Green tea's antioxidant properties protect the pancreas in diabetics: An experimental study


ABSTRACT

Diabetes mellitus is one of the most common and widespread metabolic illnesses worldwide. Hyperglycemia is a symptom of this condition, which is caused by problems with insulin secretion, insulin action, or both. Various medicinal plant species are used as a traditional treatment for diabetes mellitus, such as green tea, which is one of these plants whose extract has been used to treat diabetic patients for many years. The goal of this study was to see if green tea had any antioxidant benefits on the pancreas of alloxan-induced diabetic male albino rats. This experiment involved albino rats weighing between 110 and 120 grams. Three groups of animals were created. Control untreated normal healthy group, Alloxan-induced diabetic group and diabetic group treated with green tea. Tissue samples from diabetic and treated rats were collected and pathologically evaluated to determine the degradation of pancreatic cells in diabetic rats. In diabetic rats, biochemical data revealed a significant decrease in serum insulin, body weight, and total proteins when compared to the control group. Most of these measures improved significantly when diabetes rates were managed daily with green tea. In comparison to the control group, there was a significant improvement in morphological changes in diabetic groups after treatment with green tea in pancreatic tissues. Green tea, it may be concluded, can be used as an anti-diabetic drug to lower blood glucose levels and protect against the harmful consequences of diabetes.

Keywords: Diabetes mellitus, Pancreas, Green tea, Alloxan, Oxidative stress

1. INTRODUCTION

The pancreas is an endocrine and an exocrine gland. The endocrine part of the gland is the islets of Langerhans. It synthesizes the hormones insulin and glucagon. It plays an important role in carbohydrate metabolism. It is an
Diabetes mellitus is a metabolic condition marked by hyperglycemia, insulin resistance, and abnormal carbohydrate, protein, and lipid metabolism (Joseph & Jini, 2012). Diabetes can be treated with a wide variety of medicinal herbs. Green tea has more antioxidants in the form of quercetin than black tea. Antioxidants are essential for the body because they assist the enzyme superoxide dismutase in removing damaging free radicals from the body (Novit et al., 2021). Furthermore, the polyphenols, particularly the catechins, which account for up to 30% of the dry weight of green tea leaves, are credited with its health benefits. Because of changes in the processing of tea leaves after harvest, green tea has more catechins than black or oolong tea (El-Ghazaly et al., 2020).

Green tea has recently been extensively researched for its potential benefits in the treatment and prevention of human ailments. Consumption of green tea extract is thought to prevent or delay the onset of diseases such as diabetes. Individual oxygen and free radicals are known to be removed from the lipid peroxidation stage by flavonoids (Al-Hilfy, 2013). Green tea appears to help prevent hyperglycemia in rats in vitro by increasing insulin activity and perhaps avoiding damage to β-cells. Green tea reduced blood levels, serum creatinine, serum malondialdehyde, kidney glucose and protein excretion, as well as oxidative stress in the kidneys (Anderson & Polansky, 2002).

The goal of this study was to determine the antioxidant and hypoglycemic effects of daily oral consumption of green tea extract on histological pancreas structures in alloxan-induced diabetic rats in order to demonstrate green tea’s preventative properties. As a result, this study can serve as a scientific foundation for future research on the effects of green tea extract on the pancreas in diabetics.

2. MATERIALS AND METHODS
Our research followed the Animal Research Guideline for the Use and Care of Animals in Research, which was approved by PSA University’s Ethical Committee, Al-Kharj (PSAU-2021 ANT 10/43PI). It is an experimental study that took place between March 2021 and February 2022.

Green tea extract was purchased from a local shop in the shape of pills (300mg per tablet). Green tea extract was synthesized in this experiment by dissolving the tablets in distilled water. Gavages were used to deliver it every day. On the basis of the human dose, the dose for rats was determined using Paget's formula (Paget et al., 1983). This experiment involved thirty adult albino rats (9-11 weeks/ 110-120 g). For adaption, the rats were kept under observation for around 2 weeks before the experiment began. A single dose of alloxan (120 mg/kg) was used to cause diabetes in animals. It was injected intraperitoneally into rats to cause diabetes (Malaisse, 1982). The rats were fasted for 16 hours before receiving the alloxan injection. Then blood samples were taken from tail vein and the fasting blood glucose. The experimental animals were categorized into three groups of ten each. Non-diabetic untreated rats, Diabetic injected with alloxan and Diabetic rats treated orally with green tea extract (45mg/1ml/rat/day) for 30 days (Kurnia et al., 2015). The overnight starved animals (12-16 hours) were slaughtered under diethyl ether anesthesia at the end of the experiment. Blood was collected from the orbital vein. Trinder’s approach was used to calculate serum glucose using a commercially available kit (Trinder, 1969).

According to Reeves, (1983) serum insulin levels were tested using coat-A-count radioimmunoassay kits. The concentration of albumin and total protein was determined by the caloric method. After deducting albumin from total protein, serum globulin was determined. After one month, the rats in the control and treatment groups were slaughtered, and small portions of pancreas were removed for histological examinations. The stained sections were then viewed under a light microscope, photographed, and all differences between the three groups on the microscopic level were scientifically discussed.

3. RESULTS
In comparison to the controls, the serum insulin and glucose levels in different research groups indicated a significant decrease in serum insulin and a significant increase in fasting blood glucose (Table 1). When diabetic rats were compared to control rats, there was a significant decrease in serum insulin levels and a considerable increase in blood glucose levels. In comparison to diabetic rats, the green tea group exhibited considerable improvement in insulin and glucose levels (Table 1). In diabetic rats, the % change in body weight was reduced considerably. Furthermore, once the diabetic rats were treated with the plant extract, the % change in body weight returned to normal (Table 2). Additionally, diabetic rats’ serum total proteins, albumin, and globulin levels were
significantly lower than controls. Green tea treatment of diabetic rats resulted in changes in the assessed blood protein profile parameters. The Albumin/Globulin ratio did not differ significantly between the control and experimental groups (Table 3).

Table 1 Results of analysis of Serum glucose and insulin levels in different groups

<table>
<thead>
<tr>
<th>Group parameters</th>
<th>First Control group</th>
<th>Diabetic group</th>
<th>Diabetic with Green tea Methotrexate group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>88.33 ± 0.66</td>
<td>289.19 ± 0.91</td>
<td>89.83 ± 0.43</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>39.29 ± 0.56</td>
<td>38.65 ± 0.69</td>
<td>30.74 ± 0.29</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 2 Results of analysis of Changes in body weight in grams in different groups

<table>
<thead>
<tr>
<th>Group parameters</th>
<th>First Control group</th>
<th>Diabetic group</th>
<th>Diabetic with Green tea Methotrexate group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight at the start of the experiment</td>
<td>115.1 ± 0.06</td>
<td>115.64 ± 0.28</td>
<td>115.21 ± 0.13</td>
</tr>
<tr>
<td>Weight at end experiment</td>
<td>125.04 ± 0.11</td>
<td>100.06 ± 0.03</td>
<td>126.05 ± 0.13</td>
</tr>
<tr>
<td>Percentage</td>
<td>10.02</td>
<td>5.04</td>
<td>10.03</td>
</tr>
</tbody>
</table>

Table 3 Changes in the serum proteins profile in different groups.

<table>
<thead>
<tr>
<th>Group parameters</th>
<th>First Control group</th>
<th>Diabetic group</th>
<th>Diabetic with Green tea Methotrexate group</th>
<th>% of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>6.99 ± 0.12</td>
<td>4.99 ± 0.22</td>
<td>7.17 ± 0.13</td>
<td>-25.81%</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.98 ±0.11</td>
<td>3.24 ± 0.14</td>
<td>4.77 ± 0.11</td>
<td>-26.30%</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>3.01±0.05</td>
<td>1.75 ± 0.06</td>
<td>2.40 ± 0.08</td>
<td>-29.99%</td>
</tr>
<tr>
<td>Albumin /Globulin ratio</td>
<td>1.29 ±0.04</td>
<td>1.32 ± 0.03</td>
<td>1.33 ± 0.02</td>
<td>0000</td>
</tr>
</tbody>
</table>

The islets of Langerhans seemed normal when Hematoxylin and Eosin-stained slices of pancreas from the control group were examined. Beta cells were found in the majority of islets and acini, while alpha cells were found on the periphery (Fig. 1). The diabetic group’s islets had a significant loss in size and cellularity. Some islet cells had pyknotic nuclei, which indicated degeneration. Furthermore, significant vascular degeneration was discovered in the islet region (Fig. 2). The diabetic group treated with green tea demonstrated a partial recovery to normal cellular distribution in the islet of Langerhans, as well as enhanced cellularity and poor differentiation of the distinct cell types (Fig. 3).
**Figure 1** A, B, and C demonstrate normal histological appearance of the pancreas of first control group. The normal cellular distribution in the islet of Langerhans (H&E A, X200, B & C X400)

**Figure 2** A, B and C demonstrate sections of the Pancreas with alteration of normal histology. There is a marked degenerative change and inflammatory cell infiltration in the islet (H&E X400).
Figure 3 A, B and C demonstrate sections of the Pancreas of third group relatively return to return to the normal cellular distribution within the islet of Langerhans ((H&E; A, C X200, B X400).

4. DISCUSSION
Medicinal plants serve an essential role in diabetes therapy, especially those in developing countries. Green tea extract brings extensive serum insulin levels in diabetic mice, and green tea extract (mixed with ginseng roots) protected cells in the islets of Langerhans, according to a previous study (Karaca et al., 2010). Several pathogenic signals are triggered by the formation of reactive oxygen species (ROS) in diabetic patients (Chen et al., 2022). Apoptosis of beta-cell death, macrophages with lymphocytes and natural killer cells were all seen in histological investigations of pancreas tissues from patients with Diabetes Mellitus (Kumar et al., 2005). The differences in body weight between diabetic and treated groups were investigated in this study. The diabetic group showed a decrease in body weight, which could be due to a lack of insulin in the blood, preventing sugar from entering the cells and thus increasing the percentage of sugar in the blood. The body attempts to eliminate excess sugar through urine output. Excess urine output reduces the amount of water in the body, resulting in a reduction in body weight.

Many studies have shown that this weight loss is linked to a considerable hypoglycemic effect in diabetic rats. Glucose intolerance can be caused by a malfunction in insulin production in insulin-dependent diabetes or a defect in insulin resistance in non-insulin-dependent diabetes (Tsuneki et al., 2004; Wu et al., 2004; Ryu et al, 2006). Other studies discovered that even though rats were diabetes permanently, streptozotocin caused an incomplete loss of pancreatic beta cells (Waer & Helmy, 2012). Patrick discovered that pathological examination of streptozotocin diabetic rats revealed a reduced number of islets of Langerhans and degranulation of B-cells, hydropic degeneration, pyknosis, and necrosis, which are consistent with our findings of a reduced number of islets of Langerhans cells, presence of damaged pancreatic acini, pyknosis, and necrosis (Patrick et al., 2008).

5. CONCLUSION
Green tea’s protective properties could be attributed to the presence of antioxidant-active components in the extract, which reduced oxidative stress in the cells and resulted in normal cell structures and functions. Moreover, the results of the biological experiment were confirmed by histological inspection of the pancreas.
Acknowledgments
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Authors' Contributions
All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali, Faleh Mubarak Aldawsari and Murdhi Yousef M Alanazi participated in the study design and wrote the first draft of the manuscript. Faisal Hassan Sumaili, Abdullah Mulfy M Alanazi, and Ali Awadh Ali Alahmari collected and processed the samples. Saleh Basheer S Alanazi, Musab Sultan A Alrezehi and Ali Hassan M Alnakhli-participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

Ethics Approval
All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (PSAU-2021 ANT 10/43PI).

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Conflicts of interest
The authors declare that there are no conflicts of interests.

Data and materials availability
All data associated with this study are present in the paper.

REFERENCES AND NOTES


