOD), enzyme activities in kidney tissue were measured. Histologic sections were examined by light microscopy using Hematoxylin & Eosine. As a result, it was determined that 100 mg / kg geraniol against renal I/R injury shows more antioxidant effect and protection than 50 mg / kg geraniol.
INTRODUCTION

There is a systematic balance between the oxygen radicals and the antioxidant defense system in biological systems [1]. Free oxygen radicals (FOR) are radicals, ions, or molecules with unpaired electrons in their outermost orbitals. These mismatched electrons make FOR highly reactive because they are highly energized [2]. In a healthy organism, FOR, which is formed as a result of normal metabolism, is neutralized by the body's antioxidant defense system. However, when the metabolic equilibrium is destroyed in favor of radicals, oxidative stress occurs [3]. Oxidative stress is inevitable in ischemia reperfusion (I/R) damage [4]. Ischemia is defined as an inadequate blood supply to an organ for various reasons (especially during vascular surgical procedures and organ transplantation) [5] and reperfusion as restoring the flow of blood flow to an organ or tissue [6]. Damage caused by reperfusion causes more serious consequences in tissue or organs than ischemic damage. Among the most important causes of reperfusion injury is the emergence of oxygen radicals [7]. Oxygen is a molecule that is vital to living things and is used in the process of energy production in the cell. FOR are a natural byproduct of energy production processes and are highly oxidative and potentially harmful. However, as long as the rate of free radical formation is in balance with the rate of the endogenous antioxidant defense system, which includes enzymes such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) etc., the organism cannot be affected by free radicals [8]. Free radicals damaging DNA, proteins, and lipids in our cells [9]. In addition, to protect against the harmful effects of free radicals, daily intake of natural nutritive substances can support the antioxidant defense mechanism. One of these substances, terpene-derived geraniol, is a natural antioxidant that prevents cancer [10]. It has been reported that essential oils protect the mitochondrial membrane against free radical damage as it exhibits free radical binding properties [11].

In this study, a possible protective effect of geraniol, known to have an antioxidant impact factor, on long-term renal ischemia reperfusion injury has been tested with biochemical and histological analyses and with the native gel electrophoresis method.

MATERIAL AND METHODS

All experimental analyses were carried out with permission from the Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (HADYEK) No. 326/2013.

Test Animals

All experimental animals were supplied by the KOBAY Experimental Animal Production Laboratories Inc. The animals used in this study (and all other animals in the colony) were cared for in accordance with the Guide for the Care and Use of Laboratory Animals [12]. The test animals were enabled to adapt to the laboratory environment for one week and were kept alive during the experiment in automatically adjusted rooms; 12 h/12 h light/dark lighting, room temperature of 22 ± 2°C, and air humidity of 45-50%. During the experimental
period, all rats were fed with standard food pellet and tap water. Twenty-eight, 3-4 months old male Wistar albino rats (200-250 g in weight) were used in this study and divided into 4 groups (n = 7) by random selection.

**Geraniol Application**

The dose rate to be administered was regulated accordingly to the oral LD limits specified in the New Safety Data Sheets [13]. Two doses of geraniol (50 mg/kg and 100 mg/kg), specifically made for laboratory animals and were commercially-available from Acros Organics Thermo Fisher Scientific Geel, Belgium, were administered per intra-peritoneal injection 1 hour prior to the ischemia procedure.

**Experimental Protocol**

A right sided nephrectomy was performed in all groups under general anesthesia using xylazine and ketamine anesthetic [14]. After 15 days of recovery, only a laparotomy was performed on the rats in **Group I**, and after monitoring them for 24 h, a dissection was performed. For **Group II**, physiological serum (1 ml/kg) was administered per intra-peritoneal injection one hour prior to the ischemia procedure; the left sided renal artery was isolated, and the blood flow was interrupted for 60 minutes using an anti-traumatic vascular clamp, following reperfusion for 24 hours. The test animals in **Group III** received a 50 mg/kg single dose of geraniol, and in **Group IV** a single dose of geraniol per intra-peritoneal injection (100 mg/kg) one hour prior to the ischemia procedure; then was followed by reperfusion for 24 hours [15]. At the end of the reperfusion period, the test animals were anesthetized and humanely killed by withdrawal of the whole blood from the heart using standard method. Blood urea nitrogen (BUN) and creatinine (CRE) were analyzed in blood serum to evaluate kidney function, and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) to evaluate enzyme activities in kidney tissue samples.

**Biochemical Analysis**

BUN and CRE levels were analyzed with a commercial kit (Biolabo, France) using the Crony Airone 200 RA brand auto analyzer.

**Determination of isoenzyme activity in kidney tissue**

**Preparation of homogenate**

Total protein amount of SOD, CAT, and GPx isoenzymes, whose activities are determined in homogenates obtained from kidney tissue samples, were measured using the Qubit® instrument according to the Bradford method. To determine enzyme activity in kidney tissue samples, SOD, CAT, and GPx isoenzymes were measured according to their homogenate reaction by using the native gel electrophoresis method [16].
**Determination of CAT Activation**

CAT activity was determined according to the method by Woodbury, Spencer and Stahman [17].

**Determination of SOD Activity**

SOD activity was determined according to the method by Beauchamp and Fridovich [18].

**Determination of GPx Activity**

GPx isoenzyme activity was determined according to the method by Lin, Chen and Hou [19]. To compare the experimental groups, the areas of enzyme-induced sites were calculated using the Kodak Molecular Imaging Software package program in the Kodak Gel Logic 1500 Imaging System Gel imaging system.

**Histopathological Evaluation**

For histological analyses, 10% neutral formaldehyde was added to the kidney tissue samples taken and stained with hematoxylin and eosin (H&E stain). All prepared tissue sections were examined histologically using the Olympus brand CH40 model light microscope and the Spot Insight Color Microscope Camera 3.2.0 with SPOT Advanced™ software version 4.0.6.

**Statistical Analysis**

For the evaluation of data obtained from the experimental groups, the ‘One-way ANOVA with post hoc Tukey test’ was used in the ‘SPSS Version 20.0 for Windows’ package program. Differences between the experimental groups, which appeared as numerical value (p) as a result of all statistical applications, were accepted significantly at p≤0.05 significance level.

**RESULTS**

**Biochemical Findings**

**Biochemical findings in serum samples**

BUN and CRE values of all experimental groups obtained from the serum samples are indicated in Table 1 and Figure 1 as comparative statistics between the groups, respectively. As a result, serum BUN values are significantly higher in the I/R group than those in the Sham group. Groups II, III, and IV show a statistically significant rise contrary to Group I (p≤0.05) (Table 1). The comparison of BUN quantities of Group II to Groups III and IV, shows a statistically significant decrease in Groups III and IV (p≤0.05), whereas the comparison between Group I and Group IV shows a result of similarity (Fig. 1). Regarding the determination of CRE level in blood sera, the comparison of Group I with Groups II, III, and IV shows a level of significance in Group II contrary to Group I (p≤0.05) (Table 1), whereas Groups III and IV show no significant result (Figure 1).
Figure 1. Average and standard error graph of BUN and CRE levels (mg/dl) regarding the serum of the Groups I, II, III and IV.

Table 1. Mean values of BUN and CRE quantities in blood serum samples regarding experimental groups ± standard error values (n = 7). P <0.05 is different a: from Group I; b: from Group II; c: from Group III.

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>CRE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>58.95 ± 1.176</td>
<td>0.47 ± 0.035</td>
</tr>
<tr>
<td>II</td>
<td>289.67 ± 5.410&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.02 ± 0.200&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>205.12 ± 16.132&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.34 ± 0.206&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>136.88 ± 6.569&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.74 ± 0.036&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Isoenzyme Activities in the Kidney

Regarding the kidney tissue samples of all experimental groups, CAT isoenzyme single band, GPx isoenzyme quadrupole band, and SOD isoenzyme activities were viewed in triplicate using the native gel electrophoresis method. The measured numerical field values are shown in Table 2. Regarding the visualization of SOD, CAT, and GPx bands; relating to the kidney tissue sample of Group I, the bands of all groups are clearly represented with the highest density, contrary to Group II seen with the lowest density. In particular, the band image density of the Groups III and IV are much higher compare to Group I, whereas the band of Group IV shows a similar image to Group I (Figure 2).
Table 2. Mean values of the band images of SOD, CAT, and GPx enzyme activities determined in kidney tissue samples of all experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>CAT mm²</th>
<th>SOD mm²</th>
<th>GPx mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOD1</td>
<td>SOD2</td>
<td>SOD3</td>
</tr>
<tr>
<td>I</td>
<td>30.56</td>
<td>17.45</td>
<td>13.87</td>
</tr>
<tr>
<td>II</td>
<td>22.18</td>
<td>14.20</td>
<td>8.85</td>
</tr>
<tr>
<td>III</td>
<td>25.82</td>
<td>15.08</td>
<td>10.68</td>
</tr>
<tr>
<td>IV</td>
<td>28.82</td>
<td>14.50</td>
<td>10.68</td>
</tr>
</tbody>
</table>

Figure 2. Electrophoretic bands of CAT, SOD and GPx enzyme activity.

Histological Analyses

In this study, the protective effect of administered geraniol in a dose-dependent manner on long-term I/R injury was analyzed in rats with H&E-stained kidney tissues. The results of the histological analyses of renal tissue relating to the comparison of glomerular and Bowman’s capsule changes with glomerular and Bowman’s capsule ratio are shown in Figure 3. The examination of H&E-stained kidney sections of the control group, referred to as Group I, showed no pathological findings in the glomerular structure, Bowman’s capsule and ratio, and renal tubules (Figure 3-A).

In the kidney sections of Group II glomerular capillary hemorrhage, deterioration in the renal tubules, as well as swelling in renal tubule cells, fluid accumulation, and tubular deformation caused by cell debris were observed (Figure 3-B).

In consideration of the administration of 50 mg/kg geraniol, the renal tissue from Group III showed less swelling and loss of cytoplasm in the apical cells than those in the I/R group. However, vacuolization and partial renal tubule bleeding continued (Figure 3-C).
Considering the administration of 100 mg/kg geraniol, the histological results of the renal tissue from Group IV showed protection to a great extent on renal I/R injury. A decrease in tubular fluid accumulation and bleeding could also be observed. In addition to these results, the renal tubule cells and the glomerular structure show a similar image to Group I (Figure 3-D).

![Figure 3. Histological images of Sham and experimental groups](image)

**3-A Sham group: Normal glomerulus and tubular structure**

**3-B I/R group: Deformation in glomerular structure**

**3-C Experimental group with 50 mg/kg geraniol: Decrease in fluid accumulation in tubules (†)**

**3-D Experimental group with 100 mg/kg geraniol: Glomerulus and tubule cells near normal**

**DISCUSSION**

Renal ischemia/reperfusion injury, for various reasons, cause draining of the cellular energy storage, disruption of the intracellular communication system, and chain reactions in cells which results in the accumulation of toxic metabolites [1]. However, the main reason for this injury is thought to be FOR, the free oxygen radicals [2]. The release of FOR is due to a rapid increase in the amount of oxygen during tissue reperfusion [17]. As long as the rate of free radical formation is in balance with the rate of the endogenous antioxidant defense system, the organism cannot be affected by free radicals. The resulting FOR can be rendered harmless by the antioxidant defense mechanism via the enzymatic (SOD, GPx, CAT) or non-enzymatic pathway (polyphenols, flavonoids, terpenes, etc.) [20].
Geraniol (3.7-dimethyl-2.6-octadien-1-ol) used in this work is composed of monoterpenoid alcohol containing hydroxyl molecule. Geraniol has a good antioxidant property due to its hydroxyl (-OH) groups in its chemical structure [11]. The geraniol substance (rosa damascena) is abound in Turkey and descend from the rose family rosaceae, which can be found in natural rose oil obtained by distillation of fresh flowers [21].

In experimental I/R injury, a period of time seems to be required for ischemia effects to occur in kidneys. Several studies prevent reperfusion damage in rat kidneys from 45 min up to 60 min of ischemia [22]. It has been reported that studies with tissue samples and serum analysis that renal I/R injury occurs after 4 h at the earliest, and can take up to 24 h. To precisely see the effects of renal I/R injury in this study, the rats were subjected to a 60 min ischemia/24 h reperfusion procedure as seen in other studies [23].

Urea, a product of degradation of protein metabolism and produced with the detoxification of toxic-effect ammonia, is a marker of renal function test [24]. Hence, the term BUN is used for biochemical analysis; a BUN test measures the amount of urea nitrogen in blood that comes from the waste product urea and is compromised of 46% of the total amount of urea. An increased plasma BUN level shows a defect in kidney function. There may be several reasons for this increase, such as extreme protein intake, cardiac insufficiency, a combination of sodium and free water losses, infections, surgical interventions, burn injuries, high temperature, and/or tissue destruction [25]. Contrary to the urea cycle, 40-50% of urea is absorbed back into the proximal tubules of the kidney [26].

In this study, following an induced I/R injury, BUN values were measured in blood serum with the result of a significant increase in all groups compared to Group I (Sham group). The comparison of Group I with Group II (I/R group) shows significant differences (p≤0.05); as expected I/R procedure has an adverse effect on the kidney function. BUN values after the administration of 50 mg/kg geraniol (Group III) and 100 mg/kg geraniol (Group IV) show a statistically significant decrease compared to Group II, which shows the protective effect of geraniol.

Rather than evaluating BUN alone, evaluating it together with creatinine is more accurate in terms of renal function. Creatinine is formed by the loss of creatinine in skeletal muscles [22]. Serum creatinine level is a marker of renal (glomerular) function. Elevated serum creatinine after I/R injury is an indication of impairment of renal function in proximal tubule cells [27]. Creatinine is removed from the circulation, especially by glomerular filtration. In addition, it is not reabsorbed from the tubules [26]. Therefore, glomerular filtration and renal blood are easily affected by the current change [25].

In the Sham group the serum CRE level was statistically significant (p≤0.05) than Group II, Group III, and Group IV. However, there was a significant decrease in Group III and Group IV compared to Group II. The significant improvement in CRE level in the treatment group, Group IV (100 mg/kg geraniol), to the Group I (Sham group) CRE level showed that geraniol was protective against ischemia reperfusion injury at 100 mg/kg dose.

In the 1997 study by Williams et al [28], the performed ischemic model consisted of bilaterally clamping the renal artery and vein, lasting in a 45 min ischemic period. The effects of I/R damage on blood BUN and CRE levels and renal histology had been observed at 0, 0.5, 1, 2, 4, 6, 9, and 24 h and 1 week after reperfusion. These investigators reported that
renal injury started at 4 h at the earliest following 45 min of ischemia and that the damage had a maximum effect at 24 h.

Aydogdu, Kaymak and Yalcin [15] experiment concluded of a 60 min ischemia period in 24 h reperfusion with levels of serum BUN, and CRE recorded. This is parallel to our work. Similar results were observed in the work done by Sahin [29]. In this study, pioglitazone was used as a preservative. I/R times were determined as 60 min/24 h and the protective effect on the kidney was examined. As a result, the serum creatinine levels in I/R group were the highest, while the treatment groups reported a decrease. The renal serum BUN and CRE levels determined in our study are consistent with the knowledge of the literature and indicate that serum BUN and CRE levels are significantly elevated in long term renal I/R injury due to SOR.

Electrophoretic analysis of CAT enzyme in kidney tissue revealed a single band in all groups (Figure 2). In the kidney specimen of Group I, the band area was at high density while in Group II the band area was at low density. The low band area in Group II suggested that long-term I/R damage may have accelerated the inhibition of CAT antioxidant enzyme activity. In Group III and IV, close band areas were measured (Figure 2). When group III is compared to Group IV according to their band areas, Group III band area was seen lower than group IV band area. This suggests that the protective effect of 50 mg/kg geraniol is not sufficient and that 100 mg/kg geraniol supports the antioxidant defense system.

In the GPx enzyme in kidney tissue, four isoforms, GPx1, GPx2, GPx3 and GPx4, were detected. It was observed that Group II increased among all the groups that compared all isoforms.

In addition, the band IV of the Group IV was close to the band I. Three isoenzymes of the SOD enzyme, SOD1, SOD2 and SOD3, which have specific area measurements on the gel, were observed in the kidney (Figure 2). When all isoenzymes were examined, the band areas in Group I were found to be high whereas the band areas of Group II were found to be low in all groups. Group III and IV have similar band areas and the band areas of Groups III and IV showed an increase compared to Group II. When we examined the total SOD activity after evaluating each of the SOD isoenzymes in itself; the results of CAT and GPx are parallel to the enzyme activity results.

CAT, SOD, and GPx in living tissues are very important antioxidant defense mechanisms. While CAT is responsible for the detoxification of H$_2$O$_2$, SOD catalyzes the O$_2^-$-radical, otherwise the biological structure and membranes are damaged. In GPx, H$_2$O$_2$ converts water and oxygen. All of this plays an important role in the formation of cellular damage. Reduction of these enzymes leads to increased free radicals. Ultimately, antioxidant enzymes and free radicals must be in a certain balance for the function and integrity of cell membranes. In addition, antioxidants are inadequate in biochemical events caused by increased free radicals due to long-term tissue damage. It has been reported that inactivation of enzymes, such as SOD, CAT, and GPx, is accelerated due to oxidants in ischemic tissue and cells become more susceptible to the effects of oxygen radicals formed during reperfusion [15].

Dobashi et al [30] studied the ischemia of the left kidney (30, 60, 90 min) and reperfusion (2, 24, 72, 120 h) at different times. A significant decrease in SOD, CAT, and
GPx activities were reported and an increase in the level of lipid peroxidation in the group that received 60 min of ischemia for 24 h of reperfusion.

The study of Akkoç [22] applied calcium dobesilate which caused a significant decrease of CAT, SOD, and GPx activities in I/R group after renal I/R (45 min/24 h) in male Wistar rats compared to the other groups.

Suyani [31] performed renal ischemia in his 45-min, 24-h study in rats and found it to be the highest control group of SOD activity. This indicates that there is no increase in short-term SOD activity in the I/R models, whereas in long-term ischemia (90 min) there is significant SOD increase in applied I/R models.

Jayakumar, Thomas and Geraldine [16] reported that when assessing CAT, SOD, and GPx isoforms by native page electrophoresis, these isoforms were higher in the treatment group than in the I/R group.

These results are parallel to our study. In our study, antioxidant enzyme activity decreased significantly after ischemia. On the other hand, there are studies showing that antioxidant enzymes are elevated in I/R end. Considering these studies, it is emphasized that increased enzyme activity after I/R is related to oxidative stress.

These isoform forms play an important role in the regular functioning of cell metabolism [22]. However, during oxidative stress, this antioxidant enzyme isoforms are very important to elucidate the differences in the outcome of gene expression studies.

In Group I, kidney tissue samples with control group (HE), cortex-medulla, kidney tubule cells and glomeruli show uniform structure. Renal specimens of Group II with I/R injury were found to have vacuolization, enlargement and bleeding in the glomerulus, and deterioration of kidney tubule cell lines. In Group III, renal histological examinations of 50 mg/kg geraniol, the general appearance was normal, but bleeding was observed in the glomerular space and between the tubules. This situation raised questions about the preservation's intensity.

In Group IV kidney tissue samples given 100 mg/kg geraniol, results showed an enlargement of the glomerular space and the absence of epithelial spillage show a possible protective effect against the visible damage of the bleeding in Group II.

No scientific evidence of the use of geraniol as an agent to counteract the effects of FOR radicals in the prevention of I/R damage has been found. Only recently have researchers focused on essential oils or free radical scavengers as antioxidants.

In a study by Choi et.al [11], antioxidant activities were compared with a standard using 1-diphenyl-2-picrylhydrazyl (DPPH) on 34 citrus essential oils, and radical scavengers. The radical scavenging effect of geraniol labeled in this study against DPPH has been reported.

Tiwari and Kakkar [32] investigated the Wistar albino rats’ antioxidant potential of geraniol using tsignierteiary-butyl hydro peroxide in their study of alveolar macrophages. Geraniol significantly inhibited cell viability and showed a 45% increase in superoxide dismutase activity, a 120% increase in glutathione content, and also mitochondrial membrane potential. At the same time, compared to cells exposed to oxidative stress and geraniol cells, geraniol reduced free NO release and inhibited lipid peroxidation. These results showed that the pharmacological potential of geraniol was a critical control point in pulmonary inflammatory diseases caused by oxidative stress.
Gunes [10] in his study in the year of 2010 with H\textsubscript{2}O\textsubscript{2} in rats produced oxidative stress and geraniol synthesized geraniol xanthate substances examined the effects of trace elements. In this study, geraniol and geraniol xanthate reduced the harmful radical effect of H\textsubscript{2}O\textsubscript{2} and moreover, it played positive effects over (Fe, Ca, Zn and Mn) and played role at the level of trace elements.

There are also studies showing that geraniol has antitumor activity in vivo and in vitro against various cancer cell lines. Monoterpenes have been shown to be effective on anti-cancerogens directly and indirectly in experimental conditions [10].

Various studies have shown that different antioxidants are effective against the oxidative stress that results from I/R injury [33]. Similar studies can be carried out to investigate the effects of other monoterpane-derived substances on oxidative stress. In addition, appropriate pathways for oxidative stress induced by geraniol’s I/R injury can be studied to investigate the effect of reducing free radicals.

CONCLUSIONS

As a result, considering the biochemical and histological analysis data, it can be concluded that giving the intra-peritoneal dose of 100 mg/kg geraniol will have a protective effect on kidney I/R injury.

REFERENCES


© 2018 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY NC) license (https://creativecommons.org/licenses/by-nc/4.0/).