Habitual Green Tea Intake and Effects on Blood Glucose and Blood Lipids of Diabetic Rats

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Abstract
The effects of green tea on blood glucose and blood lipids were evaluated on alloxan-induced diabetic rats. The rats were grouped into four treatment groups: DC (diabetic group fed with rat feed and water), CON1 (diabetic group fed with rat feed and 1g/100ml of green tea intake), CON2 (diabetic group fed with rat feed and 2g/100ml of green tea intake) and CON3 (diabetic group fed with rat feed and 6g/100ml of green tea intake). The tea was brewed in boiling water for two minutes, cooled and then served. Feed, water and green tea were provided ad libitum for the respective groups for 21 days. The blood glucose was determined every seven days from the day of confirmation of diabetes to the end of the experiment while the lipid analysis was carried out at the end of the experiment. The results obtained showed that green tea did not have any reduction effect on blood glucose at 1g/100ml and 2g/100ml of tea intake. It caused 0.05% reduction in blood glucose at 6g/100ml of tea intake. However, it caused significant reduction in total cholesterol, triglyceride, LDL-cholesterol and LDL/HDL ratio.

Keywords: Green tea, diabetes, blood glucose, blood lipids.

Introduction
Tea is the most consumed drink in the world after water. The medicinal value of tea has been historically well known and since ancient times, green tea has been considered as a healthy beverage. Green tea consumption is reportedly associated with various health-promoting properties such as anti-inflammatory, anti-arthritis, antibacterial, antiangiogenic, anti oxidative, anti viral, neuro protective, cholesterol-lowering effects and so many others (Dona et al., 2003; Haqqi et al., 1999; Sudano et al., 2004; Satippour et al., 2002; Osada et al., 2001; Weber et al., 2003; Weinreb et al., 2004). The health-promoting effects of green tea are mainly attributed to its active constituent, the epigallocatechin gallate, which has also been shown to inhibit intestinal glucose and lipid uptake as well as cause vasodilation (widening of the arteries) which decreases blood pressure and prevent blood clotting. The amount of gallated catechins necessary for reduction of blood glucose concentration has been shown to be a daily dose of green tea. However, the amount required to decrease lipid uptake from the gut is much higher which has also been shown to have adverse effects in humans (Dona et al., 2003; Haqqi et al., 1999; Sudano et al., 2004; Osada et al., 2001; Weber et al., 2003; Weinreb et al., 2004). The health-promoting effects of green tea are mainly attributed to its active constituent, the epigallocatechin gallate, which has also been shown to inhibit intestinal glucose and lipid uptake as well as cause vasodilation (widening of the arteries) which decreases blood pressure and prevent blood clotting. The amount of gallated catechins necessary for reduction of blood glucose concentration has been shown to be a daily dose of green tea. However, the amount required to decrease lipid uptake from the gut is much higher which has also been shown to have adverse effects in humans (Dona et al., 2003; Haqqi et al., 1999; Sudano et al., 2004; Osada et al., 2001; Weber et al., 2003; Weinreb et al., 2004).

A study conducted in Japan showed that people who drank up to 6 cups of green tea per day were 33% less likely to develop type 2 diabetes than people who consumed less than 1 cup per week (Iso et al., 2006). In 2003, Taiwanese subjects who habitually consumed green tea for more than 10 years were shown to have lower body fat compositions and smaller waist circumferences which are important factors linking obesity to diabetes (Wu et al., 2003).

A lot of articles have suggested that green tea antioxidants may help to regulate blood glucose level, delay onset of diabetes, decrease blood pressure, low density lipoprotein cholesterol, oxidative stress and a marker of chronic inflammation (Daniel, 2015; Nantz et al., 2009; http://www.livestrong.com, 2015; http://www.pacificcollege.edu/blog, 2015). Daniel (2015) has presented green tea, as a potential inexpensive, nontoxic and pleasurable blood-sugar-lowering agent, which can be used for prevention and retardation of human diabetes and ensuing complications. However, a limited number of clinical trials using green tea, green tea extracts (GTEs), or its main ingredient catechin have shown disappointing results in controlling hyperglycemia in type 2 diabetic patients or protecting the condition in healthy subjects. McKenzie et al. (2007) showed no significant difference in glucose control after 3 months of ingestion of decaffeinated green tea in type 2 diabetic patients, Nagao et al. (2009) also showed that plasma glucose levels and Hb1c did not improve after 12 weeks of supplementation with catechin in patients with type 2 diabetes and Hsu et al. (2011) showed no difference in glycemic control or lipid parameters after 16 weeks of green tea supplementation. In addition, Ryu et al. (2006) showed that 4 weeks of green tea consumption did not affect inflammation, adiponectin levels, or insulin resistance in type 2 diabetic patients.
Based on the conflicting findings of the aforementioned researchers, there is need to provide more evidence on the beneficial effects of green tea, especially its anti-hyperglycemic and hypolipidemic effects in humans and animals. Thus, the present study was conducted on the bid to investigate more on the effects of acute ingestion of green tea on blood glucose and blood lipids concentrations.

Materials and Methods

Sample Material
The green tea sample used in this work was commercially produced and was bought from a supermarket in Owerri, Nigeria.

Animal studies
A total of 70 mature, male albino rats (Rattus norvegicus) of Wistar strain were sourced from Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. Upon arrival, the animals were allowed to acclimatize for 7 days while been maintained on regular commercial rat feed (Vital feed, growers; produced by UAC, Nigeria) and tap water. Tap water and feed were provided and libitum throughout the period.

Experimental design
A modified method of Eliakim and Obri (2009) was adopted. After 7 days of acclimatization, the rats were fasted overnight. Diabetes was induced in 63 rats using a single dose of alloxan monohydrate (125 mg/kg bwt) which was given intraperitoneally. The remaining 7 rats received saline (0.9% w/v NaCl) injection which was also given intraperitoneally (non-diabetic group). Three (3) days after injection of alloxan, the fasting blood glucose concentrations of the rats were taken, (blood glucose at confirmation of diabetes) and only rats with blood glucose above 250 mg/dl were selected as diabetic rats used in this study. The fasting blood glucose of the non-diabetic group was also measured. The rats were grouped into five treatment groups: NC (non-diabetic group fed with rat feed and water only), DC (diabetic group fed with rat feed and water only), CON1 (diabetic group fed with rat feed and 1g/100ml of brewed tea only), CON2 (diabetic group fed with rat feed and 2g/100ml of brewed tea only) and CON3 (diabetic group fed with rat feed and 3g/100ml of brewed tea only). The tea was brewed in boiling water for two minutes after which it was allowed to cool to room temperature and then served. Feed, water and the tea were provided ad libitum for the respective groups for a period of 21 days. The average fasting blood glucose concentrations of the treatment groups were measured every week from the day of confirmation of diabetes (day 0) to the final day of administration of the tea (day 21). The results of the treatment groups CON1, CON2 and CON3 were compared with those of the diabetic group DC and non-diabetic group NC that took tap water in place of the tea.

At the end of the treatment period, the animals were fasted overnight, immobilized, their jugular veins severed and their blood samples collected for lipid profile analysis.

Biochemical analysis

Blood glucose determination
Blood samples were collected from the tail arteries of the rats for the various treatment groups (NC, DC, CON1, CON2, CON3) on the day of confirmation of diabetes (day 0) and every other 7th day till the end of the treatment period (day 24) and their blood glucose concentrations were determined using One Touch Ultra glucometer, the values were expressed in mg/dl.

Percentage change in blood glucose concentration
The percentage change in blood glucose concentration of the different treatment groups was calculated using the expression below:

\[
\% \text{ Change in blood glucose} = \frac{\text{Final blood glucose conc.} - \text{initial blood glucose conc.}}{\text{Initial blood glucose conc.}} \times 100
\]

Lipid profile analysis
The reagents used for the assays were as contained in commercial test kit produced by Randox Laboratories Ltd, Antrim, United Kingdom, and Biosystems S.A. Barcelona Spain. The total cholesterol was determined as described by Allain et al. (1974). The glycerol phosphate oxidase/peroxidase method as described by Bucalo and David (1973) and Fossati and Prencipe (1982) were used for triglyceride determination. The serum HDL-cholesterol was determined using the phosphotungstate/Mg-cholesterol oxidase and peroxidase method as described by Grove (1979) and Burstein et al. (1980). The LDL-cholesterol was calculated as described by Friedewald et al. (1972) while LDL/HDL ratio was calculated using the expression: LDL value/HDL value.

Results and Discussion
The effects of different concentrations of green tea on average fasting blood glucose concentrations of the treatment groups (CON1, CON2, CON3) are shown in Figure 1. After day 0 (day of confirmation of diabetes), the average fasting blood glucose concentration of the diabetic group DC (group that did not take the green tea) continued to increase throughout the treatment period while those of the diabetic groups CON1 and CON2 increased a little from day 0 to the 7th day and then started decreasing slightly again throughout the treatment period. The diabetic group CON3 whose average fasting blood glucose concentration decreased the first week (days 0 to
7), started increasing gradually thereafter. The average fasting blood glucose level of the non-diabetic group NC remained virtually stable throughout the experimental period.

At the end of the treatment period, the groups that took different concentrations of the green tea (CON1, CON2, CON3) all showed reduced average fasting blood glucose concentrations when compared with that of the diabetic group DC that did not take the tea. Though, the effect of this tea in these groups (CON1, CON2, CON3) did not cause much remarkable reduction in their initial average fasting blood glucose concentrations, to term it anti-diabetic, it was strong enough to hold their average fasting blood glucose concentrations from rising as high as that of the diabetic group DC. This result is not far from the findings of other researchers such as Mackenzie et al., (2007), Nagao et al., (2009) and Hsu et al., (2011), who also showed that green tea did not cause significant reduction in blood glucose level.

The result of the percentage change in average fasting blood glucose concentrations of the treatment groups at the end of the treatment period is shown in Table 1. From the table, it could be observed that it was only at 6g/100ml of tea intake (CON3) that the green tea could cause 0.5% reduction of the initial value (337.0mg/dl) of CON3 group which still left the group at diabetic level of 32.4mg/dl. At other levels of tea intake (1g/100ml and 2g/100ml), no reduction was observed rather an increment of 0.12% and 14.57% respectively was noted in their initial average fasting blood glucose concentrations.

**Fig 1:** Effects of different concentrations of green tea (*Camellia sinensis*) on average fasting blood glucose concentration

*NC* (non-diabetic group that took tap water and commercial rat feed),
*DC* (diabetic group that took tap water and commercial rat feed),
*CON1* (diabetic group that took 1g/100ml of green tea and commercial rat feed),
*CON2* (diabetic group that took 2g/100ml of green tea and commercial rat feed),
*CON3* (diabetic group that took 3g/100ml of green tea and commercial rat feed).

<table>
<thead>
<tr>
<th>Tea</th>
<th>NC</th>
<th>DC</th>
<th>CON1</th>
<th>CON2</th>
<th>CON3</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Camellia sinensis</em> (Green tea)</td>
<td>+0.44</td>
<td>+60.48</td>
<td>+0.12</td>
<td>+14.57</td>
<td>-0.50</td>
</tr>
</tbody>
</table>

Positive values indicate increment in blood glucose
Negative values indicate reduction in blood glucose
All values are in percentage
*NC* (non-diabetic group that took tap water and commercial rat feed),
*DC* (diabetic group that took tap water and commercial rat feed),
*CON1* (diabetic group that took 1g/100ml of green tea and commercial rat feed),
*CON2* (diabetic group that took 2g/100ml of green tea and commercial rat feed),
*CON3* (diabetic group that took 3g/100ml of green tea and commercial rat feed).
Lipid profile or lipid panel is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids such as various disturbances of cholesterol and triglyceride levels, many forms of which are recognized risk factors for cardiovascular disease and rarely pancreatitis(http://en.wikipedia.org, (March, 2014). Increase in serum total cholesterol was associated with increased risk of atherosclerosis in the past but recent reports now indicate the LDL/HDL ratio as a stronger index of atherogenicity of the lipoproteins rather than the lipid profile of individual lipoprotein fractions, thus the lower the ratio the less atherogenic the lipoprotein profile (Wallidus et al., 2001).

Thus, from the results of the lipid profile and LDL/HDL ratios of the different treatment groups shown in Table 2, it could be observed that in all the different levels of green tea intake CON1, CON2, CON3), the tea caused significant reduction in mean serum total cholesterol, mean serum triglyceride and mean serum LDL-cholesterol when compared with the diabetic group DC that did not take the green tea. However, the tea could only cause significant increase in HDL at 6g/100ml of tea intake (CON3).These result are not far from the findings of Imai and Nakachi (1995), Bursill et al., (2001), Yokozawa et al., (2002), Sussana et al., (2006), and Haidari et al., (2012) who have associated green tea drinking with lowering serum levels of total cholesterol, triglycerides, and LDL-cholesterol but higher serum levels of HDL-cholesterol. The LDL/HDL ratio of the diabetic group DC was significantly higher than that of the non-diabetic group NC; reason being attributed to a alteration in lipid profile due to diabetes mellitus (Orchard, 1990). The LDL/HDL ratios, in all the concentrations of tea intake considered CON1, CON2 and CON3, were significantly lower than that of the diabetic group DC that did not take the green tea. This denotes the positive effect of this tea in lowering the LDL/HDL ratio which is a good step towards reducing the risk factor of cardiovascular diseases.

Table 2: Lipid Profile and LDL/HDL Ratios of the Treatment Groups

<table>
<thead>
<tr>
<th>Tea samples</th>
<th>NC</th>
<th>DC</th>
<th>CON1</th>
<th>CON2</th>
<th>CON3</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCH</td>
<td>85.1±11.5</td>
<td>101.5±6.8</td>
<td>53.3±2.5</td>
<td>63.7±1.5</td>
<td>80.3±1.2</td>
<td>11.15</td>
</tr>
<tr>
<td>TRIG</td>
<td>82.7±2.4</td>
<td>140.0±5.0</td>
<td>92.0±1.0</td>
<td>85.0±10.0</td>
<td>98.0±9.0</td>
<td>12.10</td>
</tr>
<tr>
<td>HDL</td>
<td>58.1±4.0</td>
<td>49.1±3.3</td>
<td>31.7±1.5</td>
<td>42.7±2.5</td>
<td>57.7±2.5</td>
<td>5.26</td>
</tr>
<tr>
<td>LDL</td>
<td>17.6±4.9</td>
<td>24.4±8.6</td>
<td>3.6±0.8</td>
<td>3.4±0.5</td>
<td>2.9±1.7</td>
<td>9.21</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.30±0.08</td>
<td>0.50±0.19</td>
<td>0.11±0.02</td>
<td>0.08±0.01</td>
<td>0.05±0.03</td>
<td>0.17</td>
</tr>
</tbody>
</table>

LSD: Least Significant Difference
NC (non-diabetic group that took tap water and commercial rat feed),
DC (diabetic group that took tap water and commercial rat feed),
CON1 (diabetic group that took 1g/100ml of green tea and commercial rat feed),
CON2 (diabetic group that took 2g/100ml of green tea and commercial rat feed),
CON3 (diabetic group that took 3g/100ml of green tea and commercial rat feed).

Conclusion
Green tea did not confer any anti-diabetic property on blood glucose in the three concentrations of tea intake studied but was only able to retard the rate at which the blood glucose was increasing. Thus, it can be used to delay onset of diabetes especially at per-diabetic stage. However, green tea could be one of the effective means of controlling blood lipids because of its significant reduction effects on total cholesterol, triglycerides, LDL-cholesterol and especially LDL/HDL ratio.

References


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