Nephroprotective and antioxidant effect of green tea (Camellia sinensis) against nicotine-induced nephrotoxicity in rats and characterization of its bioactive compounds by HPLC-DAD

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Nephroprotective and antioxidant effect of green tea (*Camellia sinensis*) against nicotine-induced nephrotoxicity in rats and characterization of its bioactive compounds by HPLC-DAD

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Abstract

Nicotine (NT) is a potential inducer of oxidative stress, through which it can damage numerous biological molecules. Natural antioxidants that prevent or slow the progression and severity of nicotine toxicity may have a significant health impact. The purpose of this study, conducted on Wistar rats, was to evaluate the beneficial effects of green tea (Camellia sinensis) extract on nicotine treatment induced damage on kidney.

Our results showed that nicotine significantly ($p<0.01$) increased serum and kidney malondialdehyde (MDA), the serum contents of urea, creatinine and uric acid. In addition, nicotine intoxication significantly ($p<0.01$) decreased the vitamin E and C levels in serum and kidney tissue as well as the activities of SOD, CAT and GPx. Interestingly, animals that were pretreated with green tea, prior to nicotine-administration, showed a significant nephroprotection, revealed by a significant reduction-induced oxidative damage for all tested markers. The nephroprotective activity of green tea is mediated, at least in part, by the antioxidant effect of its constituents.

Keywords: Nicotine, Green tea, kidney function, Antioxidant, oxidative damage
Introduction

Nicotine (3-(1-methyl-2-pyrrolidinyl) pyridine) is a volatile alkaloid extracted from the dried leaves of Nicotiana tabacum and Nicotiana rustica plants. It is well diffused and absorbed throughout the skin, mucosal lining of the respiratory tract, and lungs ((Mosbah et al. 2015; Abu-awwad et al. 2017). Upon absorption, it enters circulation and distributes rapidly to various tissues. The liver metabolizes most of nicotine while the rest is metabolized via the lungs and kidneys. Nicotine had recognized to induce oxidative stress throughout ROS generation, which was capable of initiating and promoting oxidative and inflammatory stresses (Chandrasekharan and Simmons 2004; Das et al. 2016). It has been reported that antioxidants appear to act against disease processes by increasing the levels of endogenous antioxidant enzymes and decreasing lipid peroxidation (Bansal et al. 2005). Lots of studies indicate that natural substances from edible and medicinal plants exhibited strong antioxidant activity that could act against nicotine-induced kidney damage, because they contain lots of free radical scavenger such as phenolic acids and flavonoid compounds (Neogy et al. 2008; Al-Malki and Moselhy 2013; Yarahmadi et al. 2017; Ben Saad et al. 2018). Green tea (Camellia sinensis) has a long history of use world-wide. Researches have investigated the potential use of naturallyoccurring dietary substances for the control and management of various chronic diseases. Since ancient times, green tea has been consumed to promote the improvement of human health. A wide range of well defined phytochemicals, which are absorbed and metabolized by the body, exhibit antinflammatory, anticarcinogenic, antiatherosclerotic and antibacterial properties (Augustyniak et al. 2005). In particular, green tea catechins and their derivatives have been characterized as antioxidants that scavenge free radicals protecting cells in normal and pathological states (Khan et al. 2006). Among all tea polyphenols, epigallocatechin-3-gallate has been shown to be responsible for great part of the
health promoting-ability of green tea. Green tea has been shown to improve kidney function in diabetic rats (Rhee et al. 2002) and has been linked to a reduced risk of cardiovascular diseases, cancer (Mukhtar and Ahmad 2002), obesity and oral health problems (Liao 2001). In addition, a recent study demonstrated a protective role of green tea against cigarette smoke induced oxidative stress (Al-Awaida et al. 2014).

To our knowledge, there are no data about the in vivo effect of green tea extract on male kidney damage and oxidative stress induced by nicotine. The present study was designed to assess in rats the protective effects of green tea (Camellia sinensis) upon nicotine induced stress in blood and kidney and the related damages. Examined parameters were urea, creatinine and uric acid in blood serum, and lipid peroxidation as well as on antioxidant enzymatic and non-enzymatic activities and, subsequently, the ability of green tea extract to improve and protect kidney function.

**Material and methods**

**Plant material**

The green tea used in this study (Lipton/Kangra-brand) was purchased from a commercial source (Jain Pan House, New Delhi, India). The green tea extract was prepared by adding 1.25 g of green tea leaves to 25 mL of boiling water (15 min). The infusion was cooled to room temperature and then filtered. The tea leaves were extracted a second time with 25 mL of boiling water and filtered; the two filtrates were then combined to obtain an aqueous 2.5% green tea extract (2.5 g of tea leaves/100 mL water) (Ben Saad et al. 2017).

**2.3. Photochemical study**

**2.3.1. Total Phenols Content**

The amount of total phenols content in green tea was determined by Folin-Ciocalteu’s reagent method (Singleton et al. 1999). 1 ml of green tea was mixed with 5 ml of Folin reagent
(10%) and the mixture was incubated at room temperature for 5 min. Then 2 mL saturated sodium carbonate (Na$_2$CO$_3$) solution was added and further incubated for 90 min at room temperature. The absorbance was then measured at 765 nm. Gallic acid was used as a positive control. All tests were carried out in triplicate and the results were expressed as µg of gallic acid equivalent (µg GA)/mL GT.

**Determination of flavonoids contents**

To determine the total flavonoids content, 1ml of green tea samples were mixed with an equal volume of 2% aluminum chloride solution (AlCl$_3$). After incubation for 30 min at room temperature, the absorbance of the reaction mixture was measured at 430 nm. The total flavonoids content was expressed as µg of rutin equivalent (µg RE)/mL GT. All tests were carried out in triplicate (Djeridane et al. 2006).

**Determination of tannins content**

Quantitative estimation of tannins was carried out using the modified vanillin–HCl in methanol method described by Price and Butler (1977). The method is based on the ability of condensed tannins to react with vanillin in the presence of mineral acid to produce a red color. GT samples (1 mL) were mixed with 20 ml of 1% HCl (in methanol) for 20 min at 30 °C in a water-bath. The samples were centrifuged at 2000 rpm for 4 min. The supernatant (1 mL) was reacted with 5 ml vanillin solution (0.5% vanillin12% HCl in methanol) for 20 min at 30 °C. Blanks were run with 4% HCl in methanol in place of vanillin reagent. Absorbance was read at 500 nm on a UV spectrophotometer. A standard curve was prepared with catechin. Results were expressed as µg of catechin equivalent (µg CE)/mL GT. Samples were analyzed in triplicate.

**Ascorbic Acid Content**

The ascorbic acid content was assayed as described by Omaye et al. (1979). An amount of 1 mL of GT was mixed with 5 ml of 10% TCA, the extract was centrifuged at 3500 rpm for 20
min. The pellet was re-extracted twice with 10% TCA and supernatant was increased 10 mL and used for estimation. To 0.5 ml of the extract, 1 mL of DTC reagent (2,4-dinitrophenyl hydrazine-Thiourea-CuSO4 reagent) was added and mixed thoroughly. The tubes were incubated at 37 °C for 3 h and to this a solution of 0.75 ml of ice cold 65% H₂SO₄ was added. The tubes were then allowed to stand at 30 °C for 30 min. The resulting color was read at 520 nm. The ascorbic acid content was determined using a standard curve prepared with ascorbic acid and the results were expressed as µg of ascorbic acid equivalent (µg AA)/mL GT. Samples were analyzed in triplicate.

**High-performance liquid chromatography (HPLC) analysis conditions**

A Varian Prostar HPLC system equipped with a ProStar 230 ternary pump, a manual injector and a ProStar 330 diode array detector were used. The HPLC separation was carried out on C-18 reverse phase HPLC column (Varian, 150 mm × 4.6 mm, particle size 5mm) on an elution gradient at 25 C. Two phases system were used as follows: mobile phase A, pure methanol, and mobile phase B was acetic acid aqueous solution (0.05%). Gradient conditions were: first 35% A and 65% B, followed by 30 min 50% A and 50% B, followed by 40 min 90% A and 10% B. The flow rate was maintained at 1 mL/min and 20ml of sample was injected. The detected compounds were recognized by comparing their retention times with those of commercially standards analyzed under the same conditions.

**Antioxidant activity**

The Hydrogen peroxide (H₂O₂) scavenging activity of green tea GT was determined according to the method reported by Liu et al. (2010). Vitamin C was used as positive control. ABTS⁺ assays were evaluated according to the method cited by Afoulous et al. (2013). The method described by Koleva et al. (2007) was used with a slight modification.

**Animals housing conditions**
This study was carried out on 32 Wistar male rats (3-4 months old), about 140-150 g body weight, purchased from the Central Pharmacy in Tunisia (SIPHAT, Tunisia). The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature and humidity controlled room. The rats were fed with a commercial balanced diet (SICO, Sfax, Tunisia) and drinking water was offered *ad libitum*. The handling of the animals was approved by the Medical Ethics Committee for the Care and Use of Laboratory Animals of the Pasteur Institute of Tunis (approval number: FST/LNFP/Pro 152012) and carried out according to the European convention for the protection of living animals used in scientific investigations (Council of European Communities 1986).

**2.7. Experimental design**

One week after acclimatization to laboratory conditions, the rats were randomly divided into four groups of eight animals each. The four groups were treated as follows:

Group 1 (C): Control rats received distilled water (0.5 mL/100 g b.w; i.p).

Group 2 (NT): injected intraperitoneally (i.p.) with nicotine (NT in aqueous solution; 1 mg/kg body weight/day) for 30 days.

Group 3 (GT): received green tea extract (GT) by intragastric gavage at a dose of 1 mL/100 g b.w for 30 days and followed by distilled water (0.5 mL/100 g b.w; i.p) during the last 30 days of GT treatment.

Group 3 (NT+GT): received green tea extract (GT) at a dose of 1 mL/100 g b.w and then injected NT (1 mg/kg body weight/day i.p.) during the last 30 days of GT treatment.

**Preparation of samples**

At the end of experimental period animals were sacrificed by cervical decapitation under light ether anesthesia. The serum was collected by centrifugation of the whole blood at 1500× g for 15 min at 4 °C and stored at –80 °C until analysis. Kidney samples were quickly removed, washed in ice-cold 1.15% KCl solution, homogenized into 2 ml ice-cold lyses buffer (pH 7.4)
and centrifuged for 30 min at 5000× g, 4 °C. The collected supernatants were stored at –80 °C until the analysis.

**Biochemical assays**

The level of creatinine, uric acid and urea in serum were determined by kit methods (Spinreact, Girona, Spain). Serum and kidney homogenate the level of lipid peroxidation was measured as malondialdehyde content (MDA) according to the method of Ohkawa et al. (1979). SOD activity was estimated according to the method described by Misra and Fridovich (1972). CAT activity was determined by measuring hydrogen peroxide decomposition at 240 nm according to the method described by Aebi (1984). GPx activity was assayed using the method described by Flohe and Gunzler (1984). In serum and kidney homogenate the levels of ascorbic acid (vitamin C) concentration was measured according to the method of Omaye et al. (1979) more so vitamin E level was determined based on the method of Desai (1984).

**Protein determination**

Serum, urinary and kidney homogenate protein contents were determined according to Lowry’s method using bovine serum albumin as standard (1951).

**Kidney histopathological studies**

Kidney tissues were cut into about 5-cm-thick slices and fixed with 10% phosphate-buffered formalin (pH 7.4). The tissue slices were dehydrated through ascending grades of alcohol, and embedded in paraffin. Tissue sections of 5–8 μm were made using microtome, and stained with hematoxylin-eosin solutions (H&E). Tissue preparations were observed and microphotographed under a light BH2 Olympus microscope.
Statistical analysis

All data were presented as means ± standard deviation (SD). Determination of all parameters was performed from six animals per group. Significant differences between treatment effects were determined using one-way ANOVA, followed by Tukey's HSD Post-Hoc tests for multiple comparisons with statistical significance of P<0.05.

Results

Total phenols, flavonoids, tannins, and ascorbic acid content

The phytochemical study of GT revealed the presence of various quantities of total phenolics, flavonoids, tannins, and ascorbic acid contents. Ours results showed that 1 mL of GT was equivalent to 730.90±4.52 µg of gallic acid, 289.30±0.23 µg of rutin, 100.03±1.02 µg of catechin, and 123±1.2 µg of ascorbic acid (Table 1).

HPLC analysis of phenolic compounds

The HPLC-DAD analysis of the extract of green tea revealed the presence of phenolic acids and flavonoids. The compounds that were identified in our extract were 6 phenolic acids: catechic, caffeic, epicatechic, vanillic, protocatechuic and coummarin with retention times of 15.10 min, 17.2 min, 21.50 min, 28.62 min, 34 min and 35.81 min, respectively. The HPLC elution profile of phenolic acids (Fig. 1 A) also showed 2 peaks of unknown compounds. The HPLC elution profile of flavonoids (Fig. 1B) showed a total of 9 compounds among which 3 flavonoids were identified: rutin, quercetin, and kaempferol. The absorbance was measured at 360 nm.

Antioxidant activity of green tea extract

Antioxidant activity determined using the (H$_2$O$_2$) scavenging assay showed results GT exhibited dose-dependent (H$_2$O$_2$) scavenging ability (Fig. 2A). The effective concentrations at
which H$_2$O$_2$ were scavenged by 50% (IC$_{50}$) was found to be 150±2.8 µg/mL but significantly lower than that of vitamin C (40±1.42 µg/mL). ABTS assay is shown in Fig 2.B. GT (300 µg/mL) exhibited the highest inhibition of 89 %. As can be clearly seen in Fig. 2. C. GT extract was efficient to inhibit the oxidation of linoleic acid. The antioxidant activity of GT extract increased with increasing extract concentration. The IC$_{50}$ value of GT (1±0.07 mg/mL) appeared significantly ($p<0.05$) lower than that of BHA (IC$_{50}$= 0.3±0.09 mg/mL) used as a positive control.

**Body weight**

Table 2 shows the effects of nicotine and green tea on the body and organ weights in different experimental groups. Nicotine alone treated rats had a significantly ($p<0.01$) reduction in body weight gain and decrease in relative kidney weights. Nevertheless, administration with green tea extract followed by nicotine treatment ameliorated the body weight near to normalcy.

**Serum markers of kidney damage**

Nicotine treatment induced severe kidney damage evidenced in serum by a significant increase of creatinine and urea and uric acid levels (Table 3). When nicotine-treated rats were also treated with green tea, all these biomarkers reverted to almost normal values.

**Oxidative damages**

Lipid peroxidation of biomembranes is one of the principal degenerative effects of free radicals. As shown in Table 4, the MDA levels was significantly increased in the nicotine-treated animals compared to the normal. Green tea significantly caused diminution of nicotine-elevated MDA level compared to those of nicotine group.
Antioxidant activities

Table 4 showed that nicotine administration was found to cause a significant decrease ($p < 0.01$) in the levels of serum and kidney vitamin E and vitamin C when compared with the control group. However GT supplement significantly ($p < 0.01$) increased vitamin E and vitamin C levels when compared with nicotine group. The extract alone did not shown significant effect on Vitamin E and vitamin C. The activities of SOD, CAT and GPx of the serum and kidney tissue are shown in Table 4. Injection of nicotine led to a lower SOD, CAT and GPx activities compared to the normal control group. However, animals treated with GT showed significant increase ($p < 0.01$) in the antioxidant enzyme activities as compared to nicotine group.

Histopathological findings

The histopathological findings on kidney for various treatment groups are presented in Figure 3. In light microscopic examinations, histopathological changes were observed in kidney of nicotine and green tea extract-nicotine-treated groups compared to control rats. The kidney in the control animals showed normal histological structure (Fig. 3A). With respect to the renal histoarchitecture of the nicotine-treated animals, showed higher degree of tubular degeneration and hypertrophy of glomeruli cells showing reduction of Bowman’s space (Fig. 3B). The sections of the animals belonging to the green tea extract alone and their combinations with nicotine treatment groups showed a normal histological appearance (Fig. 3C and D).
Discussion

Plants rich in secondary metabolites, including phenolic compounds, have antioxidant activity due to their redox properties and chemical structures. Result from this study showed that green tea extract had a high total phenolics (730.90±4.52 µg Gallic acid equivalent/mL GT), flavonoids (289.30±0.23 µg Rutin equivalent/mL GT), tannins (100.03±1.02 µg Catechin equivalent/ml GT), and ascorbic acid contents (123±1.2 µg Ascorbic acid equivalent/mL GT). Our results are in agreement with previously reported (Mada et al. 2017). The overload free radicals are continuously produced and accumulated in body which cause the oxidation of biomolecules (e.g. protein, lipid, and DNA) which leads to cell injury and apoptosis. Our results exhibited a significant radical-scavenging activity all tested concentrations. The effective concentrations at which H$_2$O$_2$ were scavenged by 50% (IC$_{50}$) was found to be 150±2.8 µg/ml. As can be clearly seen in Fig. 2. C. Same results were observed for β-carotene activity that increased with the increase of GT concentrations. GT extract was efficient to inhibit the oxidation of linoleic acid. The IC$_{50}$ value of GT (1±0.07 mg/mL) appeared significantly ($p< 0.05$) lower than that of BHA (IC$_{50}$= 0.3±0.09 mg/ mL) used as a positive control. Also, Green tea extract (300 µg/mL) exhibited the highest inhibition of ABTS. Ours results are in concordance with several studies that demonstrated the antioxidant action of green tea extract (Erkekoglou et al. 2017; Megow et al. 2017). In the present study, changes in rats body weight along with relative organ weights provided an imperative indication of nicotine-induced toxicity. Nicotine-exposure for 30 consecutive days at 1 mg/kg caused a decrease in rats body weight which might either be due to the direct cytotoxic effects (Ibrahim et al. 2016; Mosbah et al. 2015). Moreover, the decrease in the relative kidney weights following nicotine-treatment as observed in our study could be attributed to the relationship between the kidney weight increase and toxicological effects such as the reduced body weight
gain in the experimental animals (Ibrahim et al. 2016; Perkins et al. 1991). However, Supplementation of green tea to-nicotine treated animals ameliorated the body and kidney weights, which could be attributed either to the phytochemical content in green tea extract. Renal failure has been reported with nicotine intoxication. Significant increase in the level of urea, creatinine and uric acid in treated animals is indicative of kidney damage and a possible malfunction or failure of the kidneys. In rats pretreated with green tea extract at a dose of 1 mL/100 g b.w, showed a significant decrease in serum urea, creatinine and uric acid levels. This suggested an amelioration in the kidney status following nicotine treatment as compared to nicotine treated rats. These results lend credence to the use of green tea extract as a nephroprotective agent (Anwar Ibrahim et al. 2015). Our results corroborated with Xie et al. (2017) which denoted that green tea phenolic compounds had a nephroprotective effect against high-fat diet. It was obvious from our results that treatment of nicotine significantly ($p < 0.01$) increased the MDA concentration in the serum and kidney tissue and attenuates the levels of vitamins E and C. Studies have shown that nicotine may result in increased oxidative stress by the ROS superoxide overproduction which can later initiate free radicals chain reactions of lipid peroxidation (Mosbah et al. 2015; Maurya and Salvi 2005). As ROS scavengers, vitamins E and C has been shown to be effective against superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen (Al-Malki and Moselhy 2013). The observed decrease in the levels of serum and kidney vitamin E and C in nicotine rats could be the results of increased uses of these antioxidants in scavenging the ethanol-overproduced free radicals. Treatment with green tea extract significantly reversed these changes. Hence it may be possible that the mechanism of nephroprotection of extract is due to its antioxidant effect. This result is in agreement with the study of Zhang and Tsao (2016) who unambiguously shows that polyphenols, flavonoids, and catechins display significant antioxidant properties and scavenging activities on free radicals involved in vanadium toxicity. As antioxidants
(enzymatic and non-enzymatic) provide protection against free radical attacks, we investigated levels of SOD, CAT, and GPx enzymatic activities in the serum and kidney. SOD, CAT, and GPx are major enzymes protecting cells from oxidative damages. The increased formation of reactive oxygen species due to nicotine exposure would require some fully active protection mechanisms against oxidative damage (Mosbah et al. 2015; Zahran et al. 2017). It is thus likely that the decreased activity of these enzymes in kidney further exacerbate the oxidative damage. These effects are reversed by the treatment with green tea extract. However, SOD, CAT and GPx activity was significantly elevated by administration of green tea extract to nicotine-treated rats. The decrease in the activity of antioxidant enzymes suggested that the balance between the oxidant and pro-oxidant was disturbed by nicotine, and treatment of experimental rats with GT effectively reverts this imbalance and restores the level of antioxidant enzymes. The protective effect of green tea can be attributed to the presence of phenolic acids and flavonoids as revealed by the HPLC analysis. In fact, phenolic acids and flavonoids were able to release a hydrogen proton from their hydroxyl group, trap free radicals and prevent nicotine induced kidney damage. Based on the HPLC analysis of green tea extract, phenolic acids (catechic, caffeic, epicatechic, vanillic, protocatechuic and coumaric) and flavonoids (rutin, quercetin and kaempferol) were identified by Prasad et al. 2016 confirmed that green tea extract was capable of normalizing the oxidative stress in kidney.

The histopathological changes in kidney showed that treatment of rats to nicotine resulted in degenerative changes in the kidney, including tubular degeneration and hypertrophy of glomeruli cells showing reduction of Bowman’s space. The biomarkers of kidney dysfunction corroborated with histopathological lesions observed in the current study (Zahran et al. 2017). These observations indicated marked changes in the overall histoarchitecture of kidney in response to nicotine, which could be due to its toxic effects primarily by the generation of
reactive oxygen species causing damage to the various membrane components of the cell. Administration of green tea extract improved the structure of renal cells, which could be attributed to the antioxidant properties of GT in vivo (Li et al. 2017; Pereira et al. 2017).

**Conclusion**

The finding of this study showed that the administration of the green tea extract appeared to protect the kidneys due to presence of high antioxidant phytochemicals and properties of male rats from nicotine-induced oxidative stress by reducing the intensity of LPO and by enhancing the antioxidants activities. In future, work will be done to isolate bioactive constituents of green tea extract to locate potential pharmacological agents.

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**Competing interests**

The authors declare that they have no competing interests.
References


Erkekoglou, I., Nenadis, N., Samara, E., and Mantzouridou, F.T. 2017. Functional Teas from the Leaves of Arbutus unedo: Phenolic Content, Antioxidant Activity, and Detection of


Table 1: Phytochemical compounds in green tea extract

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<th>Composition</th>
<th>Contents</th>
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<tr>
<td>Total phenols (µg Gallic acid equivalent/ml GT)</td>
<td>730.90±4.52</td>
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<tr>
<td>Flavonoids (µg Rutin equivalent/ml GT)</td>
<td>289.30±0.23</td>
</tr>
<tr>
<td>Tannins (µg Catechin equivalent/ml GT)</td>
<td>100.03±1.02</td>
</tr>
<tr>
<td>Ascorbic acid (µg Ascorbic acid equivalent/ml GT)</td>
<td>123±1.2</td>
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Values are expressed as mean ±SD of triplicate measurement.

Table 2: The effects of green tea and nicotine on Body weight and relative organ weights.

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<th>GT</th>
<th>NT+GT</th>
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<tr>
<td>Initial body weight (g)</td>
<td>145.02±12.3</td>
<td>146±17.30</td>
<td>148.20±8.18</td>
<td>146.30±15.3</td>
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<tr>
<td>Final body weight (g)</td>
<td>246.2±10.3</td>
<td>163.3±6.35**</td>
<td>248.40±20.3++</td>
<td>246.89±10.3++</td>
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<tr>
<td>Body weight gain(%)</td>
<td>41.09</td>
<td>10.59</td>
<td>40.33</td>
<td>40.74</td>
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<tr>
<td>Relative kidney weight (g/ 100g)</td>
<td>0.67±0.9</td>
<td>0.34±0.36**</td>
<td>0.65±0.60++</td>
<td>0.56±0.63++</td>
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Values were expressed as mean ± SD of 8 rats in group.

**p < 0.01 compared with control (C).

++p < 0.01 compared with nicotine-treated group (NT).
Table 3: Effects of nicotine, green tea on urea (mmol/l), creatinine (μmol/l) and uric acid (μmol/l) levels in serum.

<table>
<thead>
<tr>
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<th>C</th>
<th>NT</th>
<th>GT</th>
<th>NT+GT</th>
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<tr>
<td>Urea</td>
<td>8.32±0.63</td>
<td>18.23±0.53*</td>
<td>9.36±0.23++</td>
<td>14.02±0.21+</td>
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<tr>
<td>Creatinine</td>
<td>21.4±1.25</td>
<td>32.59±1.6*</td>
<td>19.6±0.90++</td>
<td>24.31±1.06+</td>
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<tr>
<td>Uric acid</td>
<td>132±5.23</td>
<td>99.3±5.3**</td>
<td>129.30±6.3++</td>
<td>110±2.3++</td>
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</table>

Values were expressed as mean ± SD of 8 rats in group.

*p < 0.05, **p < 0.01 compared with control (C).

+p < 0.05, ++p < 0.01, compared with nicotine-treated group (NT).
Table 4: Changes in the level of MDA, on vitamin E, vitamin C, and antioxidant enzymes activities (SOD, CAT, and GPx) in the serum and kidney of control and rats treated with nicotine (NT), GT, or their combination (NT+GT). \(^1\)nmoles/mg protein, \(^2\)mg/dl, \(^3\)μg/g tissue, \(^4\)U/mg protein, \(^5\)μmol/min/mg protein and \(^6\)μmol GSH oxidized/min/mg protein.

<table>
<thead>
<tr>
<th>Groups</th>
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<th>GT</th>
<th>NT+GT</th>
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<tr>
<td>MDA(^1)</td>
<td>Serum</td>
<td>3.60±0.3</td>
<td>8.03±0.5**</td>
<td>2.6±0.12++</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>10.2±0.24</td>
<td>18.03±0.26**</td>
<td>11.02±0.5++</td>
</tr>
<tr>
<td>Vitamin E(^2)</td>
<td>Serum</td>
<td>2.5±0.01</td>
<td>0.77±0.02**</td>
<td>2.56±0.12++</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>26±0.06</td>
<td>12.03±0.2**</td>
<td>26.03±0.13++</td>
</tr>
<tr>
<td>Vitamin C(^3)</td>
<td>Serum</td>
<td>6.02±0.36</td>
<td>1.03±0.21**</td>
<td>6.23±0.23++</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>120.23±1.03</td>
<td>85.03±0.06**</td>
<td>123.2±0.04++</td>
</tr>
<tr>
<td>SOD(^4)</td>
<td>Serum</td>
<td>7.02±0.3</td>
<td>3.02±0.1**</td>
<td>7.92±0.23++</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>10.03±0.54</td>
<td>4.21±0.08**</td>
<td>10.9±0.01++</td>
</tr>
<tr>
<td>CAT(^5)</td>
<td>Serum</td>
<td>22.03±0.21</td>
<td>18.03±0.23*</td>
<td>23.03±0.1+</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>40.23±1.02</td>
<td>20.03±0.9**</td>
<td>22.03±0.12++</td>
</tr>
<tr>
<td>GPx(^6)</td>
<td>Serum</td>
<td>3.42±0.05</td>
<td>1.23±0.23**</td>
<td>3.05±0.12++</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>5.03±0.23</td>
<td>2.03±0.13**</td>
<td>6.03±1.9++</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD of 8 rats in group.

\(*p < 0.05, \ **p < 0.01\) compared with control (C).

\(+p < 0.05, \ ++p < 0.01, \) compared with nicotine-treated group (NT).
Figure captions

**Figure 1**: A. HPLC profile of phenolic acids ($\lambda = 280$ nm) from green tea extract. Peaks (2) catechic acid, (3) caffeic acid, (4) epicatechic acid, (6) vanillic acid, (7) protocatechuic acid, (8) coumaric acid and 2 unknown compounds. B. HPLC profile of flavonoids ($\lambda = 360$ nm) from green tea extract. Peaks (4) rutin, (5) quercetin, (6) kaempferol and 6 unknown compounds.

**Figure 2**: A. Hydrogen peroxide scavenging activity and positive control vitamin C at different concentrations. B. ABTS$^{•+}$ Scavenging activity (%) of green tea extract. C. Antioxidant activities (%) of GT and BHA as positive control, measured by β-carotene bleaching assay. Data are expressed as mean ± standard deviation of the mean (n=3).

**Figure 3**: Representative photographs from the kidney showing the protective effect of green tea extract on nicotine induced renal injury in rats. (A) Controls, (B) rats treated with nicotine, (C) rats treated with green tea extract, and (D) rats treated with the combination of green tea extract and nicotine. Kidney sections were stained using the hematoxylin–eosin method. Original magnifications:×400; * proximal and tubular necrosis; + tubular degeneration; hypertrophy of many glomeruli cells; ⭐ congestion.