Original Research Paper

Effect of Aqueous Extract of Guava (*Psidium guajava*) Leaf on Blood Glucose and Liver Enzymes in Alloxan Induced Diabetic Rats

Chioma Christiana Ogueri[1], Ify Elekwa[1], Victor Chibueze Ude[2]*, and Amadike Eziuche Ugbogu[1]

[1]*Department of Biochemistry Abia State University Uturu, PMB 2000, Uturu- Nigeria*

[2]*Department of Medical Biochemistry, College Medicine, Enugu State University of Science and Technology, PMB 01660, Enugu-Nigeria*

**Corresponding author E-mail: victor_chibueze@yahoo.co.uk, Tel: +234(0)8039461045*

ABSTRACT.

Aim: The aim of this study is to determine the effect of aqueous leaf extract of *Psidium guajava* leaf on blood glucose level and liver enzymes of alloxan –induced diabetic rat.

Study Design: The animals were grouped into six of 6 rats each. Groups A, B, C and E were induced diabetes by intraperitoneal injection of alloxan monohydrate with a dose of 100mg/kg body weight. The extract was administered through gastric tube per kilogram body weight as follows: group A 200mg/kg, group B 150mg/kg, group C100mg/kg, group D none (normal control), group E 150mg/kg (untreated diabetic control) and group F none (extract control).

Methodology: Blood samples were collected by cardiac puncture after fasting overnight and standard methods were used for the extraction of spices, determination of fasting blood glucose and liver enzymes.

Place and duration of study: The study was carried out at Abia State University Uturu-Nigeria and the entire experiments lasted between December 2011 and July 2012.

Result: The results showed a significant (P<0.05) decrease in the blood glucose level compared to untreated diabetic control. There was no significant (P<0.05) difference observed in alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activity compared to all the controls (normal, untreated and extract control) while alanine aminotransferase (ALT) activity decreased significantly (P<0.05) compared to untreated diabetic control.

Conclusion: Therefore, this result revealed that aqueous extract of *Psidium guajava* leaf possess anti-hyperglycaemic properties with no side effect on selected liver enzymes compared to normal control and since the leaf did not show interference with functional integrity of the liver tissues it has a better potential for treatment of diabetes.

Keywords: Psidium guajava, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood glucose

1. INTRODUCTION

Diabetes mellitus, a syndrome of disordered metabolism is usually caused by a combination of hereditary and environmental effects, resulting in abnormally high blood sugar levels (hyperglycaemia) [1]. In diabetes mellitus, the blood glucose level becomes high due to defects in either insulin secretion or insulin action in the body or both [2]. It has been estimated that World-wide prevalence of diabetes mellitus in 2008 was more than 347 million with varying prevalence among different ethnic groups [3, 4] and it is expected that in 2025 the number will rise to 500 million [5]. Cases of type 2 diabetes mellitus have been increasing in contrast of type 1 diabetes mellitus; cure and prevention of type 2 diabetes mellitus have become important concern in developed countries and in the same way, due to urbanization and life style changes toward "Western style" diet, cure and prevention of type 2 diabetes mellitus supposed to become a more serious problem in developing countries [6]. Diabetes mellitus is a chronic disease that can be managed effectively through modified life styles such as monitoring ones weight, diet, exercise to long term use of oral hypogycaemia drugs [7]. Treatment can also be achieved by the use of synthetic drugs such as sulphonylureas [8].

Herbs and vegetables have contributed significantly in providing remedies for improvement of human health in terms of prevention and/or treatment of diseases. Thousands of plant species grow wild in Africa and have both nutritional and therapeutic purposes hence, traditional doctors and leaders are the dispensers of such concoctions [9]. Guava (*Psidium guajava*) is a common shade tree or shrub in dooryard gardens in the tropics which are classified in to *Myrtaceae* family. The tree is easily identified by its distinctive thin, smooth, copper-coloured bark that flakes off, showing a greenish layer beneath [8]. There are so many reports on the phytochemical analyses of guava leaf which revealed the presence of more than 20 isolated compounds such as alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lecitins, phenols, saponins, tannins, triterpenes and vitamin C [10, 11, 12]. The decoctions made from the leaf and /or bark of *P. guajava* have been reported to be used by many countries and tribes traditionally for treatment of diarrhea, dysentery, sore throat, vomiting, stomach upsets, vertigo, haemorrhages, intestinal worms, gastroenteritis, diabetes, vaginal discharge, to regulate menstrual cycle and to tighten or tone vaginal walls after child birth [13, 14, 15,16, 17].

In recent times reports from medicinal plants research indicate that extracts from some plants are hepatotoxic or hepatoprotective. However, liver function tests are commonly used in clinical practice to screen for liver diseases, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. Individuals with type 2 diabetes have high incidence of liver abnormalities than individuals who do not have diabetes and anti-diabetic agents have generally been shown to decrease alanine aminotransferases levels as tighter blood glucose level is achieved [18].

There are reports on anti-hyperglycaemic effects of *P. guajava* leaf extracts [6, 17, 5] but, scanty reports are available on the effect on liver enzymes on using aqueous extract of *P. guajava* leaf in treatment of alloxan-induced diabetic rats. Therefore, this study was carried out to determine the effect of treatment of alloxan induced diabetic rats with aqueous extract of *P. guajava* leaf on blood glucose level and liver enzymes.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The fresh leaves of *Psidium guajava* were collected from Umuinem Village, Okigwe in Okigwe Local Government Area, Imo State-Nigeria in January, 2012. The Botanical identification of the plant specimen was carried out at the department of Plant Science and Biotechnology (PSB), Faculty of Biological and Physical Sciences, Abia State University Uturu. Samples of the specimen were deposited in the Herbarium of the same University. The fresh leaves of *P. guajava* collected were sorted and all dead matter and unwanted particles were discarded. The leaf were air dried for two weeks and grounded into powder using electric blender and the powder was stored in an air tight container in the laboratory. A total of 200g of the ground powder was weighed out and soaked in 1000mls of distilled water for two days at room temperature. The mixture was filtered using (NO. 1) Watman filter paper. The filtrate was dried at a temperature of 30^{o}C in an incubator to produce gel-like extract that weighed 20g. The extract was then diluted with distilled water into 200mg/kg, 150mg/kg and 100mg/kg body weight.

2.2 Animal Treatments

A total of 36 male albino rats weighing between 120 -135g were purchased from the animal house of Department of Biochemistry, Abia State University Uturu-Nigeria. The rats were randomly divided into 6 groups of 6 rats in each group. Groups A, B and C were the test group, group D was the normal control, Group E the untreated control and group F extract control. The rats were acclimatized for 10 days before the commencement of the experiment. The animals in both test and control group were allowed free access to food (rat pellets) and water *ad libitium*, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. The weights of the rats were taken every day throughout the period of the experiment.

The blood glucose of the rats was determined before induction of diabetes. Groups A, B, C and E were induced by a single intraperitoneal injection of freshly dissolved alloxan monohydrate (100mg/kg) using normal saline maintained at 37^{o}C as vehicle to rats fasted for 15hrs. Diabetic states were confirmed by measuring the fasting glucose concentration after 5days of injection and were compared with the initial blood glucose level. Rats with blood glucose level of13mmol/l and above were used for the study. The

groups D and F were given the same quantity of normal saline. The animals were administered aqueous extracts of *P. guajava* leaf through gastric tube for four weeks according to their body weight as follows.

i. Group A were treated with 200mg/kg body weight of the extract after the injection of alloxan.
ii. Group B were treated with 150mg/kg body weight of the extract after the injection of alloxan.
iii. Group C were treated with 100mg/kg body weight of the extract after the injection of alloxan.
iv. Group D were treated with distilled water in place of extract after no injection of alloxan.
v. Group E were treated with distilled water in place of extract after the injection of alloxan.
vi. Group F were treated with 150mg/kg body weight of the extract after no injection of alloxan.

All the animal processes involved in the handling and the experiment were carried out in accordance with the guidelines of Animal Ethical Committee of Faculty of Biological and Physical Sciences, Abia State University Uturu-Nigeria.

2.3 Collection and Analysis of Blood Specimen

The animals were fasted overnight, anaesthetized with chloroform vapour and dissected for blood collection. Blood samples were collected by cardiac puncture into plain and fluoride-oxalate treated sample bottles. The blood samples were allowed to clot and were spun in bench centrifuge (MSE England) at 3000rpm for 5min to obtain sera. The serum samples were separated into another set of plain sample tubes and stored in the refrigerator at -4^{o}C until required for the enzyme analysis. The blood glucose levels were determined by the glucose oxidase enzymatic method (Trinder, 1969). Colorimetric end point method was used to determine serum alkaline phosphatase (ALP) [20]. Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) activities were determined using Reitman and Frankel [21] method. All assays were carried out within 24hrs of the sample collection.

2.4 Statistical Analysis

All data were analyzed using Analysis of Variance (ANOVA) and means were compared for significance using Duncan's Multiple Range Test (DMRT) at $P<0.05$.

3. RESULTS

The average weights of alloxan-induced diabetic rats treated with aqueous extract of *P. guajava* leaf are as shown in table 1. A significant ($P<0.05$) decrease in average weight of the rats in test groups A, B, C and untreated diabetic control (group E) were observed after 5days post alloxan-induction. After 28 days of post treatment the average weight of the animals in groups A, B, C, D and F increased significantly ($P<0.05$) compared to group E (untreated diabetic control).

Table 1. Average weights of alloxan- induced diabetic rats treated with aqueous extract of *P. guajava* leaf measured in gram.

Duration	Group A	Group B	Group C	Group D	Group E	Group F
Pre-induction	130.72±22.31[a]	135.65±28.42[a]	140.00±26.81[a]	132.90±27.12[a]	130.92±25.32[a]	138.20±26.32[a]
5days Post-induction	121.20±21.48[a]	128.45±26.32[a]	132.05±26.21[a]	143.62±30.66[a]	122.50±21.53[a]	145.42±25.08[b]
Post-treatment	208.51±30.02[b]	210.60±28.38[b]	205.30±28.05[b]	222.32±32.86[b]	96.82±16.41 [a]	215.74±28.52[b]

Values are mean ± standard deviation of six determinations. Values in rows with different superscript alphabets are significant (P<0.05).

The effect of aqueous extract of *P. guajava* leaf on the blood glucose level of alloxan-induced diabetic rats (Table 2) show that at 5 days post-induction of alloxan, the blood glucose level of groups A, B, C and E increased significantly (P<0.05) in relation to groups D and F which were not induced with alloxan monohydrate. The blood glucose levels of the test groups decreased significantly (P<0.05) compared to untreated diabetic control (group E) after 28 days of the extract administration.

Table 2. Effect of aqueous extract of *P. guajava* leaf on the blood glucose level of alloxan-induced diabetic rats measured in mmol/l.

Duration	Group A	Group B	Group C	Group D	Group E	Group F
Pre-induction	8.89±0.10[a]	9.03±0.11[a]	9.21±0.17[a]	9.14±0.07[a]	9.28±0.12[a]	9.30±0.08[a]
5days Post-induction	13.88±0.09[b]	13.72±0.16[b]	14.40±0.09[b]	9.10±0.14[a]	14.12±0.10[b]	9.04±0.09[a]
Post-treatment	8.68±0.14[a]	8.45±0.13[a]	8.67±0.12[a]	9.31±0.17[a]	15.23±0.14[b]	8.82±0.17[a]

Values are mean ± standard deviation of six determinations. Values in rows with different superscript alphabets are significant (P<0.05).

Table 3 shows the liver enzyme activities of alloxan- induced diabetic rats treated with aqueous extract of *P. guajava* leaf. It was observed that after induction and administration of the aqueous extract, there was no significant change in ALP, AST and ALT activities of the test groups compared to both extract control (group F) and normal control (group D). However a significant (P<0.05) decrease was observed when the test groups were compared to the untreated diabetic control (group E).

Table 3. Liver enzyme activities of alloxan-induced diabetic rats treated with aqueous extract of *P.guajava* leaf (U/L).

Liver enzymes	Group A	Group B	Group C	Group D	Group E	Group F
ALP	40.33±1.76[a]	38.62±1.50[a]	39.71±1.50[a]	40.23±1.51[a]	43.87±1.35[a]	38.90±1.20[a]
AST	42.87±1.70[a]	43.70±1.78[a]	41.68±1.53[a]	44.96±2.97[a]	46.20±2.35[a]	42.08±1.25[a]
ALT	24.84±2.24[a]	26.85±2.14[a]	24.01±2.80[a]	25.73±1.86[a]	32.80±2.40[b]	25.36±2.64[a]

Values are mean ± standard deviation of six determinations. Values in rows with different superscript alphabets are significant (P<0.05).

4. DISCUSSION

Varieties of plants are known to be of economic and medicinal value and those plants that are of medicinal value are often used as herbal remedy for the restoration and maintenance of good health. Medicinal plants usually have phytochemicals as part of its constituents. The phytochemicals include alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lecithins, phenols, saponins, tannins,

triterpenes and vitamin C [10, 12]. It has been reported that the presence of phenolic compounds, gallic acid, catechins and quercetin in *P. guajava* leaf significantly inhibited the glycation of proteins such as albumin, suggesting their use for prevention of diabetes complication [22]. The presence of high amount of phenolic compounds with antioxidant activity in the leaf of *P. guajava* leaf was also reported by Haida *et al*. [23]. Deguchi and Miyazaki [6] reported that the consecutive injection of aqueous extract of *P. guajava* leaf have the potential to improve diabetes symptoms such as hyperglycaemia, nephropathy and insulin resistances in diabetic animal models and/or clinical trials.

The observed decrease in blood glucose level of the alloxan- induced diabetic rats after treatment with aqueous extracts of *P. guajava* may be attributed to the presence of tannins, flavonoids, triterpenoids, alkaloids, and other chemical compounds in the plant. These compounds which have been shown present in *P. guajava* leaf [24] have been reported to be responsible for hypoglycaemic activity in *Mormordica charantia* [25] and *Mormordica foedium* [26]. The lowering of glucose levels by aqueous extract of *P. guajava* may also be due to the high content of quercetin in *P. guajava* leaf extract thereby confirming the usefulness for diabetic patients [5] as was suggested by Cheng *et al*. [27] that quercetin in aqueous extract of *P. guajava* leaf promotes glucose uptake in liver cells, and contributes to the alleviation of hyperglycaemia in diabetes. The marked decrease in blood glucose level of the alloxan-induced diabetic rats by *P. guajava* appear to suggest that its main mechanism of action may not be due to potentiation of insulin release from pancreatic cells since alloxan induce diabetes by destroying β-cells and impairing renal function. Hence, the extract may have effect in the control of non-insulin dependent diabetes mellitus. The observed hypoglycaemic effect of aqueous extract of *P. guajava* on normoglycaemic rats (group f) is a pointer that the effect could possibly be due to increase peripheral glucose utilization and inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney [28].

Liver function tests are commonly used to screen for liver diseases, monitor the progression of known disease and monitor the effects of potentially hepatotoxic drugs [18]. Liver function test is also used to find out if a medicinal plant is hepatotoxic or hepatoprotective. The present study showed that there were no significant (P<0.05) change on alkaline phosphatase (ALP), aspartate aminotransferase (AST) activity in test groups compared to control but, significant (P<0.05) decrease was observed in alanine aminotransferase (ALT) activity in test group compared to untreated diabetic control after the administration of aqueous extract of *P. guajava* leaf on alloxan –induced diabetic rats. This may be as a result of flavonoids present in the extract. Flavonoids are indicated in the protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcer, hepatotoxins, virus and tumors [29, 30, 31].

It was also suggested that the hepatoprotective effect of *P. guajava* extracts may be due to the action of various contents of the extracts especially the flavonoids which have been found to have anti-oxidative

effects [32]. The observed decrease in ALT activity may be attributed to ant-diabetic constituents of the extract which generally decrease ALT levels as tighter blood glucose level are achieved [18]. Since the aqueous extract of *P. guajava* leaf did not show any interference with the functional integrity of the liver tissues, it is suggested that the aqueous extract be used as liver tonic.

5. CONCLUSION

This study indicated that the aqueous extract of *P. guajava* possess hypoglyccaemic properties with no side effect on the selected liver enzyme activities hence, lend pharmacological credence to the suggested folkloric, ethnomedical uses of the plant in the management of adult-onset, type 2 diabetes mellitus in some rural African communities.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration of the Authors. Authors IE and CCO designed the study and wrote the protocol. Authors VCU, AEU and CCO managed the literature search and wrote the first draft of the. Author VCU and CCO performed the statistics. Authors CCO, VCU and AEU managed manuscript the analyses of the study. All the authors read and approved the manuscript.

CONSENT

Not applicable.

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