Hypoglycemic effect of *Artemisia absinthium* and Green Tea (*Camellia sinensis*) mixture on alloxan-induced diabetic rats

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**Abstract**

This study aims to inspect the effects of green tea extract, *A. absinthium* extract, and mixture of them in diabetic rats. Forty adult male albino rats were divided into 5 experimental groups: The first group was considered as negative control group. The second group was positive control diabetic rats receiving normal diet with no treatment. The third group was diabetic rats receiving normal diet and receiving oral 50 ml of green tea extract, the fourth group was diabetic rats receiving normal diet and receiving oral 50 ml of *A. absinthium* extract. And the fives group was diabetic rats receiving normal diet and receiving oral 50 ml of green tea extract plus 50 ml of *A. absinthium* extract. The results showed decrease of serum glucose in groups (3, 4, and 5) but group No. 5 recorded the better reduction of blood glucose as compared with negative control. Treating diabetic groups fed on basal diet with different extract of green tea, *A. absinthium* led to a significant decrease in the mean value of serum cholesterol, triglyceride as compared to the positive control group. We can recommend that the extract of green tea plus *A. absinthium* has a hypoglycemic effect on alloxan-induced diabetes in rats.

**Keywords:** Bakery, Hypoglycemic, *Artemisia absinthium*, Green Tea, alloxan, glucose

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1. **Introduction**

Diabetes mellitus is defective or deficiency in Insulin secretion which occurs due to a chronic metabolic disorder and it uneartns by too much glucose in the blood and urine. Insulin, a hormone produced by the beta cells of the Islets of langerhans of the pancreas helps to utilize glucose for the production of energy by the body. Insulin helps in glucose uptake by the cells, prevents rise in blood sugar and maintains its level within normal limits [19, 37].

Tea is the second most consumed beverages in the world, next to water and well ahead of coffee, beer, wine, and carbonated soft drinks and its habitual consumption has long been associated with health benefits [6,28].

Tea is an infusion made by steeping processed leaves, buds, or twigs of tea bush, *Camellia sinensis*, in hot water for several minutes after, which it is drunk [23]. More than 300 different kinds of tea are produced from the leaves of *Camellia sinensis* by different manufacturing processes. Generally, they are divided into three types: green tea (non-fermented), oolong tea (semi fermented) and black tea (fermented). To produce green tea, freshly harvested leaves are immediately steamed to prevent fermentation, yielding a dry, stable product. This steaming process destroys the enzymes responsible for breaking down the color pigments in the leaves and allows the tea to maintain its green color during the subsequent rolling and drying processes. These processes preserve natural polyphenols with respect to the health-promoting properties [4].
The scientific community and the popular press have recently pondered that there might be beneficial properties of green tea. Its consumption has been associated with anti-inflammatory, anti-oxidative, anti-mutagenic, and anti-carcinogenic effects [20].

The constituents of C. sinensis leaves include a complex mixture of polyphenolic compounds (30% to 35%), methylxanthines such as caffeine and theobromine (2.5% to 4.0%), proteins (15% to 20%), amino acids (1% to 4%), carbohydrates (5% to 7%), and lipid components (2%); organic acids (1.5%), ash (5%), minerals and trace elements (10% to 15%); and pigments such as chlorophyll (0.5%) [3]. Among the polyphenolic compounds, flavonoids are the most abundant (80% to 90%). The largest proportion of flavonoids are catechin polyphenols (condensed tannins), which make up 30% to 40% of green tea solids. Catechin polyphenol content depends on the maturity and the processing of the C. sinensis leaves during preparation of tea. The catechin polyphenol composition of the leaves also varies with geographic location, season, and cultivation procedures [11]. Catechin polyphenols are colorless, water soluble compounds and are very stable in acidic solutions (pH less than 4). However, their solubility progressively decreases as the pH is increased from 4 to 8. A typical tea beverage, prepared in a proportion of 1 g leaf/100 mL water in a 3-minute brew, usually contains from 250 to 300 mg tea solids, composed of 30% to 42% catechins and 3% to 6% caffeine [3]. Dried concentrated extracts from green tea containing high amounts of catechins are a popular source for nutraceutical and medicinal uses.

Al-Hilfy (2012) [1] concluded that the taken doses of green tea extract are affected positively in reducing glucose level and improving the impaired kidney functions in diabetic rats. When Salim (2014) [31] showed that Zingiber officinale (ginger) and Camellia sinensis (green tea) plants share some possible mechanisms to lower glucose levels in type 1 and 2 of diabetes mellitus. Although Mostafa (2014) [22] investigated that green tea rich with catechins, especially in high amounts, reduces serum cholesterol levels, and blood glucose.

Artemisia absinthium is an aromatic plant of the family Asteraceae, subfamily Asteroideae, tribe Anthemideae and is known by the common names wormwood. Artemisia absinthium (wormwood) is an aromatic, perennial small shrub distributed in Europe and Asia [36]. Artemisia absinthium, also known as common wormwood, is a member of the Asteraceae family, which is an herbaceous perennial plant with strong sage odor [25]. The wormwood (A. absinthium) contains the monoterpene (thujone) active component and absinthen, [18, 26] beside azulenes, phenolic compounds, and flavonoids, which give antiradical and antioxidative activity [17].

Daradka et al., 2014 [8] concluded that the extract of A. absinthium has a hypoglycemic effect on alloxan-induced diabetes in rats prevents the severe reduction in body weight and improvement biochemical parameters associated with (DM) such as total cholesterol, serum protein, urea, and creatinine, and will be supportive in projecting the A. absinthium as a therapeutic agent in diabetes. Although Haidari et al., (2012) [12] concluded that green tea extract had both antihyperglycemic and hypocholesterolemic effects in diabetic rats.

Therefore, the aim of this research was to assay the effect of green tea extract and A. absinthium extract to improve glycaemic control in type 2 diabetic patients.

2. Materials and Methods

2.1. Materials

Plants:

1. A. absinthium plants were obtained from Directorate of Agriculture in Aswan, Egypt. It was left to air dry partially for 2–3 days at room temperature. It was chopped then grinded using an electrical grinder until obtained powder. Each 500 g of this powder was extracted by ethanol-water mixture (70/30V/V) for 48 h. This step was repeated for three times then the filtrate was then refluxed in 70% ethanol at 50 °C using a rotary evaporator for 36 h in continuous extraction (Soxhlet) apparatus. Pooled and concentrated ethanol extract was filtered, and it was re-concentrated under reduced vacuum pressure [8].
Green tea (*Camellia sinensis*) leaves used in this study were purchased from a local market in Aswan, Egypt. The dry green tea leaves were powdered by an electrical mill. In order to prepare the extract, 150 g of powdered green tea leaves was mixed with 1000 ml 95% ethanol and was shaken constantly for 48 hours. After filtration (Whatman filter paper No. 1), the suspension was evaporated in a rotary evaporator. The extracts were stored in 4°C refrigerator until usage [12].

2.2. Chemicals:
Alloxan monohydrate (used for diabetes induction in rats) and kits for biochemical analysis (used for biological evaluation) were obtained from Sigma Chemicals Co. (USA). The other chemicals used in this work were of analytical grade.

2.3. Experimental animals:
Healthy male albino rats weighting 200-220 g were obtained from the farm of the National Research Center, Giza, Egypt.

2.4. Induction of diabetes in experimental rats.
Rats were fasted overnight and diabetes was induced by a single intraperitoneally injection of freshly prepared solution of alloxan at a dose of (150 mg / kg body weight) in sterile saline [9]. Rats get diabetes by raise the proportion of hyperglycemia gradually during fed on basal diet and injection of Alloxan for three days. Development of diabetes was confirmed by measuring fasted blood glucose levels. Only rats with fasting blood glucose levels greater than 250 mg/dL were considered diabetic and then included in the experiment.

2.5. Experiment design:
Forty male albino rats were randomly divided into five groups each of eight rats as following:

- **Group 1**: Negative control, normal healthy rats, receiving normal diet with no treatment.
- **Group 2**: Positive control, diabetic rats receiving normal diet with no treatment.
- **Group 3**: Diabetic rats receiving normal diet and receiving oral 50 ml of green tea extract.
- **Group 4**: Diabetic rats receiving normal diet and receiving oral 50 ml of A. absinthium extract.
- **Group 5**: Diabetic rats receiving normal diet and receiving oral 50 ml of green tea extract plus 50 ml of A. absinthium extract.

2.6. Body weight:
Rats were recorded changes of body weight during the experimental period (4 weeks). Blood samples were collected sequentially from the eye plexuses of rats before and during the experimental period.

2.7. Biochemical parameters:
Blood samples were collected from the eye plexuses of animals by a fine capillary glass tubes during the first day, 10 days, 20 days and at the end of experimental period. Blood serum samples were separated by centrifugation for 10 min at 1500 xg and kept at −20°C until analysis [33]. Serum glucose (mg/dL) concentration was determined according to the methods described by Young and Friedman (2001) [38] using Spectrophotometer adjusted at 500 nm.

Total triglyceride and Cholesterol were determined according to Fossati and Prencipe (1982) [10] and Roeschlau et al. (1974) [29], respectively.

2.8. Statistical analysis
The results were statistically analyzed according to statistical analysis system SAS (1999) [32]. Duncan’s at 5% level of significance was used to compare between means according to Snedecor and Cochran, (1980) [35].

3. Results and discussions:

3.1. Changes in body weight
Table (1), illustrates that the average body weight (BW) of diabetic rats was significantly (p< 0.05) lower than that of the normal control (group1), Drastic reduction was obtained in the BW of diabetic rats group2 (control+), the body weight of the diabetic rats (group3 and group4) treated with green tea extract and A. absinthium extract, respectively, was significantly (p< 0.05) lower than the normal control (group1). On the other hand (group3) diabetic rats treated with 50 ml of green tea extract plus 50 ml of A. absinthium extract improved their body weight loss when compared to the diabetic control (group2).
Body weight gain of rats on group 5 recorded (36.83 g) was slightly lower than group 1 control (-) (41.15 g), this indicates that group 5 diabetic rats treated with 50 ml of green tea extract plus 50 ml of A. absinthium extract was slightly less efficient than control (-) in promoting growth.

These results are consistent to those obtained by Al-Hilfy (2012) [1], Mostafa (2014) [22], and Daradka et al., (2014) [8].

3.2. Serum glucose

Groups of rats recorded different level of serum glucose in Table (2). Results illustrates that induction of diabetic in rats with alloxan (150 mg /kg body weight) significantly increased serum glucose from 89.12- 108.88 mg/dl to 234.12 – 250.23 mg/ dl after 10 days of alloxan injection. Kumar et al. (2011) [21] reported that alloxan acts as diabetogenic by destruction of ß-cell of the islets of langerhans and causes massive reduction in insulin release, thereby inducing hyperglycemia. Serum glucose of diabetic rats in group 2 (control +) was significantly (p ≤ 0.05) increased to 299.22 mg/ dl at the end of experiment. Diabetic rats on different groups (control –, group 3, group 4, and group 5) significantly reduced serum glucose gradually. The lowest serum glucose level was significantly obtained in the group of rats number 5 (154.11 mg/ dl), followed by group 3, then group 4 (192.11, 198.10 mg/ dl), respectively, as compared with the control positive due to alloxan producing oxygen radicals that destroy pancreatic ß-cells and cause severe hypoinsulinaemia that is responsible for the hyperglycemia seen in alloxan-treated animals [16].

Group 5 diabetic rats which treated with 50 ml of green tea extract plus 50 ml of A. absinthium extract were recorded low of glucose level significantly in alloxan-induced rats during experimental period. These observations may be due to the high level catechin on glucose tolerance which may be mainly due to the two epicatechin (EC), Epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG) [27]. The significant antihyperglycemic may be due to the presence of active components of A. absinthium like α- and β-thujones, thujyl alcohol, azulenes, bisabolene, cadinene, sabinene, pinene, and phellandrene [2,7,24].

Several studies have proved that the hypoglycemic effect of green tea attributed to the presence of polyphenols, catechins and a water-soluble polysaccharide fraction Sabu et al., (2002) [30] and Shimizu et al., (2000) [34]. It has been reported that green tea polyphenols increase insulin activity in diabetic rats [6]. It also is documented that the water-soluble polysaccharides fraction of green tea is responsible for its anti diabetic effect Isigaki et al., (1991) [15]. Other studies also confirmed that green tea epigallocatechin gallate promotes pancreatic ß-cells regeneration in alloxan-treated rats, has insulin-like and insulinotropic activities, and inhibits gluconeogenesis through inhibition of liver phosphoenolpyruvate kinase synthesis Chemler et al., (2007) [5].

Similar results were obtained by Chemler et al., (2007) [5], Park et al., (2009) [27], Al-Hilfy (2012) [1], Mostafa (2014) [22], and Daradka et al., (2014) [8].

3.3. Serum Triglycerides (TG)

Induction of diabetic in rats significantly (p≤ 0.05) raised triglycerides to 88.08 - 128.00 mg/ dl compared to 63.13 - 68.22 mg/dl in the control negative group (Table 3). The best results in serum triglycerides were observed in hyperglycemic group (groups 5) fed on basal diet and treated with 50 ml of green tea extract plus 50 ml of A. absinthium extract, because this treatment showed no big significant differences in serum triglyceride as compared to the negative control group. The lowering of triglyceride observed in all groups, the more pronounced lowering effect was observed in the groups No. 5 (71.01 mg/dl) then group No.4 (78.25 mg/dl), and group No. 3 (80.22 mg/dl) as compared to the negative control group.

The reduction of triglycerides may be attributed to catechin suppress postprandial hypertriacylglycerolemia through the inhibition of pancreatic lipase, which, therefore, delayed the absorption of fat [14].

These results are close to which obtained by Ikeda et al., (2005) [14], Haidari et al. (2012) [12], and Mostafa (2014) [22].
Table 1. Body weight and body weight gain (g) of normal and diabetic rats.

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Body weight(g) during experimental period(days)</th>
<th>Body weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial weight</td>
<td>10</td>
</tr>
<tr>
<td>control (-) Group 1</td>
<td>219.25 ±7.55a</td>
<td>235.11 ±10.33a</td>
</tr>
<tr>
<td>control (+) Group 2</td>
<td>211.22 ±5.46c</td>
<td>182.23 ±11.20c</td>
</tr>
<tr>
<td>Group 3</td>
<td>204.22 ±11.05bc</td>
<td>211.21 ±11.64b</td>
</tr>
<tr>
<td>Group 4</td>
<td>220.50 ±11.32bc</td>
<td>219.13 ±11.22b</td>
</tr>
<tr>
<td>Group 5</td>
<td>215.59 ±14.00a</td>
<td>217.34 ±21.17a</td>
</tr>
</tbody>
</table>

Mean (n=8) = SD in the same column with different letters are significantly different (p≤0.05).

Figure 1. Body weight (g) of normal and diabetic rats.
Table 2. Serum glucose (mg/dL) of normal and diabetic rats.

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Serum glucose (mg/dL) during experimental period(days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>control (-) Group 1</td>
<td>102.12 ±0.60DE</td>
</tr>
<tr>
<td>control (+) Group 2</td>
<td>108.88 ±0.50DE</td>
</tr>
<tr>
<td>Group 3</td>
<td>101.12 ±0.11DE</td>
</tr>
<tr>
<td>Group 4</td>
<td>89.12 ±0.15DE</td>
</tr>
<tr>
<td>Group 5</td>
<td>99.11 ±0.25DE</td>
</tr>
</tbody>
</table>

Mean (n=8) ± SD in the same column with different small letters are significantly different (p<0.05) based on animal group. Mean (n=8) ± SD in the same row with different capital letters are significantly different (p<0.05) based on experimental period.

Figure 2. Serum glucose (mg/dL) of normal and diabetic rats

Table 3. Serum triglycerides (mg/dL) of normal and diabetic rats

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Serum triglycerides (mg/dL) during experimental period(days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>control (-) Group 1</td>
<td>68.22 ±0.004A</td>
</tr>
<tr>
<td>control (+) Group 2</td>
<td>65.10 ±0.104C</td>
</tr>
<tr>
<td>Group 3</td>
<td>63.13 ±0.204D</td>
</tr>
<tr>
<td>Group 4</td>
<td>65.11 ±0.504C</td>
</tr>
<tr>
<td>Group 5</td>
<td>66.10 ±0.124D</td>
</tr>
</tbody>
</table>

Mean (n=8) ± SD in the same column with different small letters are significantly different (p<0.05) based on animal group. Mean (n=8) ± SD in the same row with different capital letters are significantly different (p<0.05) based on experimental period.
Figure 3. Serum triglycerides (mg/dL) of normal and diabetic rats.

Table 4. Serum cholesterol (mg/dL) of normal and diabetic rats.

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Serum cholesterol (mg/dL) during experimental period</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (-) Group 1</td>
<td>109.01 ± 0.00 A</td>
<td>112.06 ± 3.12 A</td>
<td>105.11 ± 3.06 A</td>
<td>108.04 ± 1.14 A</td>
<td></td>
</tr>
<tr>
<td>control (+) Group 2</td>
<td>109.01 ± 0.00 B</td>
<td>153.00 ± 5.15 B</td>
<td>161.13 ± 4.60 B</td>
<td>166.67 ± 1.46 B</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>109.01 ± 0.00 C</td>
<td>14.00 ± 2.12 C</td>
<td>136.66 ± 4.12 C</td>
<td>126.12 ± 1.3 C</td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>109.01 ± 0.00 D</td>
<td>134.00 ± 2.14 D</td>
<td>122.45 ± 3.46 D</td>
<td>128.00 ± 3.11 D</td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>109.01 ± 0.00 E</td>
<td>133.02 ± 3.15 E</td>
<td>128.06 ± 2.23 E</td>
<td>116.55 ± 3.55 E</td>
<td></td>
</tr>
</tbody>
</table>

Mean (n=8) ± SD in the same column with different small letters are significantly different (p< 0.05) based on animal group. Mean (n=8) ± SD in the same row with different capital letters are significantly different (p< 0.05) based on experimental period.
3.4. Serum cholesterol.

Data in (Table 4) revealed that treating diabetic groups fed on basal diet with different levels of green tea extract, A. absinthium extract, and green tea extract plus A. absinthium extract led to a significant decrease in the mean value of serum cholesterol as compared to the positive control group. The value of total serum cholesterol in rats suffering from hyperglycemia decreased gradually with increasing the level of green tea extract and A. absinthium extract. Statistical analysis in this table showed that no significant change in serum cholesterol was observed between the groups treated with 50 ml of green tea extract plus 50 ml of A. absinthium extract as compared to the negative control group.

Ikeda, 2008 [13] confirmed that the reasons of reduction of serum cholesterol may be attributed to the inhibition of intestinal absorption of cholesterol by green tea and catechin, resulting, therefore, to reducing the serum cholesterol concentrations.

4. Conclusion

The extract of green tea plus A. absinthium has more a hypoglycemic effect on alloxan-induced diabetes in rats. It has prevents the reduction of body weight and improvement biochemical parameters associated with diabetes such as total cholesterol, triglycerides, and will be supportive mixture as a therapeutic agent in diabetes. This work suggests that green tea plus A. absinthium mixture could be used as therapeutic nutritional supplement for the management of diabetes.

Compliance with Ethics Requirements. Authors declare that they respect the journal’s ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References


13. Ikeda, I., Multifunctional effects of green tea catechins on prevention of the metabolic syndrome. Asia Pac J Clin Nutr., 2008, 17(S1), 273-274.


