Trop J Nat Prod Res, May 2022; 6(5):795-800 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antidiabetic and Hepatoprotective Effects of *Psidium guajava L* Fruit Puree on Alloxan-induced Diabetes in Wistar Rats

Parker E. Joshua[1], Ogechukwu C. Iloka[1], Emmanuel S. Okeke[1,2]*, Emmanuel C. Aham[1,2], Samson A. Oka[3], Tochukwu N. Onyemuche[4], Okwesilieze F. C. Nwodo[1]

[1]*Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State 410001, Nigeria*
[2]*Natural Science Unit, School of General Studies, University of Nigeria, Nsukka, Enugu state, Nigeria*
[3]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Kogi State, University, Anyigba.Kogi state, Nigeria*
[4]*Department of Biochemistry, Faculty of Biological Science, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria*

ARTICLE INFO

Article history:
Received 30 March 2022
Revised 25 May 2022
Accepted 30 May 2022
Published online 04 June 2022

ABSTRACT

Psidium guajava L. (fam. Myrtaceae) is a semi-deciduous tree endemic to tropical and subtropical areas. The leaves, roots and bark have long been used for folkloric and medicinal purposes such as in the treatment of hypertension, fever, toothache and diarrhoea. The purpose of this study was to evaluate the antidiabetic and hepatoprotective effects of *Psidium guajava* fruits in alloxan-induced diabetic rats. Fresh guava fruits were chopped and blended into a smooth paste (purée). The purée was weighed, dissolved in freshly prepared normal saline in appropriate stock concentrations and used in a 21-day treatment. Acute toxicity studies of the fruit purée conducted in mice showed no toxicity up to 5000 mg/kg. Experimental animals were acclimatized for 7 days while diabetes was induced using alloxan monohydrate (150 mg/kg) intra-peritoneally. After a 21-day treatment of diabetic rats with 200 and 400 mg/kg of *Psidium guajava* fruit purée, the fasting and random blood glucose levels decreased significantly ($p < 0.05$) relative to that of the diabetic control. Also, there was a significant reduction in serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase levels of rats treated with 200 and 400 mg/kg of *Psidium guajava* fruit purée compared to the diabetic control. Thus, it could be inferred that *Psidium guajava* fruit purée is safe at high doses, suppresses hyperglycemia and protects the liver by decreasing lipid peroxidation and improve antioxidant status in alloxan-induced diabetic rats. These results provide the scientific evidence for its use as a nutraceutical agent in the management of diabetes and hepatotoxicity.

Keywords: Diabetes, *Psidium guajava*, Hepatoprotection, Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase.

Introduction

Diabetes mellitus (DM) is a metabolic disorder caused by defects in insulin secretion and/or insulin resistance and is characterized by elevated and sustained blood glucose levels over prolong periods of time.[1] It is a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins and an increased risk of complications from vascular disease,[2] and it is associated with a reduced quality of life and increased risk of morbidity and mortality. Globally, 422 million adults were diabetic in 2014 and without interventions to reduce the prevalence of DM, it is estimated that at least 629 million people will be affected by 2045.[1] The United Nation estimates that DM prevalence in Nigeria is 5.77%. This implies that 1 out of every 17 adults are diabetic and that 11.2 million Nigerians live with the disease.[3] In recent years, there is emphasis on utilizing traditional medicines that have a long and proven history of treating various ailments.[4]

*Corresponding author. E mail: emmanuel.okeke@unn.edu.ng
Tel: +2348035277554

Citation: Joshua PE, Iloka OC, Okeke ES, Aham EC, Oka SA, Onyemuche TN, Nwodo OFC. Antidiabetic and hepatoprotective effects of *Psidium guajava L* fruit puree on alloxan-induced diabetes in Wistar rats. Trop J Nat Prod Res. 2022; 6(5):795-800.

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Alloxan monohydrate is an analogue of glucose which when injected in rodents results in destruction of the β-pancreatic cells. Alloxan, is selectively toxic to the β-cells as it piles up in these cells via uptake by the glucose transporter-2 (GLUT2).[5] The cytotoxic action of Alloxan on the β-pancreatic cells is initiated by reactive oxygen species (ROS) formed in this redox reaction with dialuric acid, resulting in a simultaneous and rapid increase in cytosolic calcium concentration which triggers rapid destruction of the β-cells and insulin secretion or action is hampered.[6] Thus, injection of Alloxan monohydrates to rodents' increases serum glucose concentration, an indication of induction of rodent DM.

Psidium guajava L. known as guava belongs to the Myrtaceae family having 133 genera and 3,800 species is a semi-deciduous tree which is native to tropical and subtropical countries. Traditionally, the leaves and bark of *P. guajava* tree have a folkloric and medicinal uses till today.[7] Much of the traditional uses have been validated by scientific research.[4] The plant has been exploited extensively in terms of pharmacological activity of its major components, and the results indicate potent anti-diarrheal, antihypertensive, antimicrobial, and anti-mutagenic activities, both *in vitro* and /or in animal models.[8] Various research have shown that guava leaves extract has antioxidant action as a result of its high phenolic constituents which help to lower/ or prevent free radicals-mediated liver damage caused by alloxan administration in diabetic rats, consequently conferring antidiabetic as well as hepatoprotective benefits.[9,10] Notwithstanding the long-term use of the extracts of the leaves, bark and root for therapeutic purposes, little information is available on the anti-diabetic and hepatoprotective effects of *Psidium guajava* fruit. It is against this backdrop that this study is aimed at assessing the anti-diabetic and

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

hepatoprotective effects of *Psidium guajava* fruit purée in alloxan induced diabetic rats.

Materials and Methods

Plant material and extraction procedures

Fresh *Psidium guajava* L. (Guava) fruits were obtained from Ogige market Nsukka, Enugu State, Nigeria in July 2019. The fruit sample was authenticated by Mr. Alfred Ozioko, a taxonomist of the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State Nigeria. The guava fruits (whole) were chopped and blended to a smooth paste (purée) with a manual grinder. The fruit purée was weighed and dissolved in freshly prepared normal saline in appropriate stock concentration.Voucher specimen of the plant with No. INTERCEED/009 was deposited at the InterCEED Herbarium.

Experimental animal

Ethical clearance

The approval (approval number UNN/FBS/EC/1069) for the experimental protocols and humane use and handling of laboratory animals were given by the Faculty Ethics and Biosafety Committee, Faculty of Biological Sciences, University of Nigeria Nsukka.

Acute toxicity study (Determination of LD_{50})

Thirteen (13) Swiss mice (20-25g) were housed at the Animal House of Department of Zoology and Environmental Biology, University of Nigeria Nsukka, and fed with poultry starter feed and drinking water *ad libitium.* They were acclimatized to laboratory conditions for seven (7) days. Acute toxicity study of *Psidium guajava* fruit puree was carried out according to the previously described method .[11] The study was carried out in two phases.

Phase I: The mice were divided into three (3) groups of three mice (3) each and were given 10, 100, and 1000mg/kg body weight of *P. guajava* puree respectively by oral intubation method. The outcome of this experiment determined what happened in the phase.

Phase II: In this phase, four (4) groups of 1 mouse each were given 1600, 2900, 3500 and 5000 mg/kg body weight of *P. guajava* puree by oral intubation method. The treated mice were closely observed for 72 hours for abnormal behavior and lethality. The LD_{50} was calculated as the geometric mean of the maximum non-lethal dose and minimum toxic dose.

Experimental design

Twenty (20) male Wistar rats (120 -150 g) were housed in the Animal House of the Department of Biochemistry University of Nigeria Nsukka, and maintained with Vital Feed (growers mash) and water *ad libitum* and allowed to acclimatize to laboratory conditions for seven days. The baseline blood glucose levels were determined before the induction of diabetes. The rats had free access to water and were faster overnight prior to experimentation. The experimental animals were divided into five groups of four rats each as follows.

The first group consists of normal control rats (i.e., rats that are not induced with alloxan monohydrate) treated with normal saline.

The second group consists of diabetic untreated rats (i.e.,rats induced with alloxan monohydrate) without treatment.

The third group consists of diabetic rats (i.e., rats induced with alloxan monohydrate) treated with 25mg/kg b.w. Metformin (standard drug).

The fourth group consists of diabetic rats (i.e., rats induced with alloxan monohydrate) treated with 200 mg/kg b.w. *Psidium guajava* purée.

The fifth group consists of diabetic rats (i.e., rats induced with alloxan monohydrate) treated with 400 mg/kg b.w. *Psidium guajava* purée

Induction of diabetes in rats

After a period of 7 days acclimatization, the grouped rats were fasted overnight, followed by induction of diabetes using alloxan monohydrate (150 mg/kgb.w.) intra-peritoneally.[12, 13] Rats with fasting blood glucose (FBG) level greater than 200 mg/dl 72 h post induction were considered diabetic and included in the investigation.

The treatment lasted for 21 days during which the FBG and random blood glucose (RBG) levels, respectively concentration of the rats was measured on days 1,4,8,15 and 21 using Accuchek active glucometer (Roche Diagnostics, Germany) with the corresponding test strips.[11] *Psidium guajava* fruit puree was dissolved in fresh normal saline and administered orally once daily by oral intubation. After the experimental period, blood samples were collected from the rats by ocular puncture to assay for serum liver enzyme activities.

Assay for liver enzyme activities

The serumaspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were assayed by the method of [14] as described by. [15]

Assay for serum alanine aminotransferase (ALT) activity

Principle: ALT catalyzes the donation of an amino group from alanine to α-oxoglutarate to yield glutamate and pyruvate. The activity of ALT was determined by measuring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Reagents: Reagent 1 (ALT substrate solution) made up of 100 mmol/l phosphate buffer at pH 7.4, α- ketoglutarate (2.0 mmol/l) and L-alanine (200 mmol/l). Reagent 2 (color developer) was 2, 4 dinitrophenyl hydrazine (2.0 mmol/l).

Procedure: Reagent 1, 0.5 ml was added to 0.1 ml of the blank test tube, and 0.1 ml of the sample. Distilled water, 0.1 ml was added to the sample only. The blank and the sample tubes were incubated simultaneously for 30 minutes at 37ºC. Reagent two (0.5 ml), was then mixed with the blank and the sample respectively. Thereafter, the tubes were mixed and left to stand for 21 minutes at 25ºC. Sodium hydroxide (5.0 ml), was each added to the blank and sample tubes. The content of the tubes was mixed again, and the absorbance of the reaction mixture in each tube was read against the blank after 5 minutes at 546 nm. The activity of ALT was determined using the standard ALT activity table provided.

Assay for serum aspartate aminotransferase (AST) activity

Principle: The enzyme catalyzes the movement of an amino group from aspartate to α-oxoglutarate to produce glutamate and oxaloacetate. The activity of AST was determined by checking the quantity of oxaloacetate hydrazone produced with 2, 4-dinitrophenylhydrazine.

Reagents: Reagent 1: AST substrate solution with 100 mmol/l phosphate buffer at pH 7.4, 100 mmol/l L-aspartate and 2 mmol/l α-oxoglutarate. Reagent 2: 2, 4-dinitrophenylhydrazine.

Procedure: The test tubes labelled sample, received 0.1 ml of serum/tissue homogenate, 0.5 ml of Reagent 1, and 0.1 ml of distilled water were added respectively whilethe blank contained 0.5 ml of Reagent 1, and 0.1 ml of distilled water. The mixtures in both tubes were incubated at 37ºC for 30 minutes followed by addition of 0.5 ml of Reagent 2. The tubes were shaken and allowed to stand for 20 minutes at 25ºC. Thereafter, 5 ml of sodium hydroxide was added to both tubes, mixed and allowed to stand for another 5 minutes. The absorbance of the sample at 546 nm was read against the reagent blank. The activity of AST (IU/L) was determined from the standard AST activity table provided.

Assay for serum alkaline phosphatase (ALP) activity

Serum ALP activity was determined by the methods of [16,17]

Principle: ALP catalyzes the hydrolyses of a colorless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein, and a pink colored complex in alkaline environment, which can be monitored spectrophotometrically at 405 nm.

Reagents: ALP chromogenic substrate solution, phenolphthalein monosulfate, and a standard solution of ALP in water/ethanol (equivalent to 30 IU/L).

Procedure: Phenolphthalein monosulfate (color developer) was prepared by adding one vial of phenolphthalein monosulfate salt to 250 ml of deionized water. One drop of ALP chromogenic substrate solution was added to 100 µL of deionized water in a clean test tube, mixed and incubated at 37ºC for a period of 5 minutes. Subsequently, 100 µL of the sample or standard was added to the mixture and

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

incubated at 37ºC for 20 minutes in a water bath. After incubation, 5 ml of phenolphthalein monosulfate was added, and the absorbance of the reaction mixture was compared to the blank at 550 nm.

Statistical analysis
The results obtained were expressed as mean ± standard deviation and tests for statistical significance done using one way and two-way analyses of variance (ANOVA) at 95% confidence level. The statistical Product and Service Solutions (SPSS) version 20 was used and mean values with $p \leq 0.05$ was taken to be significant.

Results and Discussion

Qualitative phytochemical composition of white Psidium guajava fruit purée
Table 1 shows the qualitative phytochemical composition of *Psidium guajava* fruit purée. Reducing sugars are relatively high; alkaloids, flavonoids, glycosides, tannin, terpenoids and soluble carbohydrates are present in moderate amounts; steroids, saponins, fats and oil are relatively low in amounts while proteins and resin were not detected.

Quantitative phytochemical composition of white Psidium guajava fruit purée
Table 2 shows the quantitative composition of white *Psidium guajava* fruit purée. White guava fruit purée contained high quantity of reducing sugars and lower contents of alkaloids, steroids, reducing sugars, soluble carbohydrates, tannins, glycosides, terpenoids, and flavonoids.

Acute toxicity study (median lethal dose LD_{50})
Table 3 shows that there were no lethality or behavioral changes occurred in the three groups of mice that received 10, 100 and 1000 mg white guava purée /kg body weight at phase I level. No death was observed within 24 hours among the groups of mice treated respectively with 1600, 2900, 3500 and 5000mg white guava purée/kg body weight orally. The animals were active throughout the experiment and thus, white guava fruit purée may be regarded as safe.

Effect of white Psidium guajava purée on the fasting blood glucose concentration of alloxan-induced diabetic rats
Figure 1 shows the results of the Effect of white *Psidium guajava* purée on the fasting blood glucose concentration of alloxan-induced diabetic rats. The data showed that prior to introduction of diabetes with alloxan; the mean baseline fasting blood glucose concentrations of the animals in all the groups were within the normal range (below 110 mg/dl). On day 1 (i.e. 5 days post induction), the animals in groups 2,3,4 and 5 had mean fasting blood glucose concentrations above 200 mg/dl, showing significant ($p<0.05$) elevations in fasting blood glucose concentrations relative to that of group 1 rats; evidently the animals were judged diabetic. On day 4, animals in Group 3, 4 and 5 the fasting blood glucose concentrations decreased non significantly ($p>0.05$) relative to that of the diabetic untreated rats, the control group. However, there were significant decreases ($p<0.05$) in the fasting glucose concentrations of rats in Group 3,4 and 5 on days 8,15 and 21 when compared to the diabetic control group. The fasting blood glucose concentration of rats that received normal saline (normal control) remained unchanged and was not significant ($p>0.05$) statistically during the study (Figure 1).

Effect of white Psidium guajava purée treatment on random blood glucose concentration of alloxan-induced diabetic rats
The mean baseline random blood glucose concentrations of 122, 127, 131, 139 and 124mg/dl observed for groups 1,2,3,4 and 5 respectively were within the normal range (<140mg/dl), hence not diabetic. On day 1 (5 days post alloxan treatment), group 2, 3, 4 and 5 showed significant ($p<0.05$) elevation in random blood glucose concentrations relative to the of group 1 animals thus indicating that the rats were diabetic. On day 4, rats in groups 3 and 5 witnessed significant ($p<0.05$) reductions in their random blood glucose concentrations compared to group 2 animals (diabetic controls) but non-significant ($p>0.05$) reduction was observed in the random blood glucose concentrations of group 4 animals relative to that of group 2. On day 8, animals in groups 3, 4 and 5 showed significant ($p<0.05$) decreases in their random blood glucose concentrations compared to that of group 2 rats. Also, on day 15, rats in groups 3, 4 and 5 showed significant ($p<0.05$) decreases compared to that of group 2.

Table 1: Qualitative phytochemical composition of white *Psidium guajava* fruit purée

Test	Inference
Alkaloids	+
Flavonoids	+
Glycosides	+
Tannins	+
Steroids	+
Reducing sugar	+
Terpenoids	+
Proteins	ND
Soluble carbohydrates	+
Saponin	+
Fats and oil	+
Resin	ND

+ = present; ND =not detected

Table 2: Quantitative phytochemical composition of white *Psidium guajava* fruit purée

Constituents	Composition (mg/100g)
Alkaloids	3.256 ± 0.004
Flavonoids	2.813 ± 0.003
Glycosides	2.624 ± 0.004
Tannin	5.244 ± 0.003
Steroid	0.621 ± 0.004
Reducing sugar	256.525 ± 0.004
Soluble carbohydrates	1.436 ± 0.003
Saponin	0.835 ± 0.004
Terpenoids	2.235 ± 0.004

Results are presented as mean±standard deviation of replicate measurements. n=3

Table 3: Acute toxicity studies

Dose (mg/kg body weight)	Number of animals	Number of deaths
Phase I		0
10	3	0
100	3	0
1000	3	0
Phase II		
1600	1	0
2900	1	0
3500	1	0
5000	1	0

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

On day 21, the random blood glucose concentrations of groups 3, 4 and 5 decreased more significantly ($p<0.05$) relative to that of group 2 but did not show significant ($p> 0.05$) decrease when compared to that of group 1 rats (normal control) (Figure 2). The result of the anti-diabetic effects of white *Psidium guajava* purée on alloxan induced diabetic mellitus showed that after a 21-day treatment schedule guava fruit puree in a dose dependent manner significantly ($p < 0.05$) reduced both fasting and random blood glucose concentrations of rats in the treated group when compared with the diabetic control group. Polyphenols such as phenolics and flavonoids present in guava puree possess antioxidant properties and could be responsible for inhibiting the scavenging effect of free radicals produced by alloxan administration, the regeneration of the beta cells, release of insulin and consequently a decline in the blood glucose level.[13] Also, the guava purée could have facilitated the uptake of glucose by the peripheral cells, leading to a decline in the blood glucose level,[18] or promoting insulin secretion by closure of K+ ATP channels, membrane depolarization, and stimulation of calcium influx into the cells, which is an important step in secretion of insulin.[19] This finding is in consonance with the report of [20, 10]. Norazmir and Ayub[21] showed that white guava fruit puree lowered fasting and random blood glucose concentrations of alloxan-induced diabetic rats, thus, indicating its anti-diabetic property.

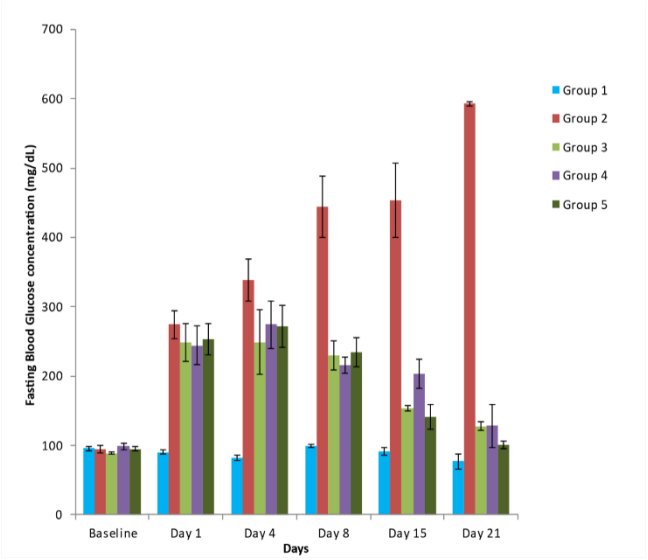

Figure 1. Effect of white *Psidium guajava* purée on fasting blood glucose concentrations of alloxan-induced diabetic rats

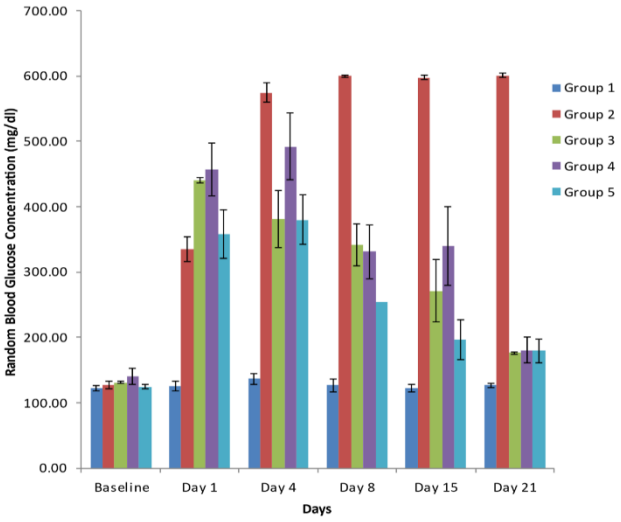

Figure 2. Effect of white *Psidium guajava* purée on random blood glucose concentration in alloxan-induced diabetic rats.

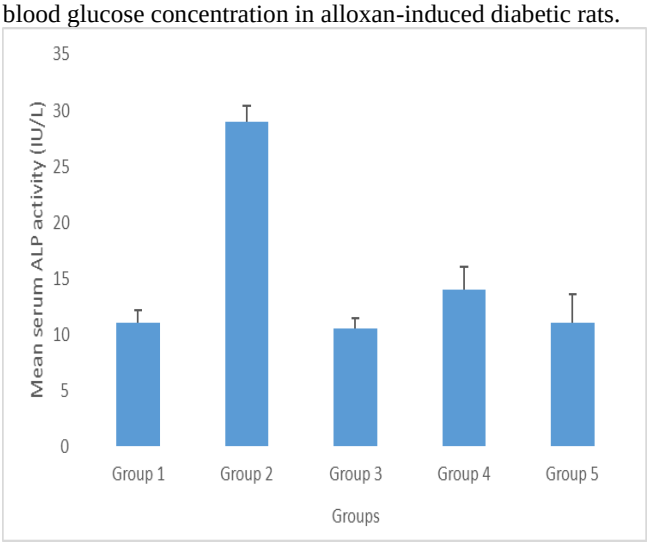

Figure 3: Effect of white *Psidium guajava* Linnaeus purée on serum ALP activity of diabetic rats

Effect of white Psidium guajava purée treatment on serum ALP activity of alloxan-induced diabetic rats

The ALP activity (29.00 IU/L) of Group 2 (diabetic untreated) animals was significantly ($p<0.05$) elevated relative to the value obtained for group 1 rats (normal control). Rats treated with metformin (Group 3) showed significant ($p<0.05$) decreases in their serum ALP (10.50 IU/L) relative to that of Group 2 rats. Similarly, guava puree treatment of 200 and 400 mg/kg b.w. (groups 4 and 5 respectively) caused significant ($p<0.05$)decrease in ALP activities (14.00 and 11.00 IU/L respectively) of the animals in the groups relative that of group 2 animals but did not cause any significant ($p<0.05$)decrease relative to that of group 1 (10.59 IU/L) (Figure 3).

Effect of white Psidium guajava treatment purée on serum AST activity alloxan-induced diabetic rats

Figure 4 presents the Effect of white *Psidium guajava* treatment purée on serum AST activity alloxan-induced diabetic rats. The result showed a significant ($p<0.05$) increase was observed in the serum AST activity of group 2 (diabetic untreated) animals with mean activity of 73.50 IU/L relative to that of group 1(non - diabetic) animals which had mean AST activity of 42.50 IU/L. Also, relative to the serum AST activity of group 2 (diabetic untreated) rats with mean AST activity of 73.50 IU/L, animals in groups 1 (normal non-diabetic), 3 (25 mg/kg b.w.Metformin), 4 (200 mg guava puree /kg b.w.) and 5 (400 mg/kg b.w.guava puree) exhibited significant ($p<0.05$) decreases in their serum AST activities with mean activities of 42.50, 46.25, 43.33 and 35.00 U/L respectively. Furthermore, non-significant ($p>0.05$) decrease was observed in serum AST activities of animals in groups 3,4 and 5 (diabetic treated groups) relative to group 1 (non-diabetic) rats (Figure 4).

Effect of white Psidium guajava purée treatments on serum ALT activity alloxan-induced diabetic rats

The serum ALT activity of animals in group 2 (diabetic untreated rats) was significantly($p<0.05$) elevated with mean activity of 64.00 IU/L compared to group 1 rats (normal control) which had mean serum AST activity of 34.50 IU/L. Also, guava puree treatments with 200 and 400mg/kg b.w. significantly ($p<0.05$) reduced the serum ALT activities with mean activities of 50.47 and 34.50 IU/L respectively relative to groups 2 (64.00IU/L) but showed significant($p<0.05$) increases relative to metformin treatment (27.50 IU/L) (Figure 5). The liver is a major body organ responsible for insulin clearance and release of inflammatory cytokines that helps in maintaining post-prandial and normal fasting glucose level.[19]Alterations in liver enzyme activities which leads to liver diseases is a major complication of DM.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

In this study, a significant ($p < 0.05$) increase in the activities of serum AST and ALT was observed in diabetic control relative to normal control and the treated groups (Figures 3 and 4). This could indicate damage of the hepatic tissue leading to leakage of these cellular enzymes from the cytosol to the bloodstream.[12] Also, a corresponding increase in serum ALP activities was observed in diabetic control which confirms that the structural integrity of the plasma membrane was compromised following alloxan-administration to rats. This is because in diabetic conditions, hyperglycemia is an underlying cause for the generation of ROS by the non-enzymatic glycation of proteins, amino group of phospholipids and DNA, via pro-inflammatory cytokines which activate cyclo-oxygenase. Most of the complications of diabetes mellitus are due to ROS generation.[22] Furthermore, ROS activates NF-kb (transcription factor), which increase the mRNA level of tumor necrosis factor (TNF) alpha and IL -12 (hepatic proinflammatory cytokines), resulting in liver cell injury. Also, superoxides act as cellular messengers and elicit inflammatory response, which induces gene expression encoding inflammatory proteins (e.g., proteinases like collagenases and elastases), leading to tissue destruction.[23] Treatment with the guava fruit puree led to a significant ($p < 0.05$) decrease in serum AST, ALT and ALP activities in groups 4 and 5 which receive 200 and 400mg/kg white guava fruit puree, respectively in a dose-dependent manner compared to the mean values recorded for rats in group 2 (diabetic untreated) animals. The decrease in serum ALP and AST activities observed in the groups treated with guava puree was similar to the observed decreased in serum ALP and AST activities in group 3 animals (standard control) treated with Metformin. These findings are in accordance with[11] who reported treatment related declines in liver enzyme activities following alloxan induction. Since raised levels of AST and ALT enzymes activities are associated with heart and liver diseases, decreased in these serum enzyme levels in diabetic animals treated by white guava fruit puree indicated that the risk of liver and heart diseases may possibly be reduced in diabetic patients by eating raw white guava fruits. Increased level of ALP indicates bone disease, liver disease or bile tract blockage,[24] therefore, reduction in ALP level by guava fruit puree revealed its protective effect on liver and improvement in liver function efficiency.[9] Since diabetes is associated with increased oxidative stress, the observed hepato-protective effect of white guava puree may be as a result of high level of vitamin C,[25] flavonoids and other phenolic compounds present such as rutin, quercetin, naringin, catechins, chlorogenic acids and gallic acid [26, 27] present in guava fruit which exhibit high level of antioxidant activity through the mechanism of hydrogenelectron-donation. Thus, an improved antioxidant status with a decrease in lipid peroxidation may be some of the mechanisms by which dietary treatment prevents diabetic complications.[26]

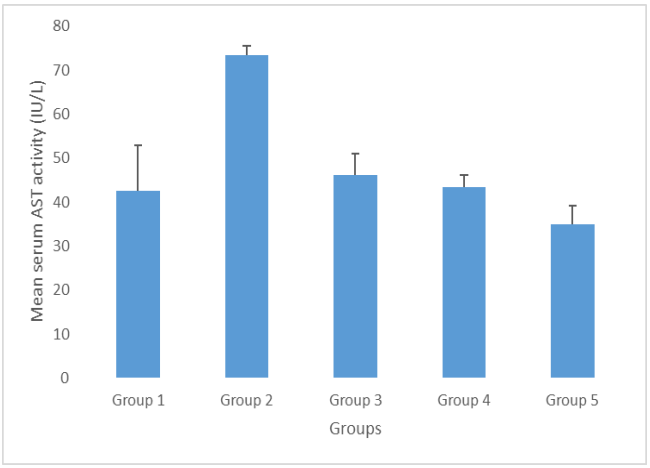

Figure 4: Effect of white *Psidium guajava L.*purée on serum AST activity of diabetic rats.

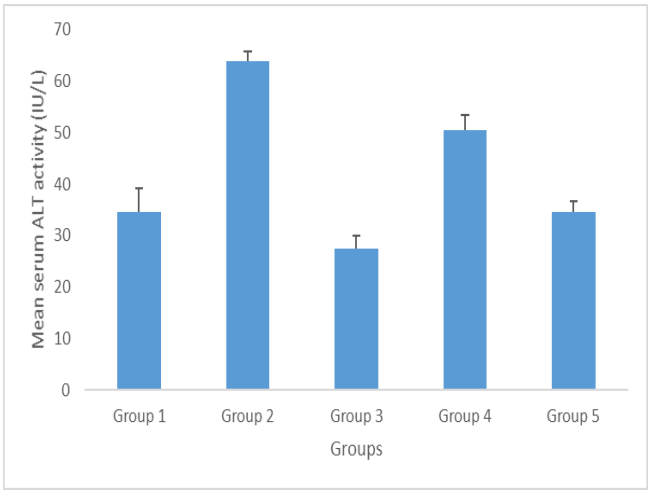

Figure 5. Effects of white *Psidium guajava*purée treatment on serum ALT activity of diabetic rats.

Conclusion

The study has provided evidence that white *Psidium guajava* purée has antidiabetic effects and exerts protective effect in alloxan-induced diabetic rats. White guava purée effectively lowered fasting and random blood glucose levels and liver marker enzymes concentrations. Therefore, oral administration of white *Psidium guajava* fruits purée equivalent to eating the fruit can be used as a potential nutraceutical therapy for the management of diabetic conditions and post prandial hyperglycemia.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors acknowledge Covenant University Centre for Research, Innovation and Discovery (CUCRID) for the payment of the processing charge

References

1. World Health Organization (WHO). Classification of diabetes mellitus, World Health Organization, Geneva. 2019. https://apps.who.int/iris/handle/10665/325182. Accessed 25th May 2021.
2. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018; 138:271–281.
3. Uloko AE, Musa BM, Ramalan MA, Gezawa ID, Puepet FH, Uloko AT, Borodo M M, and Sada KB. Prevalence and Risk Factors for Diabetes Mellitus in Nigeria: A Systematic Review and Meta-Analysis. Diabetes Ther. 2018; 9(3):1307–1316.
4. Bhavsar RB and Pagar HJ. Review: *Psidium Guajava* – Phytochemical constituents and pharmacological actions. J Emerg Tech Innov Res. 2021; 8(7):c652-655.

5. Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward WK. Induction of type 1 diabetes mellitus in laboratory rats by use of alloxan; route of administration, pitfalls, and insulin treatment. Commun Med. 2004; 54:252–257.
6. Muhammad IU, Jarumi IK, Alhassan AJ, Wudil AM, Dangambo MA. Acute toxicity and hypoglycemic activity of aqueous fruit pulp extract of *Adansoniadigitata*L. (AFPEAD) on alloxan induced diabetic rats. J Adv Med Pharm Sci. 2016; 6(3):1–6.
7. Amadi LO, Wanabia D, Amadi V. Synergistic effects of alum and guava (*Psidium guajava)* leaf extracts on some pathogens from clinical samples. Int'l J Curr Res. 2016; 8(5):31354-31358.
8. Ugbogu EA, Emmanuel O, Uche ME, Dike ED, Okoro BC, IbeC, Ude VC, Ekweogu CN, Ugbogu OC. The ethnobotanical, phytochemistry and pharmacological activities of Psidium guajava L. Arab J Chem. 2022; 15:103759
9. Nasir M, Tahir-Nadeem M, Saeed F, Ahmad T, Imran M. Assessment of renal and hepato-protective potential of guava leaves in male Sprague dawley rats. Cell Mol Bio. 2021; 67(1):142-146.
10. Eze UN, Eze AA, Ugwu CV, Onuoha M, Ubenyi A, Olunuga O A. Anti-hyperglyceamic Effects of Psidium guajava LINN Crude Leaf Extracts and Fractions in Alloxan-induced Diabetic Mice. J Chem Nutr Biochem. 2021; 2(2):1-27.
11. Lorke D. A new approach to practical acute toxicity testing. Arch Biochem Biophysics. 1983; 225:175-270.
12. Eguavoen C, Ekpo DE, Ebeire EN. Effect of seven keys herbal formulation on plasma concentrations of liver transaminases of alloxan-induced diabetic rats. J Pharm Res Int. 2016; 11(4):1-11.
13. Idakwoji PA, EkpoDE, Joshua PE, Njoku OU, Nwodo OFC. Ethanol extract of *Tephrosiabracteolata* leaves and its fractions ameliorates alloxan-induced diabetes and its associated complications in Wistar rat model. Int J Diabetes Dev Ctries. 2021; 41:456-468.
14. Ekpo DE, Joshua PE, Odiba AS, Nwodo OFC. Flavonoid-rich fraction of *Lasianthera africana* leaves alleviates hepatotoxicity induced by carbon tetrachloride in Wistar rats. Drug ChemToxicol. 2021; 7:1-17.
15. Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28(1):56-63.
16. Babson AL, Greeley SJ, Coleman CM, Philips GE. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. Clin Chem.1966; 12(18):482-90.
17. Klein B, Read PA, Babson AL. Rapid method for the quantitative determination of serum alkaline phosphatase. Clin Chem. 1960; 6:269-275.
18. Tefesse TB, Hymete A, Mekonnen Y, Tadesse M. Antidiabetic activity and phytochemical screening of extracts of the leaves of *Ajugaremota*Benth on alloxan-induced diabetic mice. BMC Compl Altern Med. 2017; 17:243.
19. Morajhar AS, Hardikar B, Sharma B. Hepatoprotective effects of crude extracts of *Pongamiapinnata* in alloxan-induced diabetic albino Wistar rats. Int J Zool Res. 2015; 11(2):37-48.
20. Díaz-de-Cerio E, Verardo V, Gómez-Caravaca A M, Fernández-Gutiérrez A, Segura-Carretero A. Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int J Mol Sci. 2017; 18:897-927.
21. Norazmir MN and Ayub MY. Beneficial lipid-lowering effects of pink guava puree in high fat diet induced-obese rats. Malay J Nutr. 2010; 16(1):171-185.
22. Wolff SP. Diabetic mellitus and free radicals. British Med Bull. 1993; 49:642-6.
23. Oldenburg B, Kats-Renaud H, Koningsberger JC, Henegouwen GP, Asbeck B. Chemiluminescence in inflammatory bowel disease patients: a parameter of inflammatory activity. Clin Chem Acta. 2001; 310(2):151-6.
24. Mayne PD. Clinical chemistry in diagnosis and treatment. Edward Arnold (A division of Hodder Headline Plc), London.1996; 224-41:23p.
25. Croft KD. The chemistry and biological effects of flavonoids and phenolic acids. Ann New York Acad Sci.1998; 854:435-442
26. Paganga G, Miller N, Rice-Evans CA. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Rad Res. 1999; 30:153-162.
27. Armstrong AM, Chestutt JE, Gormley MJ, Young IS. The effect of dietary treatment on lipid peroxidation and antioxidant status in newly diagnosed non-insulin dependent diabetes. Free Rad Biol Med.1996; 21:719-26.