Evaluation of the effectiveness of *Camellia sinensis* on fat peroxidation and Ox-LDL in rats.

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Abstract

It has been believed that Green tea (GT) drinking has an antioxidant property and have beneficial effects in the treatment of some disease. However, few findings were found on the impact of GT on oxidative stress. In this study, the protective effect of green tea against oxidative stress caused by hydrogen peroxide in rats was evaluated. For this purpose, research groups in four weeks include, the control (con) and five groups supplemented (G1) with 10g GT, (G2) with 20g GT, (P) with 1% H₂O₂, (GP1) with 1% H₂O₂ with 10g GT and (GP2) with 1% H₂O₂ with 20g GT. The results of the administration of GT in H₂O₂ rats on serum biochemical parameters such as lipid profile, Malondialdehyde (MDA) and oxidized low-density lipoprotein (OX-LDL) were estimated. The findings obtained from this research revealed that the usage of GT lowered the level of cholesterol (CHO), triglyceride (TG), low-density lipoprotein (LDL), coronary risk index (CRI), MDA Ox-LDL concentrations; it also demonstrated Increases in high-density lipoprotein and very-low-density lipoprotein (VLDL) compared to rats supplied with H₂O₂ (P) group, the baseline lipid profiles and tea consumptions with or without H₂O₂ were equivalent across the con and GT treated groups. The usage of GT found to be advantageous in lowering OX-LDL and lipid peroxidation in rats. These findings validate the traditional use of GT to protect against lipid peroxidation and atherosclerosis.

**Keyword:** *Camellia sinensis*, Green tea, Lipid peroxidation, Ox-LDL, Rat
1. Introduction

Diet and beverages are essential in the body's protection mechanism against oxidative damage, which is linked to various diseases. Tea is the wide beverage consumed by humans globally and has also received considerable attention for its potential protective effect on improvement of coronary artery disease and development of vascular resistance as well as inhibitory effect on tumor progression and cardiovascular disease also diabetes and hypertension (1, 11, 12). All type of tea (green, oolong and black) is the dried leaves of the *Camellia sinensis* vine, which belongs to the *Theaceae sp.* genus (2, 3). GT, unlike black and oolong tea, is not fermented, so the active constituents in the plant stay untouched (4, 5). The main GT components are polyphenols, particularly catechins (6). *Camellia sinensis* polyphenols have been shown to minimize plaque formation and lipid oxidation, suggesting an anti-atherosclerotic effect (7). Many studies have shown the effect of GT compounds on the removal of reactive oxygen species (ROS) as well as the inhibition of free radicals (8-10). It is now possible to calculate the amount of oxidized LDL (Ox-LDL) in circulation with several methods for measuring circulating Ox-LDL have been established (13, 14). The role of Ox-LDL in circulation as a valuable predictor for atherosclerosis and coronary heart disease is considered (15, 16). Given that GT has a large number of antioxidants (17), as yet few studies have been done on the impact of antioxidants on circulating Ox-LDL levels (18). As we all realize, antioxidants help minimize oxidative stress and work with free radicals to prevent the progression of oxidation reactions (17, 19). Drinks that are rich in antioxidants can assist in the prevention of chronic diseases. GT, a common antioxidant beverage among Orientals, is one of the most well-known antioxidant beverages (20).

Given the limited research on effect of increased antioxidants due to the amount of GT consumed, in this study, the analysis to clarify the effect of antioxidants on the circulation GT on Ox-LDL did the best. Also investigated GT intake's effect on circulating biochemical parameters lipid profile, oxidative stress marker and Ox-LDL.

2. Materials and Methods

2.1. Animals

Thirty-six male Sprague Dawley albino rats weighed 250-300 g were randomly divided into six groups. The rats were housed in a polypropylene cage and held under normal controlled conditions (temperature 25±2°C; natural light-dark cycle) in Kirkuk university- veterinary
faculty. The rats were supplied with food and water ad libitum. The industrial rat diet after seven day, the rats clustered as follows:

Group1: supplied for one month with regular distilled water (control, n=6).
Group2: given for one month with 10g GT prepared solution / 1L of distal water (G1, n=6).
Group3: given for one month with 20g GT prepared solution / 1L of distal water (G2, n=6).
Group 4: given for one month with 1% H$_2$O$_2$ (P, n=6).
Group5: given for one month with 1% percent H$_2$O$_2$with GT10g / 1L of distal water (GP1, n=6).
Group6: given for one month with 1% percent H$_2$O$_2$with GT20g / 1L of distal water (GP2, n=6).

2.2. Sample investigations
GT was sold at a local farmer's market in the Turkish governorate of Konya. GT solutions (10-20 g) were boiled in one liter of distill water to extract different concentrations. After cooling, the solutions were filtered via filter paper then provided to rats as their only drinking water source(21, 22). Hydrogen peroxide was obtained from Sigma Company for chemicals. (St. Louis, MO, USA).

2.3. Collections of blood examples
After the experimental duration of 30 days, under ether anesthesia, the blood sample was taken from the cardiac using 5 cc syringes by observing the principles of research ethics then collected in the Non-coagulant tube for biochemical examination. The samples were then centrifuged at centrifuged for 10 minutes 3000rpm, and the serum was extracted, numerated then held freezing at -20°C for estimation of the biochemical parameters.

2.4. Biochemical parameters
Cholesterol (CHO), triglyceride TR, high and low-density lipoproteins (HDL) (LDL) were measured using reagent kits manufacturer's instructions which were purchased from BIOLABO (SA, France), diagnostic kits. The concentration of very low density-lipoprotein (VLDL) was calculated as TG/5; also the coronary risk index (CRI) was calculated (23, 24). Oxidized LDL (Ox-LDL) was measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA, Mercodia, Uppsala, Sweden) and Malondialdehyde (MDA) was determined according to thiobarbituric acid-reactivity (25).
2.6. Statistical analysis

All results are expressed as means ± SD (standard deviation) of the number of experiments. One-way analysis of variance (ANOVA) with SPSS version 18.0 (SPSS, Cary, NC, USA) was used to improve statistical validity, and Duncan's multiple range test (DMRT) was used to achieve individual comparisons (26). A significant difference among groups was described as a value of (p≤0.05).

3. Results

Based on the results listed in Table one, the effects of GT extracts on serum lipid profiles and serum concentrations of CHO, TR, HDL, LDL, VLDL, and CRI in rats that drink GT for four weeks versus the control rat, there was no significant difference (p≤ 0.05) in CHO, TR, HDL, LDL, VLDL, CRI between the control and GT groups G1 and G2. However, the peroxide treated group reported a significant increase (p ≤ 0.05) in CHO, TR, LDL, VLDL, CRI compared to the control groups, although HDL decreased significantly in the P group (p ≤ 0.05) compared to the control, G1, G2, GP2, while there is no significant difference between GP1 and GP2 compared to the other groups. Also, GP1 and GP2 show a significant decrease (p ≤ 0.05) in CHO, TR, LDL, VLDL, CRI compared to the peroxide treated group.

Table 1. Effects of GT extracts on serum lipids profile in treatment groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Con</th>
<th>G1</th>
<th>G2</th>
<th>P</th>
<th>GP1</th>
<th>GP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>82.5±5.2a</td>
<td>80.0±7.4a</td>
<td>75.8±3.9a</td>
<td>173.3±11.6b</td>
<td>116.3±12.5c</td>
<td>99.8±10.3c</td>
</tr>
<tr>
<td>TR</td>
<td>71.3±8.5a</td>
<td>76.0±7.4a</td>
<td>70.5±2.6a</td>
<td>123.3±10.4b</td>
<td>98.5±9.8c</td>
<td>94.3±5.9c</td>
</tr>
<tr>
<td>HDL</td>
<td>47.3±6.1a</td>
<td>52.8±9.1a</td>
<td>60.0±9.5a</td>
<td>32.5±6.3b</td>
<td>44.5±6.7a,b</td>
<td>50.8±6.5a</td>
</tr>
<tr>
<td>LDL</td>
<td>49.5±10.7a</td>
<td>42.5±7.3a,c</td>
<td>29.9±8.9a</td>
<td>162.2±13.9b</td>
<td>91.45±16.7d</td>
<td>67.9±14.7c,d</td>
</tr>
<tr>
<td>VLDL</td>
<td>14.3±1.7a</td>
<td>15.2±1.5a</td>
<td>14.1±0.5a</td>
<td>24.7±2.1b</td>
<td>19.7±1.9c</td>
<td>18.9±1.2c</td>
</tr>
<tr>
<td>CRI</td>
<td>1.1±0.3a</td>
<td>0.8±0.25a</td>
<td>0.5±0.2a</td>
<td>4.6±0.8b</td>
<td>2.1±0.7c</td>
<td>1.4±0.4a*c</td>
</tr>
</tbody>
</table>

*The different letters in the same column indicate that there were a significant differences value (P≤ 0.05).
Figure 1 shows the effects of GT extracts on serum ox-LDL; in comparison between the groups, it was found that only peroxide treated group had a highly significant difference with the G1, G2, and GP2 groups and no statistically significant difference was observed in the other treatment groups (p ≤ 0.05).

Figure 2. The level of MDA in the serum of treatment groups.

According to the results shown in figure 2, a significant increase in serum MDE was shown in GT-treated rat in peroxide, GP1 and GP2 groups (p ≤ 0.05), but there is no significant
difference (p \leq 0.05) between GP2 and Gp1, and the GP2 group did not have a statistically significant difference with the control group (p \leq 0.05).

### Table 2. Effects of GT extracts on serum MDA and Ox-LDL in treatment groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Con</th>
<th>G1</th>
<th>G2</th>
<th>P</th>
<th>GP1</th>
<th>GP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA mmol/L</td>
<td>0.4±0.03a</td>
<td>0.3±0.01a</td>
<td>0.3±0.6a</td>
<td>0.8±0.07b</td>
<td>0.5±0.1c</td>
<td>0.43±0.07a,c</td>
</tr>
<tr>
<td>OXLDL ng/ml</td>
<td>34.1±1.3a</td>
<td>30.9±6.1a</td>
<td>28.9±9.0a</td>
<td>54.9±7.4bc</td>
<td>38.8±10.8ac</td>
<td>37.4±3.6a</td>
</tr>
</tbody>
</table>

The different letters in the same column indicate that there were a significant differences value (P \leq 0.05)

### 4. Discussion

Oxidative stress caused by H2O2 is associated with potent ROS and nitrogen-oxygen species in laboratory animals (27). The copper and iron will catalyze via the Fenton reaction in cells, is responsible for most of the harmful effects of the drug on tissues, such as lipid peroxidation (28). Whenever the amount of free radicals exceeds the cellular ability to eliminate them, it leads to oxidative stress, which causes severe cell toxicity; exposure to excessive oxidative stress causes DNA, enzymes, and proteins damage, particularly lipid peroxidation enzymes (29, 30). As a result of this degeneration, dead cells, resulting in various pathological conditions (28). Medicinal plants are an important part of the pharmaceutical industry that is extracted directly and indirectly from herbs. (31).

Tea is the common consuming beverage after water worldwide. In this study, it was found that GT has an antioxidant effect on oxidative stress in rats (32, 33). The findings of this research show that GT intake reduced MDA concentration; in addition, the results suggest that the treatment of GT reduces the lipid peroxidation and oxidative stress found in experimental rats. The level of lipid peroxidation is generally assessed by measuring MDA. These findings are compatible with the theory that antioxidants can reduce markers of oxidative harm (34). Lipid peroxides are the product of many lipid peroxidation reactions, which are caused by biological processes and have harmful effects on cell membranes and DNA. (35). Several potential
Pathways of behavior have been suggested for the antioxidative activity of GT. It is likely that GT inhibits iron-induced lipid peroxidation by chelating iron. The identification of Ox-LDL of oxidative phase rise in net negative charge and increase in buoyant mass. The concentration of MDA in circulation is used as a measure of oxidized LDL.(35).

The efficiency and performance of the circulating Ox-LDL are still uncertain. LDL is the main lipoprotein carrier in the blood. Three-quarters of overall total CHO is worked out in LDL. Circulatory LDL is vulnerable to oxidation. Researchers believe that low-density oxidized lipoproteins play a very important role in the initial vascular endothelial attack before lesion formation. Pathophysiological findings have shown that Ox-LDL is involved in atherosclerotic lesions(36). It is also conceivable that LDL-C is transformed to Ox-LDL by free radicals or many other compounds in the blood. Ox-LDL exerts several results that may be atherogenic, including chemotaxis of monocytes, up-regulation of endothelial adhesion molecules, activation of growth factor, chemokine expression, and proliferative influence on smooth muscle cells and monocytes (37, 38).

The current study reports on the inhibitory effects of GT on circulating Ox-LDL, which agrees with Inami, Takano (39), who found that using Japanese GT (500 mg per day) resulted in an 11.7 percent reduction in Ox-LDL concentration in plasma.

GT produce excellently metal-chelating structures, researches have shown that GT possesses antioxidant activities and effectively inhibits low-density lipoprotein (LDL) oxidation and lipid peroxidation in vitro. GT polyphenols are not easily absorbed, with small proportions of orally administered GT emerging in the blood in rats; leading to very poor absorption and greater abundance of GT in the intestinal lumen, it is likely that the lipid-lowering effect of GT is mediated largely control on the intestinal processes involved in digestion and absorption of lipids According to available data, GT and interacts with lipid luminal emulsification, hydrolysis, and micellar solubilization(40).GT can potentially affect the absorption and intracellular metabolism of lipids, as well as the assembly and release of chylomicrons. GT polyphenols are quickly absorbed into LDL particles and play a function in reducing LDL oxidation, which means that taking GT is beneficial in reducing atherosclerosis risk correlated with oxidative stress(41). This result confirms our results that GT specifically contributed to the defense against LDL oxidation its association with proteins and phosphatidylcholine. Importantly, the hypocholesterolemic behavior of GT has also been identified in laboratory animals and humans; these previous studies, together with our present findings, indicate that
GT can be preventing the progression of oxidized LDL–triggered atherosclerosis via means of the synergistic action between both the cholesterol-lowering effect and the antioxidative property in humans(42). The results indicate a significant increase in CRI in the peroxidation community relative to the remaining groups; this result is consistent with a study showing that CRI-1 or CRI-2 are significant indicators of vascular risk and their predictive utility is better than independent parameters(43). GT, on the other hand, has been shown in animal experiments to reduce CHO levels in the circulation, as well as TG levels in the plasma, in cholesterol-fed hamsters, rats, and mice (44).

GT was found to have an antioxidants impact in rats (45). The results indicate that the administration of GT decreases the lipid peroxidation and oxidative stress observed in experimental rats. Lipid peroxides are a combination of highly reactive components of lipid peroxidation, a natural mechanism in all biological processes, and negative consequences for the cell membrane and DNA (46).

In conclusion, the present work has shown that providing GT extract in drinking water to experiment rats for four weeks with or without H2O2 reduces the concentration of the lipid peroxidation products in serum. Bioactive ingredients in GT play a significant role against lipid peroxidation, enhancing the activity in this way. Drinking GT may be highly beneficial as an alternative therapeutic agent in atherosclerotic cases.

**Ethical Clearance:** Observance of the principles of ethics of working with laboratory animals in the present study was done based on the principles of University of Kirkuk ethical committee.

**Reference**


