

Full Length Research

Effect of Aqueous Extract of *Ocimum Gratissimum* on Glycaemic Index of Starch Meal

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Diabetes mellitus is a chronic metabolic disorder characterised by hyperglycaemia, which leads to many complications such as nephropathy, neuropathy, eye problems and many others. High glycaemic index foods have been implicated in post-prandial hyperglycaemia, the control of which might be helpful in the management of diabetes mellitus. Glycaemic index is a measure of power of food to raise blood glucose level after its ingestion. In the present study, lowering effect of aqueous extract of *Ocimum gratissimum* leaves (AEOGL) and its acute toxicity were investigated using white albino rats. The result of oral acute toxicity (LD₅₀) revealed that the AEOGL is relatively safe by having oral LD₅₀ greater than 5000mg/Kg body weight. Co-administration of AEOGL and food, and administration of AEOGL before and after food were investigated to know the best order of treatment. On the glucose response curve, AEOGL brought the blood glucose level back to the baseline before the expiration of 2-hour period of investigation. The blood glucose level was still above the baseline in the subjects treated with meal only without the AEOGL at the end of 2-hour period of investigation. Delaying the ingestion of food for five minutes after administration of AEOGL brought the glucose response curve to the baseline fastest. On the lowering the glycaemic index of food, all the orders of administration of AEOGL and food lower the glycaemic index of the food significantly ($p > 0.05$) when compare to the standard foods only (Starch and glucose separately). Delaying the ingestion of food for five minutes after AEOGL administration lowers the GI best. It lowers the GI of the starch from 82.11% to 19.51%. This strategy can be employed in the control and management of diabetes.

Keywords: Glycaemic index, Diabetes mellitus, Aqueous extract, *Ocimum gratissimum*, blood glucose, Acute toxicity.

INTRODUCTION

Diabetes is a metabolic disorder characterised by rise in blood glucose level (hyperglycaemia). It occurs when the pancreas cannot produce enough insulin (a hormone that regulates blood sugar) or when the body cannot efficiently use the insulin produced (WHO, 1999). Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

Over time, diabetes can damage heart, blood vessels, eyes, kidneys and nerves. According to the World Health Statistics (2012), one in ten adults worldwide has diabetes and WHO (2014) projects that diabetes will be the seventh leading cause of death by 2030.

Glycaemic index (GI) of a food is a measure of how the carbohydrate-containing food raises blood glucose level after meal. Foods are ranked based on how they raise blood glucose level when compare to a

reference food — either glucose or white bread (ADA, 2014). ADA, 2014 categorised food into three, based on their GI values: Low GI Foods (55% or less), Medium GI (56% – 69%) and High GI (70% or more). Glucose or white bread are classified as standard food and assumed to have GI of 100%.

Diets that composed of carbohydrates which digests quickly and absorbed rapidly into the blood stream are frequently termed as high GI foods (ADA, 2014). Besides increasing blood glucose level, diets with high GI also increase insulin responses following consumption (Radulian *et al.*, 2009). In contrast, diets with a low glycaemic index usually contain carbohydrates that digest slowly and are slowly absorbed (Ojo *et al.*, 2018). Consequently, these low GI foods have a sluggish influence on blood glucose levels and insulin response.

Hyperglycaemia is the medical term for a high blood glucose level which is a common problem for people with diabetes (Radulian *et al.*, 2009). The aim of diabetes treatment is to keep blood sugar levels as near to normal as possible. After consumption of high GI foods, there is a large, rapid rise in the level of blood glucose (Ojo *et al.*, 2018). LowGI foods may also delay the return of hunger, by slowing gastric emptying. Frequent consumption high GI foods have been implicated in postprandial hyperglycaemia, the control of which might be helpful in monitoring diabetes mellitus (Radulian *et al.*, 2009).

Ocimum gratissimum(OG) is a family of *Lamiaceae*, which is herbaceous plant commonly found in the savannah, tropical rain forest and coastal areas of West Africa and tropical Asia (Okonet *al.*, 2012). It is commonly called 'scent leaf' in Nigeria and it is commonly used as a condiment in cooking (Ugonna, 2013). The local names are Efinrin, Efirinaaja, Erumaba (Yoruba), Daidoyatagida (Hausa), Esewon (Edo-Akoko), Nehonwu, Nchanwu or Ahuji (Igbo), Nton (Ibibio/Efik), Aramogbo (Edo) and Menthesauvage (French) (Nwinyi *et al.*, 2009; Okonet *al.*, 2012; Ugonna, 2013). Reports showed that various extracts of this plant were effective in the treatment of diabetes mellitus (Oguanobi *et al.*, 2012). Aqueous extract of OG reduced blood sugar level in streptozotocin-induced diabetic rats and alleviated the prime symptoms of diabetes mellitus namely; polydipsia, polyphagia and weight loss (Okon *et al.*, 2012). OG is alleged for several therapeutic properties including hypoglycaemic, anti-helminthic effect, antifungal properties, antibacterial activities and anti-convulsant activity (Orafidiya *et al.*, 2000). Phytochemical analysis of OG revealed important constituents as tannins, alkaloids, saponins, flavonoids, steroids, phlobatannin, terpenoids, cardiac glycosides and phenolic compounds (Afolabi *et al.*, 2007; Abdullah, 2012). It also contains anti-nutrient

phytin phosphorus, oxalate, phytic acid and polyphenols (Fagbohun *et al.*, 2012; Abdullah, 2012). No research on the effect of OG on GI of food has been reported.

In the present study, effect of aqueous extract of *Ocimum gratissimum* on glycaemic index of a standard food was investigated

MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Equipment used for this study includes, centrifuge, spectrophotometer, water bath, weighing machine, heater and glucometer

2.1.2 Medicinal Plant Used

Ocimum gratissimum leaves were obtained locally from Ilorin, Kwara state, Nigeria. The leaves were identified in herbarium unit of Biological department of Bayero University Kano, Nigeria and was assigned herbarium number BUKHAN00306. A sample of the leaves were deposited in the herbarium for record purpose.

2.1.3 Reagents

Soluble starch was purchased from Hopkin & Williams Chadwell Health Essex England.

3.1.4 Animals

A total of 41 Wistar albino rats (25 males and 16 females) were purchased from zoological garden of Biological Science Department, Bayero University Kano, Nigeria.

2.2 METHODOLOGY

2.2.1 Preparation of the Aqueous Extract of *Ocimum gratissimum* Leaves (AEOGL)

Aqueous extract of *Ocimum gratissimum* leaves (AEOGL) was prepared by washing and air-dried the leaves in shade for seven days and minced to powder using mortar and pestle. A quantity of 50g of the powder was soaked in 500mL of deionised water. The mixture was allowed to stand for 48 hours with intermittent shaking and thereafter filter with mesh cloth. The liquid mixture was then centrifuged at 8000 rpm for 10 minutes. The extract was evaporated at

45°C in oven to obtain a solid residue. The solid residue extract was reconstituted in deionised water in appropriate concentration before administration.

2.2.2 Acute Toxicity Studies (LD₅₀ Determination)

The median lethal dose (LD₅₀) of the plant extract was determined by method of Lorke (1983)

LD₅₀ test of the plant extract was carried out in two phases (shown below) using sixteen (16) Wistar albino rats as design below. The actual amounts (concentrations) of the extract (AEOGL) were delivered into the animals' gastrointestinal tract through the use of plastic cannula. However, the feeds were provided freely to the animals.

First Phase

Group I (n = 2) + Feed + Distilled water (oral cannulation) + 24-hour Observation -Control

Group II (n = 2) + Feed + AEOGL (1000mg/Kg.b.w, oral cannulation) + 24-hour observation

Group III (n = 2) + Feed + AEOGL (1600mg/Kg.b.w, oral cannulation) + 24-hour observation

Group IV (n = 2) + Feed + AEOGL (2000mg/Kg.b.w, oral cannulation) + 24-hour observation

Second Phase*

Group I (n = 2) + Feed + AEOGL (3000mg/Kg.b.w, oral cannulation) + 24-hour observation

Group II (n = 2) + Feed + AEOGL (3500mg/Kg.b.w, oral cannulation) + 24-hour observation

Group III (n = 2) + Feed + AEOGL (4000mg/Kg.b.w, oral cannulation) + 24-hour observation

Group IV (n = 2) + Feed + AEOGL (5000mg/Kg.b.w, oral cannulation) + 24-hour observation

*The outcome of phase I determined the actual doses of Phase II and occurred because no animal died at Phase I.

Where n is the number of animals (rats) per group. The median lethal dose (LD₅₀) was calculated using maximum dose of the second phase. The median lethal dose was calculated as follows:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where D₀ = Dosage of 0% mortality

D₁₀₀ = Dosage of 100% mortality

2.2.3 Effect of AEOGL on GI of Starch Meal

Effect of AEOGL on glycaemic index (GI) of a

starch diet was determined using twenty-five (25) white albino rats. The rats were divided into five (5) groups of 5 rats each. Overnight fasting blood glucose concentrations of the rats were taken at zero time before given amount of extract (one-tenth of LD₅₀) and a starch meal containing 0.15g carbohydrates by oral cannulation as designed below.

Group I (n = 5): Overnight fasting (12-hour) + (0.15g glucose) only + Equivalent volume of AEOGL as water. (This is the standard used to standardise the starch used as meal).

Group II (n = 5): Overnight fasting (12-hour) + (0.15g Soluble Starch) only –Control

Group III (n = 5): Overnight fasting (12-hour) + (0.15g Soluble Starch + AEOGL, one-tenth LD₅₀) simultaneously.

Group IV (n = 5): Overnight fasting (12-hour) + AEOGL (one-tenth LD₅₀, before) + 5minutes + (0.15g soluble starch)

Group V (n = 5): Overnight fasting (12-hour) + (0.15g soluble starch) + 5minutes + AEOGL (one-tenth LD₅₀)

Animals' blood glucose levels were measured at 0, 15, 30, 45, 60, 90 and 120 minutes of AEOGL and starch administration.

A plot of blood glucose concentration (mg/dL or mmol/L) on vertical axis against time (in minutes) on horizontal axis was constructed. This plot is called blood glucose response curve. Before the plots were constructed, the starting point glucose levels of the rats under investigations were deducted from their respective glucose levels observed over 120 minutes. That is, the baseline values were zeroed by deducting the respective baseline values from all the values obtained under investigations.

Glycaemic Index (GI) of each group was determined by calculating the incremental area under two hours of blood glucose Curve (iAUC) and compared with the iAUC for standard diet (control) using the following equation:

$$GI = \frac{iAUC \text{ for (Diet + Extract)}}{iAUC \text{ for Standard Food}} \times 100$$

iAUC = incremental area under the curve over 2-hour

RESULTS AND DISCUSSION

The result of acute toxicity (LD₅₀) is presented in table 1. For the two phases (phase I and phase II of AEOGL administration), no mortality case was recorded. Also, throughout the course of 24-hour of investigation, no sign of toxicity was recorded. This indicates that the extract is relatively safe. Moreover, the extract have

LD₅₀ higher than 5000mg/Kg body weight (table 1), which makes it to be relatively non-toxic.

Table 1: Result of LD₅₀ of AEOGL on Albino Rats

Extract	Experiment	Group	Dose (mg/Kg.b.w) orally	Number of rats/groups	Mortality	LD ₅₀
AEOGL	Phase I	I	water	2	0/2	>5000mg/Body weight (Kg)
		II	1000	2	0/2	
		III	1600	2	0/2	
		IV	2000	2	0/2	
	Phase II	I	3000	2	0/2	
		II	3500	2	0/2	
		III	4000	2	0/2	
		IV	5000	2	0/2	

Outcome of effect of AEOGL on the blood glucose response curve and GI of starch meal are indicated in figure 1 and figure 2 respectively. Co-administration of AEOGL and food, and administration of AEOGL before and after food were investigated to determine the most effective order of administration. The blood glucose response curve of all the animals treated with AEOGL fall back to baseline before the expiration of 2-hour of investigation. The blood glucose level was still above the baseline in the animals treated with starch meal only without the AEOGL at the end of 2-hour period of investigation. Delaying the ingestion of food for five minutes after administration of AEOGL brought the glucose response curve to the baseline fastest. Here, the blood glucose level was brought to baseline at 78 minutes after administration of meal. The second-best administration is the administration of both meal and AEOGL simultaneously, this brought the blood glucose level to baseline at 84 minutes. The least effective order of administration is by delaying the

administration of AEOGL for five minutes after ingestion of meal, this brought the blood glucose level to baseline at 100 minutes.

On the effect on GI of food, all the orders of administration of AEOGL lower the GI of the food significantly ($p > 0.05$) when compared to the standard foods only (Starch and glucose separately). Delaying the ingestion of food for five minutes after AEOGL administration lowers the GI best. It lowers the GI to 19.51%. The second-best order of administration is the simultaneous administration of both AEOGL and food, it lowers the GI to 25.83%. The least effective order of administration is delaying administration of AEOGL for five minutes after ingestion of food, it lowers the GI of starch meal from 82.11% to 46.20%. The most effective order of administration is to delay the ingestion of food for five minutes after administration of AEOGL. This strategy can be employed in the control and management of diabetes.

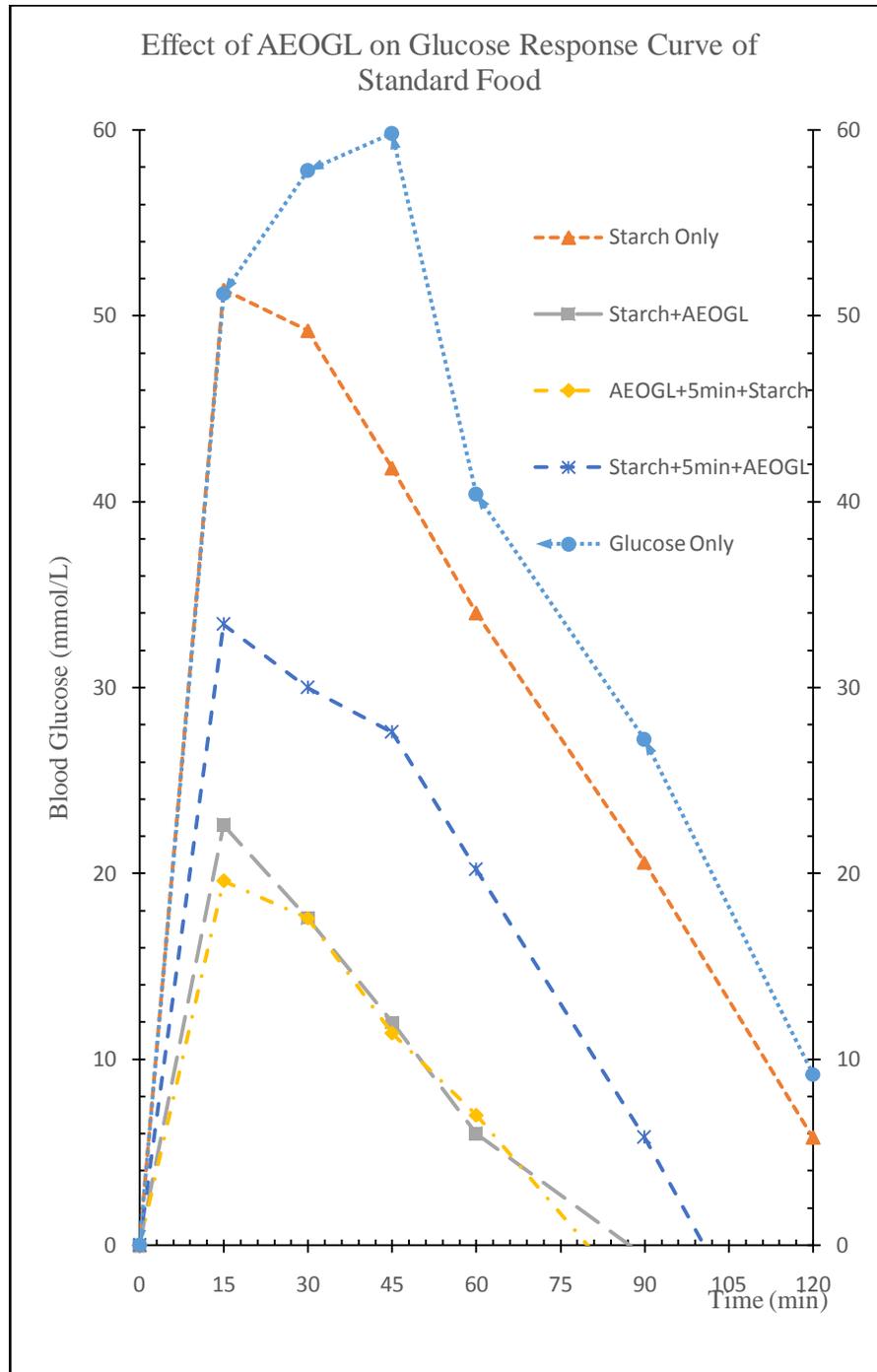


Figure 1: Effect of AEOGL on Glucose Response Curve of a Standard Diet

Investigation has shown that low-GI meal helps to manage glucose levels in people with Type 2 diabetes and according to the ADA 2016, one of the most effective ways of managing diabetes is through the consumption of low GI diets. Consumption of diets rich in low GI is a great means to safeguard your blood sugar. If you have diabetes, high GI diets can make it harder to control diabetes. Consumption of low GI diets

can assist you gain tighter control over your blood sugar. Paying much consideration to the GI of foods can be helpful in the management of diabetes. Following a low-GI diet may also be useful in weight loss. Any therapy that can bring a high GI diet to a low GI can be strategized to curb or manage diabetes mellitus.

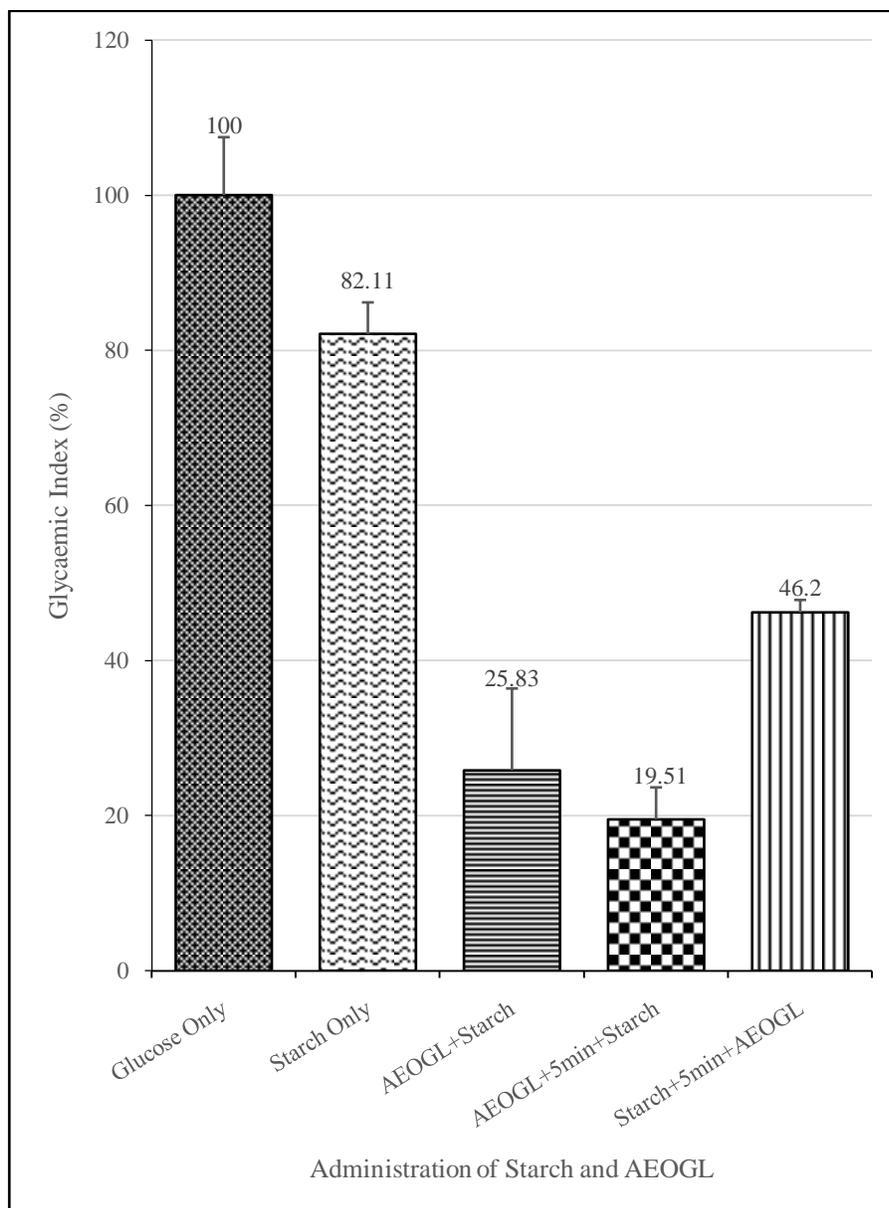


Figure 2: Effect of AEOGL on GI of StarchMeal

In conclusion, when properly planned, consumption of high GI food together with a therapy that lower its GI might be beneficial in controlling diabetes mellitus. One of such therapy might be AEOGL, which has been tested in albino rats and found potent. AEOGL, when administered orally five minutes before the intake of high glycaemic index food, might slow down the digestion of carbohydrate and hence, retard the absorption of glucose into the blood stream.

A research needs to be conducted to investigate the effect of the extract on the activities of digestive

enzymes of carbohydrate i.e. α -amylase and α -glucosidase.

Conflict of Interest: No conflict of interest is associated with this article

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