Effects of Green Tea on Activity of α-Glucosidase in Vitro and Glycaemic Index of White Bread in Vivo

Lawal Tajudeen Afolayan¹, Ononamadu Chimoabi James², Okonkwo Emmanuel Krist³, Adedoyin Halimat Jumai, Shettima Muhammad Liman⁴, Muhammad Ibraham Usman⁵, Alhassan Adamu Jibrin⁶

¹Department of Biochemistry and Forensic Science, Faculty of Science, Nigeria Police Academy, Wudil-Kano, Kano State. Nigeria (Corresponding author)
²³⁴⁵Department of Biochemistry and Forensic Science, Faculty of Science, Nigeria Police Academy, Wudil-Kano, Kano State. Nigeria
⁶Department of Medical Biochemistry, College of Medical Sciences, Yobe State University, Damaturu, Yobe. Nigeria
⁷Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University Kano, Kano State. Nigeria

Abstract: Activities of α-glucosidase enzyme and high glycaemic index diet have been implicated in postprandial hyperglycaemia, the control of which might be useful in curbing diabetes mellitus. Some herbs have been reported to show inhibitory activities against carbohydrate digesting enzymes (such as α-glucosidase) and lower the glycaemic index of a carbohydrate-rich diet. In the present study, lowering effect of green tea (Camellia sinensis) on α-glucosidase activity and on the glycaemic index of white bread were investigated in vitro and in vivo respectively. For in-vitro study, α-glucosidase enzyme was incubated with substrate (p-nitrophenyl glucopyranoside) in the absence and presence of inhibitor (green tea) and its activity was investigated. Thirty (30) healthy participants were involved in the in vivo study of glycaemic index. Ten-hour fasting blood glucose levels of the participants were taken before they took bread and tea and then the postprandial glucose levels were taken at 15, 30, 45, 60, 90 and 120 minutes accordingly. The results of this study revealed that the green tea lowered the activity of α-glucosidase to 17.59% with IC₅₀ of 200.30µg/mL and Ki of 113.04µg/mL. It also lowers the glycaemic index of white bread (85.57%) to 33.95% when bread consumption was delayed for 5 minutes after taking the tea. In conclusion, this study shows that green tea can be used to lower the postprandial blood glucose level which might be beneficial in the control and management of diabetes mellitus.

Keywords: Diabetes mellitus; Hyperglycaemia; Glycaemic index; α-Glucosidase; Green tea

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterised by hyperglycaemia due to lack of or insufficient insulin production by the pancreas or ineffective use of the insulin produced (WHO, 2018). Activities of carbohydrate digesting enzymes such as α-glucosidase has been implicated in post prandial hyperglycaemia (Alhassan et al., 2017), the control of which might be helpful in the management of diabetes mellitus. Uninhibited hyperglycaemia leads to diabetes mellitus, which also lead to many complications such as nephropathy, retinopathy, neuropathy, ischaemic heart disease, stroke and peripheral vascular disease (Alhassan et al., 2017; WHO, 2018). Increasing prevalence of diabetes worldwide has called for new approaches to its management and diets low in glycaemic index (GI) have been proposed as a useful means for managing glucose response (Ojo et al., 2018; Lawal et al., 2019).

Glycemic Index (GI) is a scientific ranking of how carbohydrate-rich foods affect blood sugar levels in 2-hour of consumption (Ojo et al., 2018; ADA 2019). GI of Food is evaluated against pure glucose, which has a value of 100 on the index, the index ranges from 0 to 100 with 0 – 55 (low), 56 – 69 (medium) and 70 – 100(high) (ADA 2018). Carbohydrates that digest quickly increase blood sugar rapidly and is classified as high GI, the ones that digest moderately and mildly release sugar into the bloodstream is classified as medium GI, while that digest slowly release sugar sluggishly into the bloodstream are referred to as low GI (Venn et al., 2011; Greenwood et al., 2013; Ojo et al., 2018; Lawal et al., 2019). GI is a measure of percentage of incremental area under curve (IAUC) with respect to 2-hour blood glucose following the ingestion of a test diet compared with a standard diet (glucose or bread) (Venn et al., 2011, Lawal et al., 2017, Ojo et al., 2018; Lawal et al., 2019). GI value of food is not based on the features of the individual that consumed the food but, on the food itself (Venn et al., 2011). Hence, dietary management tactics that target weight loss and improved glycaemic control in patients with type-2 diabetes may rely on the use of diets with low glycaemic index (Ojo et al., 2018; Lawal et al., 2019).

Green Teas a worldwide herbal beverage made from the prepared leaves and leaf buds of Camellia sinensis (L.), it belongs to the plant family Theaceae and is a species of evergreen shrub or small tree (Sinha and Mishra, 2008). Tea from Camellia sinensis can be categorised into three types depending on the level of formulations i.e. green (unfermented), oolong (partially fermented) and black (fermented) tea. Here, the term fermentation means oxidation, which means exposure of the leaves to air while drying without any addictive during the process (Sinha and Mishra, 2008; Ahmed and Stepp 2013; Reygaert 2017). The major chemical components of interest in green tea was
reported to be polyphenols which are majorly flavonoids and the four major flavonoids in the green tea are the catechins, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Graham 1992; Sinija and Mishra, 2008). Out of these, epigallocatechin gallate is regarded as the most significant active component. The concentration of total polyphenols in dried green leaves was found to be about 10% and other components of interest in the dried tea leaves are gallic acid, quercetin, kaempferol, myricetin, caffeic acid and chlorogenic acid (Katiyar and Elmets, 2001; Sinija and Mishra, 2008; Reygaard, 2017). The uniqueness of green tea is the way its leaves are processed, green tea leaves are steamed, which prevent the epigallocatechin gallate compound from being oxidised (Katiyar and Elmets, 2001). Researches have proved the health benefit of green tea which include; anti-cancer, anti-fungal, anti-viral, anti-HIV, antioxidant and anti-inflammatory effects. Other benefits are skin treatment, cholesterol reduction (Ahmed and Stepp, 2013; Reygaard, 2017) and prevention of diabetes (Takatoshi et al., 2006). In the present studies, effects of green tea to inhibit α-glucosidase catalysis and to lower the glycaemic index of a white bread were investigated in vitro and in vivo respectively.

2. Materials and Methods

2.1 Materials

2.1.1 Equipment
Equipment used for this study includes, centrifuge, spectrophotometer, water bath, weighing machine, heater, oven, micropipettes, test-tubes, test-tube rack and ACCU-CHEK Active glucometer’.

2.1.2 Green Tea
Green tea (source: Bilje Borca, Lot no: L002 and best before 2021) was purchased from SAHAD STORE along zoo road in Kano metropolis of Nigeria. The tea was prepared according to the instruction on the tea pack label. One sachet was soaked in 200mL boiling water for 10 minutes. The concentration of the tea was then determined.

2.1.3 Reagents
Saccharomyces cerevisiae-α-glucosidase, p-nitrophenyl glucopyranoside (pNPG) was purchased from Sigma-Aldrich USA, D-glucose, p-Nitrophenol, sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate were purchased from Hopkin & Williams Chadwell Health Essex England. Sodium carbonate was purchased from BDH Chemicals Ltd Poole England.

2.2 Methodology

2.2.1 α-Glucosidase Inhibitory Assay
The effect of green tea on α-glucosidase activity was determined according to the method of Kang et al., (2011) with little modifications. Various concentrations of the tea; 10, 25, 50, 100 and 150 µg/mL were prepared in 20 mM of sodium phosphate buffer (pH 6.9). Aliquot amount (100µL) of each concoction was pre-incubated with 100µL of α-glucosidase enzyme (1.0U) at 37°C for 10 minutes. The mixture was then incubated with 100 µL of 10 mM p-nitrophenyl-α-glucopyranoside (pNPG) solution at 37°C for another 10 minutes. The reaction was put to halt by adding 2 mL of 0.2M Na₂CO₃ solution to the reacting mixture. The mixture was diluted with 5mL of deionised water and the absorbance of the mixture was taken at 405 nm. The control sample without the concoction (negative control) was treated in the same manner but buffer was substituted for the tea. Acarbose was also used in place of tea as positive control and treated in the same way.

The experiments were run in triplicates and the absorbance was converted to the amount of p-nitrophenol released using p-nitrophenol standard curve and the enzyme activities were determined by using the formula;

\[
\frac{\text{mg of p-nitrophenol released x Dilution factor}}{\text{Time of incubation x mg of enzyme in the reaction mixture}}
\]

Percentage of enzyme activities was determined from the formula:

\[
\% \text{Enzyme Activity} = \frac{\text{Activity of the Enzyme with Tea x 100}}{\text{Activity of the Enzyme without Tea}}
\]

A plot of percentage enzyme activity was made against the concentrations of the tea concoction and the concentration of the concoction that inhibits 50% enzyme activities (IC₅₀) was determined graphically.

2.2.2 Mode of α-Glucosidase Inhibition
The mode of inhibition of the tea was determined using the modified method of Ali et al (2006) and the experiment was run in triplicate. Two different concentrations of the tea were prepared; 100 and 200µg/mL, and five different concentrations (2.0, 4.0, 6.0, 8.0 and 10.0 mM) of pNPG solutions were prepared as well. 100µL of each extract concentration was pre-incubated with 100µL of α-glucosidase (1.0U) separately for 10 minutes at 37°C in the five pairs of test-tubes. The mixtures were then incubated with 100µL of the different concentration of substrate (pNPG) at 37°C for catalysis to commence for another 10 minutes. The reactions were put to halt by the addition of 2mL of 0.2M Na₂CO₃ and the absorbance of the mixtures were taken at 405 nm. The control sample was also prepared and treated likewise, but the concoction was replaced by buffer solution.

The amount of p-nitrophenol released was determined spectrophotometrically using a p-nitrophenol standard curve and converted to reaction velocities (v).

\[
v = \frac{\text{Amount of p-nitrophenol (µg) x Dilution Factor}}{\text{MWp-nitrophenol x Time x Volume of Enzyme (mL)}}
\]

A Lineweaver-Burk (double reciprocal) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted. The mode of inhibition of the concoction on α-glucosidase activity was determined by...
analysis of the double reciprocal plot using Michaelis-Menten kinetics.

2.2.3 Effect of Green Tea on Glycaemic Index (GI) of White Bread

A total of 30 participants aged 20 – 24 years voluntarily took part in this study, using a modified method of Gilian, 2011. Participants were composed of five groups of six healthy cadets each. Before each test day, participants were instructed to consume their evening meal latest by 8:00PM and to refrain thereafter from food and beverages and were asked to report to lab the next morning following an overnight fast of at least 10 hours. Each group was given the following:

Group 1: Glucose only (take glucose only)
Group 2: Bread only (take bread only)
Group 3: Bread + Teas (taken simultaneously)
Group 4: Bread + 5 minutes + Tea (take bread and wait for 5 minutes before tea)
Group 5: Tea + 5 minutes + Bread (take tea and wait for 5 minutes before bread)

Fasting blood was taken by venepuncture for the determination of glucose using ACCU-CHEK Active instrument. Baseline blood samples were collected before bread (50g) and tea consumption and postprandially at 15, 30, 45, 60, 90 and 120 minutes. Bread (50g) and tea were consumed at an even pace over 15 minutes and participants remained seated throughout the test periods. Incremental area under the curve (iAUC) was calculated using trapezoid method as described by FAO, 1998. The glycaemic index was then calculated as shown in the equation below;

\[
GI = \frac{iAUC_{\text{Bread} (+ \text{Tea})}}{iAUC_{\text{Glucose}}} \times 100
\]

Where GI is the glycaemic index; iAUC_{\text{Bread} (+ \text{Tea})} is the incremental area under the curve of bread only, bread before tea or tea before bread as applicable while iAUC_{\text{Glucose}} is incremental under the curve of glucose only. Note that glucose is used to standardise GI of bread.

2.2.4 Statistical Analysis

The values or the characteristic of the samples were compared using t-test

3. Results and Discussions

3.1 Results

Inhibitory effect of green tea concoction on α-glucosidase catalytic activity is depicted in table 1. Here, the enzyme activity decreases as the concentration of the concoction increases and the decrease became significant (p<0.05) at 300µg/mL and above when compare to the control group without the tea concoction.

Table 1: Concentrations effect of Green tea concoction on α-glucosidase activity

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Control</th>
<th>100</th>
<th>300</th>
<th>400</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase Activities (µmol p-nitrophenol/min/mg enzyme)</td>
<td>15.43±0.95</td>
<td>10.58±0.83*</td>
<td>6.14±0.89*</td>
<td>3.99±0.52*</td>
<td>2.70±0.37*</td>
</tr>
</tbody>
</table>

Values are mean plus standard deviation (of Triplicate)

* The difference is statistically significant (p<0.05) when compare to the control group

Figure 1 depicts how the inhibitory concentration of 50% enzyme activity (IC\(_{50}\)) was determined from the plot of percentage α-glucosidase activity against the concentration of green tea concoction. Here the IC\(_{50}\) is 200.03±3.87µg/mL.

Figure 2: Lineweaver-Burk plot of α-glucosidase with and without green tea

Figure 3 depicts a plot of slope of Lineweaver-Burk plot against the concentration of the green tea concoction, the intercept on x-axis is -K_i. Here the K_i is 113.04 µg/mL.
The result of the effect of green tea concoction on glucose response curve was depicted in figure 4. Here, blood glucose level of glucose only group increased from the baseline to 4.10µmol/L which is the highest peak. The second peak is the bread only group which rose from baseline to 2.6 µmol/L. The third peak (1.9µmol/L) was obtained from the group in which both bread and tea were co-administered, the fourth peak (1.5µmol/L) was from the group in which taking tea was delayed for 5 minutes after the administration of bread, and the last peak (1.1µmol/L) was from the group in which taking bread was delayed for 5 minutes after the administration of tea.

Figure 5 depicted the result of effect of the green tea on the glycaemic index of white bread. GI of glucose is 100% and was used to standardise the white bread which was 85.51%. Co-administration of both bread and tea, taking bread and wait for 5 minutes before taking tea and delaying intake of bread for 5 minutes after intake of tea, decreased GI of the white bread to 51.06%, 38.11% and 33.95% respectively.

4. Discussion

The rising occurrence of diabetes mellitus worldwide demands for new tactics to its management and diets with low glycaemic index have been anticipated as a usual means for managing glucose response. High glycaemic index food and activity of α-glucosidase enzyme have been apprehensive in raising blood glucose level rapidly. If not properly controlled, this rapidly rising in blood glucose level (postprandial hyperglycaemia) may lead to diabetes mellitus, which is associated to many complications (Lawal et al., 2017). Ethnobotanical survey has revealed many herbs with great potential in lowering blood glucose level and less toxic (Alhassan et al., 2017) Thus, it is imperative to search for any herbal concoction that can reduce the glycaemic index of this food or inhibit the activity of this enzyme in order to curb postprandial hyperglycaemia. When hyperglycaemia is properly controlled, it may diminish the likelihood of diabetes mellitus incidence. Evidence has suggested that dietary GI is positively associated with type-2 diabetes (Venn et al., 2011).

The results of this study indicate that green tea (*Camellia sinensis*) concoction, when taken in the right doses and in the correct order may be useful in cutting down the prevalence of postprandial hyperglycaemia. This finding may be due to the presence of the active component of the tea (*Camellia sinensis*), epigallocatechin gallate (EGCG), which might inhibit α-glucosidase activity and thereby slow down the release of glucose molecules into the blood stream. Thus, reduces the glycaemic index of the diet. These findings are in line with that of other researchers that components of some medicinal plants have anti-hyperglycaemic properties. Lawal et al., 2017 revealed the glycaemic index lowering effect of aqueous extract of *Persea americana* seeds (AEPAS). In their research, AEPAS reduced GI of starch diet from 82.11% to 18.49%. Alhassan et al., 2017 investigated the inhibitory effect of AEPAS on α-glucosidase activity and found out that AEPAS inhibited activity of the enzyme significantly (p < 0.05) with IC<sub>50</sub> of 13.52µg/mL. In this same vein, aqueous extract of *Ocimum gratissimum* leaves (AEOGL) has been shown to
reduce the GI of starch food from 82.11% to 19.51% (Lawal et al., 2019). Findings have suggested that epicatechin gallate (ECG) may be a potential inhibitor of α-amylase (IC$_{50}$= 45.30±0.30µg/mL) and α-glucosidase (IC$_{50}$= 4.03±0.01µg/mL) which could be used as a nutrient supplement for the prevention of diabetes (Wu et al., 2019). Gao et al., (2013) confirmed the beneficial effects of green tea, green tea polyphenols and epigallocatechin gallate in the treatment of diabetes mellitus and this was ascribed to their inhibitory effect against α-amylase and α-glucosidase in gastrointestinal tract. IC$_{50}$ value of EGCG against α-glucosidase was reported to be 5.27±0.01µg/mL and was much lower than the IC$_{50}$ of acarbose against α-glucosidase (4822.78±26.04 µg/mL) (Gao et al., 2013). This suggests that EGCG strongly suppressed the activity of α-glucosidase and that this maybe be utilized for the control of postprandial hyperglycaemia. Research conducted by Xu et al., (2019) also supported that α-glucosidase inhibitory activity of EGCG (IC$_{50}$= 19.50±0.30µM) was much higher than that of acarbose (IC$_{50}$= 278.70±1.10µM) and the mode of inhibition is reversible non-competitive. Molecular docking results of the research of Xu et al., (2019) showed that binding sites of α-glucosidase for EGCG were close to the active site pocket of the enzyme.

5. Conclusion

In conclusion, results of this findings indicate that green tea concoction inhibited α-glucosidase significantly (p<0.05) and lowered significantly (p<0.05) glycaemic index of white bread. This strategy may be helpful in deterrence of postprandial hyperglycaemia in order to caution diabetes mellitus and its complications.

6. Acknowledgement

The authors of this article appreciate the efforts of all cadets of Nigeria Police Academy who voluntarily participated in this research. We also thank the medical laboratory scientists of Nigeria Police Academy Clinic for their roles in this study.

7. Conflict of Interest

All the authors of this work declare no conflict of interest.

8. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

List of Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNPGP</td>
<td>para-nitrophenyl glucopyranoside</td>
</tr>
<tr>
<td>GI</td>
<td>glycaemic index</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Inhibitory concentration of 50% activity</td>
</tr>
<tr>
<td>iAUC</td>
<td>incremental area under the glucose response curve</td>
</tr>
<tr>
<td>iAUC$_{bread}$</td>
<td>incremental area under the glucose response curve after consuming bread only</td>
</tr>
<tr>
<td>iAUC$_{Glucose}$</td>
<td>incremental area under the glucose response curve after consuming glucose only</td>
</tr>
<tr>
<td>Km</td>
<td>Michaelis-Menten constant</td>
</tr>
<tr>
<td>Ki</td>
<td>inhibitory constant</td>
</tr>
<tr>
<td>AEPAS</td>
<td>aqueous extract of <em>Persia americana</em> seeds</td>
</tr>
<tr>
<td>AEOG</td>
<td>aqueous extract of <em>Ocimum grattissimum</em> leaves</td>
</tr>
<tr>
<td>ECGG</td>
<td>epigallocatechin gallate</td>
</tr>
</tbody>
</table>

References


Volume 9 Issue 1, January 2020

www.ijsr.net

Licensed Under Creative Commons Attribution CC BY

Paper ID: ART20204148
DOI: 10.21275/ART20204148
928
OcimumGratissimum mon Glycaemic Index of Starch Meal. Global Journal of Medicinal Plants Research: 5 (3) 009 – 015


**Figure Legend**

**Figure 1:** Plot of percentage α-glucosidase activity against concentration of green tea from which IC₅₀ was determined. X-axis is the concentration of green tea (mg/mL) and y-axis is the percentage of α-glucosidase activity (i.e. residual activity). The concentration of the green tea that corresponds to 50% of α-glucosidase activity was determined as IC₅₀ as shown with broken line in the figure.

**Figure 2:** Lineweaver-Burk plot of α-glucosidase with and without green tea. Lineweaver-Burk plot (also known as double reciprocal plot) is the plot of inverse of initial velocities (1/v) against inverse of concentration of substrate, pNPG, (1/[pNPG]). The blue line is the control i.e. without green tea, orange colour line is with 100µg/mL of green tea while the light black line is with 200µg/mL of green tea. The inverse of intercept on the x-axis is -Km and -Km.app

**Figure 3:** Secondary plot from slope of Lineweaver-Burk (y-axis) versus the concentration of green tea (µg/mL) on x-axis. The intercept on the x-axis is –Ki

**Figure 4:** Effect of green tea on glucose response curve of white bread. The plot of rise in blood glucose level (mmol/L), after the baseline glucose level has been deducted, was plotted against time (minutes). Orange colour line (with …V…) is the glucose response curve for consumption of bread only group; blue line (with ---o---) is the glucose response curve for consumption of glucose only group; yellow line (with ---0---) is the glucose response curve for consumption of green tea after 5 minutes of green tea consumption; deep blue line (with ---*---) is the glucose response curve for consumption of green tea after 5 minutes of taken bread; while light black line (---c---) is the glucose response curve for consumption of both tea and bread simultaneously.

**Figure 5:** Effect of green tea on glycaemic index of white bread. Values are mean ± standard deviation, n = 6 for each treatment, significant difference (p < 0.05 student’s t-test) between glycaemic index of control and the treatments are indicated by a (*).