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Effect of White Guava (*Psidium guajava* L.) Fruit Puree in Rats Injected with 2,4,5,6(1H,3H)-Pyrimidinetetrone

Victor Eshu Okpashi*, Kate Mlumun Ucho, Emmanuella Ekpenyong-Anwan Oyo-Ita and Bridget Bassey Jones

Department of Biochemistry, Cross River University of Technology, Calabar, Nigeria

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Abstract. There is a concern about finding alternate herbs for diabetic mellitus dysregulation. A focus on herbal treatments with proven anti-diabetic properties is being investigated. The effects of white guava fruit puree on diabetic Mellitus and its ability to protect hepatocytes in rats injected with 2,4,5,6(1H,3H)-pyrimidinetetrone were studied. The screening of qualitative phytochemicals in white guava fruit puree was done. The puree was tested for acute and sub-acute toxicity in mice at a dose of 5000 mg/Kg body weight. In different amounts, flavonoids, steroids, alkaloids, tannins, terpenoids, glycosides, saponins, reducing sugar and soluble carbohydrates were found. Resins and proteins were found to be undetectable. For twenty-one (21) days, rats were given 200 and 400 mg/Kg b.w of white guava fruit puree, where a significant ($P \geq 0.05$) reduction in body weight was seen. There was a decrease in fasting and randomized blood glucose levels. They had reduced serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphates activities (ALP), which was suggestive ($P \geq 0.05$). Non-diabetic rats were matched with diabetic rats given 200 and 400 mg/Kg b.w of white guava fruit puree. The results show that white guava fruit puree extract is both safe and effective in the treatment of diabetic Mellitus.

Keywords: *Psidium guajava* L., hepatoprotective, diabetes mellitus, herbal, wistar rats

Introduction

Diabetes mellitus is a metabolic disorder characterized by defects in the control of insulin production and insulin activity (Jacobson *et al.*, 2007). It is a group of disorders characterized by hyperglycemia, altered lipid, carbohydrate and protein metabolism and increased the risk and difficulty of vascular disease (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ECDTDM), 2003). Diabetes is connected to a lower quality of life and a higher risk of morbidity and mortality (Misra and Khurana, 2009). Recently, there has been a focus on the use of medicinal plants that have a long history of being used to treat diabetes (Joseph and Mini, 2011). *Psidium guajava* L. belongs to the Myrtaceae family, which has approximately 133 species. It's a semi-deciduous tree that grows in hot and humid climates. Traditionally, the leaf and bark of *P. guajava* have been used for a wide range of medicinal purposes (Nwinyi *et al.*, 2008). Some traditional herbs have been proven to be effective (Joseph and Mini, 2011). The plant's components have been implicated in the treatment of diarrhoea, hypertension, antimicrobial and anti-mutagenic activity, for *in vitro* and animal studies (Tripathy *et al.*, 1981). Despite the long-term use of this plant's parts for therapeutic purposes, the fruit, bark and root have not been adequately recorded. The anti-diabetic and hepatoprotective benefits of white guava fruit are supported by limited evidence. As a result, the goal of this study is to see if white guava fruit puree has anti-diabetic and hepatoprotective properties in rats induced by 2,4,5,6(1H, 3H)-pyrimidinetetrone. Thus, alloxan is a popular name for the chemical compound 5,5-dihydroxyl pyrimidine-2,4,6-trione. It's made up of urea, which has carcinogenic characteristics and a cytotoxic glucose analog (Osasenaga *et al.*, 2017). Alloxan has the following chemical formula: $C_4H_2N_2O_4$ and a molecular mass of 142.06. It is a common diabetogenic drug that is used to evaluate the antidiabetic potential of both pure chemicals and plant extracts in diabetes research. Marcela *et al*. (2007) studied the severity and prevalence of periodontal disease in diabetes patients. They claimed that even in the absence of aggressive causes like ligatures, diabetes induction stimulates or perhaps co-induces the beginning of periodontal disease like changes. As a result, diabetes induction transforms a previously resistant host into a susceptible phenotypic and diabetes develops as a result, it might be regarded as a major risk factor for the onset of periodontal disease. The exact reason why alloxan is widely employed in debates induction is demonstrated in this report and many others. To ameliorate the effect of alloxan, guava leaves are steeped in heated water

*Author for correspondence; E-mail: vic2reshu@gmail.com

before being consumed in various Asian countries (Parker *et al.*, 2020). It is a diabetes traditional treatment. These leaves are high in antioxidants like vitamin C and flavonoids like quercetin, as well as other nutrients. Pre-diabetic and diabetic individuals benefit from drinking Guava leaf tea with every meal. Guava leaf tea has been approved in Japan for specific health purposes, including the treatment and prevention of diabetes (Paganga *et al*., 1999). In reality, multiple clinical investigations in Japan have shown that guava tea has anti-diabetic characteristics; drinking the tea frequently helped individuals with Type 2 diabetes lower blood glucose (after eating). The participants in the first study drank guava leaf tea after eating white rice. They saw higher drops in blood sugar levels after 30 min, 90 min and 120 min respectively. The same participants in the same trial drank only hot water after eating white rice again and exhibited no advantage. Patients with prediabetes or moderate diabetes Type 2 were given guava leaf tea for 12 weeks and their fasting blood sugar levels were lower than before they started drinking the tea. Two forms of sugars, maltose and sucrose are inhibited by the tea components, which helps to manage blood sugar levels after eating (Farnazzi-Machado *et al.*, 2012). In addition, the effects of guava leaf extracts in rats with type 2 diabetes found that guava leaf extract consumption resulted in a significant reduction in the rat's blood sugar levels. Guava leaf extracts and entire plant extracts were evaluated for chemotherapeutic potential. Its effects on cancer cell lines from the colon, prostate and epidermal malignancies are still being investigated (Osasenaga *et al.*, 2017). Guava leaves have the ability to suppress a variety of cancer cell lines. Lycopene and the flavonoid quercetin are abundant in the leaves. Lycopene and quercetin, both of which have anti-cancer effects, as demonstrated in studies to help reduce the risk of cancer. Guava leaf extract has been shown to inhibit the growth of cancer cells in the breast and prostate (Gutierrez *et al.*, 2008). In 2010, a human study was conducted. Guava's potential health advantages for prostate cancer were explored. The findings suggested that guava extract can reduce the size of a prostate cancer tumor. Guava leaf has potent antibacterial properties, according to various research. In addition, dried leaves are used in traditional medicine to treat viral infections. Guava is also used as an herbal treatment for cholera. Guava leaf and bark have antibacterial efficacy against multi-drug resistant Vibrio cholera, according to the International Center for Diarrhoeal Disease Research in Bangladesh.

Materials and Method

Plant material and extraction procedure. Fresh white *Psidium guajava* L. (Guava) fruits were procured from Ikom main market in Cross river state, Nigeria, for this study. Dr. H.R. Omehie of Cross river university of technology's forestry and wildlife department recognized the plant materials. The leaves were ground into a fine powder and stored in a jar. In a soxhlet, 500 g of crushed leaves were extracted with two solvent systems: n-hexane and methanol. To obtain the crude extracts, the extracts were concentrated.

Acute toxicity (determination of LD_{50}). Thirteen (13) Wistar albino mice with a body weight of 20 to 25 g were used. The rats were housed at Cross river University's rat house of biochemistry. The rats were fed poultry starter and given free access to water. The rats were subjected to a seven day adaptation period. Lorke (1983) method was used to investigate the acute toxicity of *Psidium guajava* fruit puree extract on 13 albino mice.

Experimental phases. During the investigation, there were two phases,

Phase I. The mice were divided into three groups, each with three mice; they were given 10, 100 and 1000 mg/Kg b.w. of *P. guajava* puree through oral intubation, respectively. This phase was used to track and investigate the extract's toxicity in rats.

Phase II. Oral intubation was used to administer 1600, 2900, 3500, and 5000 mg/Kg b.w. of *P. guajava* puree to four (4) groups of one mouse each. The medicated mice were monitored for 72 h to see if they died. The LD_{50} was calculated as the average of the maximum non-lethal dose and the minimum poisonous dose.

Injection of 2,4,5,6(1H,3H)-pyrimidinetetrone. Male Wistar albino rats weighing 120-150 g were used in this study. Before the injection of 2,4,5,6(1H,3H)-pyrimidinetetrone, the blood glucose level was tested. The rats were given free access to drink but were fasted for 24 h before being injected with 2,4,5,6(1H,3H)-pyrimidinetetrone. It was dissolved in ice-cold normal saline as a carrier and given intraperitoneally at a dose of - 150 mg/Kg body weight. Blood was drawn from the tail vein five days after induction and placed on the glucometer sensor pad that had previously been introduced into the glucometer strip. Diabetic rats with blood glucose levels of greater than 200 mg/dL are tested and examined (Frode and Medeiros, 2008).

Experimental design. The rats were separated into five groups.

Group 1. Normal rats given normal saline; **Group 2.** Diabetic (untreated) control; **Group 3.** Diabetic given 25 mg/Kg b.w of metformin; **Group 4.** Diabetic given 200 mg/Kg b.w of *Psidium guajava* puree; **Group 5.** Diabetic given 400 mg/Kg body of *Psidium guajava* puree.

For the next 21 days, the treatment was continued. The rats' fasting and random blood glucose (FBG and RBG) levels were monitored using an Accuchek active glucometer (Roche Diagnostics, Germany) in the following order: 1, 4, 8, 15 and 21 days after receiving the extract (Trinder, 1969). Oral intubation was used to administer the *Psidium guajava* fruit puree, which was dissolved in fresh normal saline. Using a spectrophotometric approach and Randox commercial diagnostic kits, blood was taken through ocular puncture and serum liver enzymes - alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activity were tested.

Data analysis. The data are expressed as mean S.D and statistical significance was determined using one-way and two-way analyses of variance (ANOVA) at a 95% confidence level (P>0.05).

Results and Discussion

The mean baseline fasting blood glucose concentrations in all groups were within the normal range (below 110 mg/dL) before the introduction of diabetes with 2,4,5,6(1H,3H)-pyrimidinetetrone, as shown in Fig. 1. The rats in groups 2, 3, 4 and 5 exhibited mean starving blood glucose concentrations above 200 mg/dL on day 1 (i.e. 5 days after induction), indicating significant elevations in starving blood glucose concentrations compared to group 1 rats. Clearly, the rats were diagnosed as diabetic. When compared to the untreated group, rats in groups 3, 4 and 5 experienced a non-significant (P>0.05) drop in fasting blood glucose concentrations on day 4. (control). Despite a significant decrease in fasting glucose readings in the 3, 4 and 5 groups, in comparison to the diabetes group on days 8, 15 and 21 (control). Fasting blood glucose levels in rats given normal saline solutions (normal control) remained rather constant and were not statistically significant (P>0.05).

The mean baseline random blood glucose concentrations for groups 1, 2, 3, 4 and 5 were 122, 127, 131, 139 and 124 mg/dL, respectively and were within the normal range (140 mg/dL), indicating that they were not diabetic. On day 1 (5 days after treatment with 2,4,5,6(1H,3H)-pyrimidinetetrone), rats in groups 2, 3, 4 and 5 had significantly higher random blood glucose values (P>0.05) than rats in group 1, indicating that the rats were diabetic. On day 4, group 3 and 5 rats had significant reductions in their random blood glucose concentrations when compared to group 2 (diabetic controls), however the drop in group 4 rats' random blood glucose concentrations was non-significant (P>0.05). On day 8, rats in groups 3, 4 and 5 were divided into three groups' revealed significant reductions in their random blood glucose concentrations, which were comparable to group 2. Animals in groups 3, 4 and 5 also showed significant declines (P>0.05) on day 15 when compared to group 2. On day 21, the random blood glucose concentrations of groups 3, 4 and 5 reduced significantly (P>0.05) when compared to group 2, but not significantly when compared to group 1 (normal controls) Fig. 2.

Effect of white *Psidium guajava* puree treatment phosphatase (ALP) activity. The alkaline phosphatase (ALP) activity (29.00 IU/L) of group 2 rats (diabetic untreated) was considerably higher (P>0.05) than that of group 1 (non-diabetic) rats (10.50 IU/L). Furthermore, when compared to group 2 mice, metformin-treated animals (group 3) had a significant drop in blood ALP (10.50 IU/L). Similarly, guava puree treatment at 200 and 400 mg/Kg b.w. (groups 4 and 5 respectively) resulted in a significant drop in ALP actions (14.00 and 11.00 IU/L) in the groups compared to group 2 animals, but not a significant decrease (P>0.05) compared to Group 1 (10.59 IU/L) Fig. 3.

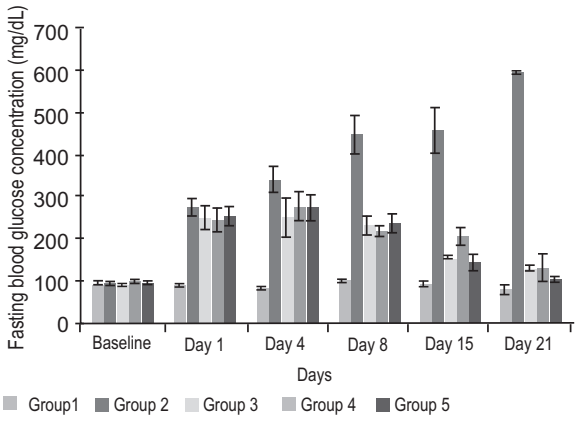

Fig. 1. Effect of white *Psidium guajava* puree on the fasting blood glucose concentration in diabetic rats.

The serum AST activity of group 2 (diabetic untreated) was found to be significantly higher than that of group 1 (non-diabetic) animals, with a mean activity of 73.50 IU/L compared to 42.50 IU/L. Animals in groups 1 (normal non-diabetic), 3 (25 mg metformin/Kg b.wt), 4 (200 mg guava puree/Kg b.wt) and 5 (400 mg guava puree/Kg b.wt) showed substantial decreases in serum AST activities, with mean activities of 42.50, 46.25, 43.33 and 35.00 IU/L, respectively, compared to group 2. Furthermore, serum AST activities of groups 3, 4 and 5 (diabetic treatment groups) were non-significantly lower (P>0.05) than those of group 1 (non-diabetic) rats (Fig. 4).

When compared to group 1 (non-diabetic) animals with a mean serum AST activity of 34.50 IU/L, the serum ALT activity of (diabetic-untreated) animals in group 2 was enhanced (P>0.05) with a mean activity of 64.00 IU/L. In addition, guava puree treatments of 200 and 400 mg/Kg b.wt significantly reduced (P>0.05) serum ALT activities, with mean activities of 50.47 and 34.50 IU/L respectively, when compared to group 2 (64.00 IU/L), but showed significant increases (P>0.05) when compared to metformin treatment (27.50 IU/L). Fig. 5.

On 2,4,5,6(1H,3H)-pyrimidinetetrone induced diabetic Mellitus, the anti-diabetic and hepatoprotective properties of white guajava puree were investigated. *Psidium guajava* extracts have long been prized as anti-diabetic treatments. When compared to the control group, *Psidium guajava* fruit puree significantly reduced both fasting and random blood glucose concentrations in rats after a 21-day therapy (diabetic). The anti-diabetic activities of *Psidium guajava* puree were attributed to flavonoids, which include glycosides and terpenoids (Vertichenvan and Jegadeesan, 2002; Cetto and Wiedenfiend, 2001). Their findings are similar to those of (Norazmir and Ayub, 2010; Maryuma *et al*., 1985). Evidence that *Psidium guajava* fruit puree reduces fasting and random blood glucose levels in rats with 2,4,5,6(1H,3H)-pyrimidinetetrone induced diabetes implies that guava puree has anti-diabetic properties. This could be due to the reduction of glucose absorption, the high sensitivity of insulin receptors and the stimulation of peripheral glucose uptake, all of which are experimentally verified. Because the liver detoxifies xenobiotics, the liver function assay is crucial (Heywood, 1983). Enzymes are the most reliable indicator of tissue damage in general. The release of amplified enzyme measurements into the blood stream is used to detect organ or tissue harm (Uroko *et al*., 2020). According to Vanghn (1999), the activities of most enzymes that can be measured in blood are pretty

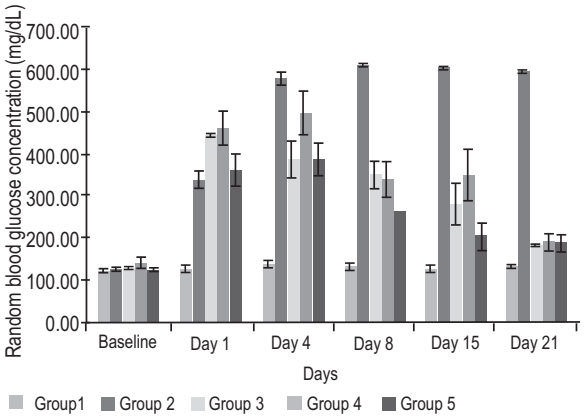

Fig. 2. Effect of white *Psidium guajava* puree on the random blood glucose concentration in diabetic rats.

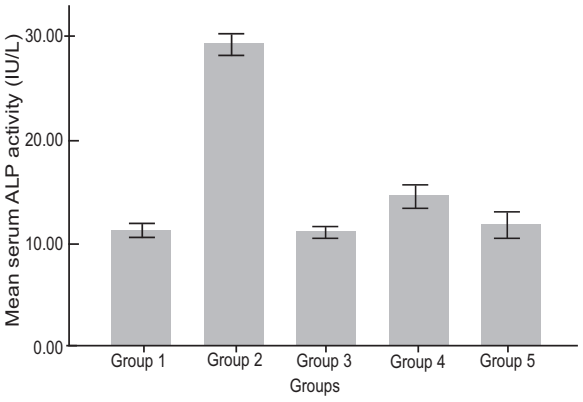

Fig. 3. Effect of white *Psidium guajava* Linnaeus puree on serum alkaline phosphatase (ALP).

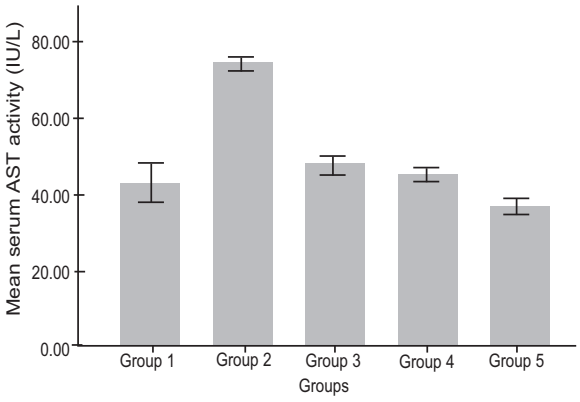

Fig. 4. Effect of white *Psidium guajava* L. puree on serum aspartate aminotransferase (AST).

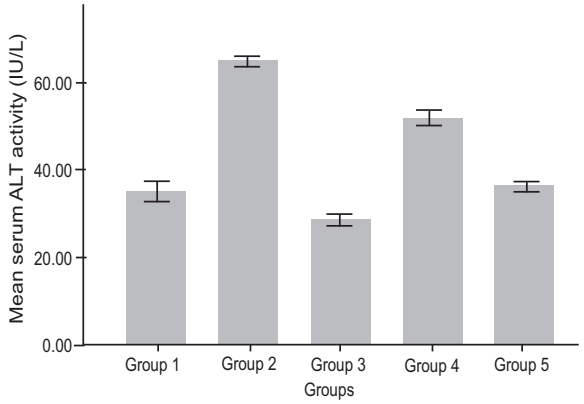

Fig. 5. Effect of white *Psidium guajava* puree treatment on serum alanine amino-transferase (ALT) activity in diabetic rats.

consistent in healthy people. These results show that serum ALP, ALT and AST actions in rats in group 1 (normal control) were significantly enhanced. This is because, in diabetic conditions, hyperglycemia is an underlying cause of reactive oxygen species by non-enzymatic glycation of proteins, the amino group of phospholipids and DNA, *via* pro-inflammatory cytokines that activates cyclo-oxygenase. Most difficulties associated with diabetes Mellitus are due to the generation of ROS (Wolff, 1993). Further, ROS activates NF-Kb (transcription factor), increases the level of mRNA, TNF-alpha and IL-12 (hepatic pro-inflammatory cytokines), resulting in liver cell injury. Also, superoxides which act as a cellular messenger can as well elicit an inflammatory response and also induce gene expression encoding inflammatory proteins such as proteinases (collagenases and elastases), leading to tissue destruction (Oldenburg *et al*., 2001). The serum activities of groups 4 and 5 which received 200 and 400 mg *Psidium guajava* fruit puree/Kg b.w., respectively, in a dose-dependent manner were significantly lower than those of group 2 (diabetic untreated) rats. The decrease in blood ALP and AST activities seen in groups given guava puree was comparable to the decrease in serum ALP and AST activities shown in group 3 (standard control) given metformin, while (Okpashi *et al*., 2014; Farinazzi-Machado *et al*., 2012) published similar findings. Because increased AST and ALT activities are associated to heart disease, diabetic rats administered white guava fruit puree had lower serum enzyme levels, indicating a lower risk of liver and heart disease in diabetic patients if raw white guava fruits are prescribed to diabetic people. Because higher ALP levels indicate liver illness or bile duct blockage (Joshua *et al*., 2019), the preventive effect of guava fruit puree on liver cells was established (Rai *et al*., 2010; Armstrong *et al*., 1996). The hepato-protective effect of white guava puree may be due to a high level of vitamin C (Msihra and Seshadri, 1967). Flavonoids subtypes - quercetin, rutin, naringin, catechins, gallic acid and chlorogenic acids (Papanga *et al*., 1999; Croft, 1998) present in guava fruit do exhibit high level of antioxidant activity *via* the electron and as a result, improved antioxidant activity and lipid peroxidation deregulation could be a potential method for dietary supplementation to help with diabetic complications.

Conclusion

This study shows that white *Psidium guajava* puree has anti-diabetic properties and can preserve the liver of rats given 2,4,5,6(1H,3H)-pyrimidinetetrone to cause diabetes. Fasting and random blood glucose levels, as well as liver marker enzyme concentrations, were successfully reduced by white guava puree. As a result, oral administration of white *Psidium guajava* fruit purees equal to eating the fruit could be employed as a viable nutraceutical therapy for diabetes and postprandial hyperglycemia management.

Conflict of Interest. The authors declare that they have no conflict of interest.

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