The effect of aqueous extract of green tea (**Camellia sinensis**) on liver enzymes of experimentally induced diabetes mellitus in mature female rabbits

Maher M.S. ALardy
Hayder A.N. ALzamely
Coll. of Vet. Med./ Univ. of AL-Qadiysia
Coll. of Vet. Med./ Green Univ. of AL-Qassim
email: Mahermahdi3@gmail.com

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Abstract
This study was carried out to find the effect of aqueous extract of green tea on liver enzymes in alloxan induced diabetic adult female rabbits. Thirty-five female rabbits weighted 1-1.5 kg were divided into five groups and treated for 8 weeks as follows. Group 1: intact rabbits were drenched with 2ml of drinking water for 8 weeks as control group. Group 2 (T1): diabetic rabbits were drenched with 2ml of drinking water as placebo. Group 3 (T2): Diabetic rabbits were drenched with single daily dose of green tea extract (200mg/kg body weight). Group 4 (T3): Diabetic rabbits were daily injected with insulin (3 I.U subcutaneous) for 8 weeks. Group 5 (T4): intact rabbits were drenched with single daily dose of green tea (200mg/kg body weight).

At the end of experiment the blood samples were taken by cardiac puncture, the blood serum was separated to measure liver enzymes. The results revealed that significantly decreased (p< 0.05) was observed in aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) levels in all treated groups, while increased significantly in diabetic group. This study concluded that aqueous extract of green tea can act as hepatoprotective agent.

Key words: rabbits, diabetes, green tea, liver enzymes, polyphenol, alloxan.

Introduction
Diabetes mellitus can be defined as a group of metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both resulting in impaired function in carbohydrate, lipid and protein metabolism.
Also characterized by hyperlipedemia, hyperaminoacidemia, and hypoinsulinaemia it leads to decrease in both insulin secretion and insulin action (1). It is frequently associated with the development of micro and macro vascular diseases which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (2). There are two forms of diabetes mellitus: type 1 and 2. In type 1 diabetes or, insulin-dependent diabetes mellitus the pancreatic β-cells are progressively destroyed and secrete little or no insulin. Type 2 diabetes or non-insulin-dependent diabetes mellitus, is a heterogeneous disorder of insulin resistance and pancreatic β-cells dysfunction (3). In developing countries as products are expensive and not easily accessible. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus. So the traditional herbal medicines are mainly used which are obtained from plants, it plays important role in the management of diabetes mellitus (4).

Tea is the most consumed beverage in the world. Green tea is made from the leaves of the warm weather evergreen Camellia sinensis L. (Theaceae). The major components of interest are the polyphenols. The major polyphenols in green tea are flavonoids. The four major flavonoids in green tea are the catechins, epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechin gallate (EGCG). Epigallocatechin gallate is viewed as the most significant active component. Other compounds of interest in dried green tea leave include gallic acid, quercetin, kaempferol, myricetin, saponins, caffeic acid and chlorogenic acid, recently green tea is being widely studied for its beneficial effects in the treatment and prevention of human diseases. It has been demonstrated that catechins can reduce blood glucose level. Therefore, it has been expected that an intake of green tea extract will prevent or delay the onset of diseases such as diabetes. Polysaccharides of green tea have also been reported to have hypoglycemic activities (5).

**Materials and methods**

Green tea extract: green tea extract powder obtained from United State America (True Nutrition Company) and the active ingredients is polyphenol in concentration (98%).

**Animals:**

Thirty five adult males of domesticated rabbits weighting about (1-1.5) kg were used and housed in individual cages. The animals were divided into five groups, seven in each group (intact control group (CG), diabetic control group (T1), diabetic group (T2), diabetic insulin group (T3), green tea (T4)

**Induce of Diabetes mellitus:**

Diabetes mellitus was induced in three groups (T1,T2,T3) over night fasting rabbits by single injection of alloxan (alloxan monohydrate) at dose 100 mg / kg into marginal ear vein each 100mg of alloxan was diluted in 1ml of 0.9% normal saline (10). Immediately after alloxan injection 10 ml of 20% glucose IP was given to the rabbits in order to overcome sudden decrease in blood glucose level. The rabbit were prevented from feeding for 12 hours and drenching water replaced by 5% glucose for 24 hours. After 4-5 days from injection, samples of blood taken to assess serum glucose concentration to make sure the incidence of diabetes in animals.

**Experimental design:**

The control animals (CG) were administered 2 ml of normal saline by mouth and injected with normal saline (100 μl s.c) daily for 8 weeks. Group 2 (T1) in which rabbits were induced diabetes mellitus then drenched with DW (2ml) and injected with normal saline (100 μl s.c) daily for 8 weeks. Group 3 (T2) in which rabbits were induced diabetes mellitus then treated with green tea (200 mg/ kg.BW) and injected with normal saline (100 μl s.c) daily for 8 weeks. Group 4 (T3) in which rabbits were induced diabetes mellitus then drenched with distal water (2ml) and injected with insulin (100 μl contain 3 IU /s.c) daily for 8 weeks. Group 5 (T4) in which rabbits (healthy animals) were give green tea only for 60 days. At the end of experiment, blood samples were taken from the heart of overnight fasted animals into plastic syringes, and allowed to clot, then
centrifuged for 10 minutes and serum samples were stored in polyethylene eppendorff tubes at -20°C until analysis. The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the method (6) and serum alkaline phosphatase (ALP) was determined by the method of (7).

**Statistical analysis:**

The results of the present study were analyzed by univariate analysis of variance. The data were expressed as means ± standard error (M ± SE). Least significant different test (LSD) was used to test the difference between means (groups) by using statistical program for social science SPSS. (P< 0.05) was considered significant.

**Results**

The results of effect of green tea extract on the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as serum alkaline phosphatase (ALP) were revealed increased the levels of all estimated enzymes in this study in group T1 significantly when compared with control group and other treated groups as (13.69 ± 0.07, 35.47± 2.16 , 44.29 ± 3.07) respectively , while the levels of these enzymes in group T2 and group T3 were reach to their levels of control group as(14.04 ± 0.06, 13.25 ± 0.31, 30.29 ± 1.10) and (13.84 ± 0.04, 12.87 ± 0.28, 29.65 ± 1.37) of T2,T3 respectively.(Table1).

Also table (1) appeared there was no significant differences among (C, T2, T3, T4) groups in levels of these enzymes. The result of (T2) group revealed the effect of green tea (camellia sinensis) extract (200mg/ kg ) on ALT,AST,ALP enzymes level which were significantly (P<0.05) reduced the levels of these three enzymes of rabbits suffering from diabetes. Diabetic animals in group (T1) which induced by single dose of alloxan 100 mg /kg body weight (positive control) lead to significant increase in liver enzymes when compared with control rabbit (group C),but there was no significant changes in liver enzymes of rabbits of normal control group (group C), but diabetic animals which treated with insulin group (T3) revealed improvement the mean values of liver enzymes (increase) when compared with non-treated diabetic rabbits (group T1) and control group (CG). While rabbits which treated with green tea only showed no significant values when compared with T2 and T3 groups and control group (CG) after 8 weeks of treatment.

**Discussion**

These findings are generally in agreement with previous experimental diabetes studies (8). Some investigators have suggested that the increase in enzyme levels in patients with diabetes mellitus resulted from the influence of insulin on liver tissue and The rise in ALT activity is almost always due to hepatocellular damage and usually accompanied by arise of AST (9). The injection with alloxan induced decrease in both liver glycogen and total serum protein contents (10). The levels of ALT, ALP, AST are altered thereby damaged structural integrity of the liver, as they are present in cytoplasm and are released in blood.

### Table(1): The effect of the aqueous extract of green tea on AST and ALT and ALP enzymes (IU/ L) in alloxan diabetic rabbits.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>13.69 ± 0.07</td>
<td>10.27 ± 0.41</td>
<td>27.39 ± 0.54</td>
</tr>
<tr>
<td>T1</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>30.73 ± 0.21</td>
<td>35.47 ± 2.16</td>
<td>44.29 ± 3.07</td>
</tr>
<tr>
<td>T2</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>14.04 ± 0.06</td>
<td>13.25 ± 0.31</td>
<td>30.29 ± 1.10</td>
</tr>
<tr>
<td>T3</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>13.84 ± 0.04</td>
<td>12.87 ± 0.28</td>
<td>29.65 ± 1.37</td>
</tr>
<tr>
<td>T4</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>13.53 ± 0.18</td>
<td>10.01 ± 0.46</td>
<td>27.31 ± 0.22</td>
</tr>
</tbody>
</table>

CG = Intact control rabbits: drenched with D.W. (2ml) and injected with N.S. (100 μl s.c) daily for 8 weeks.
T1 = Diabetic control rabbits: given single dose of alloxan (100 mg / kg,B.W.) drenched with D.W. (2ml) and injected with N.S. (100 μl s.c) daily for 8weeks.
T2 = Diabetic rabbits: drenched with green tea (200 mg/ kg,B.W) and injected with N.S. (100 μl/ s.c) daily for 8 weeks
T3 = Diabetic rabbits: drenched with D.W. (2ml) and injected with insulin (100 μl contain 3 IU) daily for 8 weeks
T4= Rabbits drenched with green tea extract (200 mg/kg) and injected with N.S. (100 μl s.c) daily for 8 weeks.
circulation after cellular damage (11). In the assessment of liver damage certain biomarkers via AST and ALT level are measured because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication for the health status of the liver. When inject of insulin hormone will act as mechanism action of green tea in reduced liver enzymes. Green tea catechin may reduce hepatic necrosis by suppressing oxidative stress and controlling the transcription factor expression involved in stellate cell activation (12). The antioxidant property of flavonoidal compounds of GT extract contributes to decrease the oxidative stress in liver and increase the levels of antioxidant enzymes, superoxide dismutase, catalase and glutathione (13). The hepatoprotective activities of green tea are attributed to its catechins that scavenge ROS/RNS in vitro (14). The drinking of green tea with high catechin content may help to prevent and/or attenuate the development of a certain type of hepatitis (15). The green tea possesses preventive effects of liver damage in rats (16). Recently, Noori et al. (2009) (17) investigated the effect of green tea against liver cirrhosis in rodents modeling. Plasma alanine aminotransferase (ALT) was much lower in orally treated green tea group confirming its vitality against liver dysfunctions. Also green tea provides protection against oxidative damage thereby lowering aspartate aminotransferase (AST) level after treatment with green tea. (20) determined the role of tea catechins in rats with hepatic oxidative abnormalities and highlighted that intraperitoneal injection of tea catechins decreased activities of serum AST, ALT and ALP. From these result it could be concluded that treating with green tea extract slowed significant decrease (p≤0.05) in the mean value of ALT, AST and ALP when compared with diabetic rabbit and control groups.

References


