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Alloxan-Induced Diabetes: The Synergistic Effects Of Combined Ethanolic Leaf Extract Of *Dacryodes edulis* and *Psidium guajava*

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ABSTRACT

The purpose of this study was to evaluate the synergistic antidiabetic and possible hepatoprotective effects of combined ethanolic leaf extract of *Psidium guajava* and *Dacryodes edulis* in alloxan-induced diabetic Wistar rats. Using ethanol at the proper stock quantities, fresh leaf extract of *Psidium guajava* and *Dacryodes edulis* was produced and employed in a 14-day therapy. Studies on the combined extract's acute toxicity in rats at doses up to 6000mg/kg revealed no harm. Using alloxan monohydrate (150mg/kg) intraperitoneally, diabetes was developed after 14 days of acclimation in experimental rats. The blood glucose levels in diabetic rats treated for 14 days with various concentrations of extract—10% (10ml of extract was diluted in 90ml of ethanol), 15% (15ml of extract was diluted in 85ml of ethanol), and 20% (20ml of extract all decreased significantly ($p<0.05$) in comparison to the diabetic control rats. Additionally, rats treated with extract concentrations of 10%, 15%, and 20% showed significantly lower serum levels of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) compared to the diabetic control group. Thus, it may be concluded that the combined ethanolic leaf extract of *Psidium guajava* and *Dacryodes edulis* reduces lipid peroxidation, improves antioxidant status, and decreases hyperglycemia in alloxan-induced diabetic rats while also protecting the liver. In order to control diabetes and hepatotoxicity, *Psidium guajava* and *Dacryodes edulis* leaf extracts can be mixed.

Key Words: *Psidium guajava*, *Dacryodes edulis*, combined ethanolic leaf extract, diabetes, hepatotoxicity, Phytochemicals

I. INTRODUCTION

In various nations of the world, including Nigeria, China, and India [1], plants have been the primary source of medications for the treatment of diabetes. The World Health Organization [2], acknowledged the significance of anti-diabetic plants in the development of affordable and efficient treatments for diabetes, which is currently thought to afflict 30 million people worldwide. According to Loew and Kaszkin [3], the majority of anti-diabetic plants contain compounds such as glycosides, alkaloids, terpenoids, and flavonoids, among others, that give them their anti-diabetic properties.

In West Africa, the plant *Dacryodes edulis* (Lam.), sometimes known as the African Black Pear, is well recognized. It is a medium-sized, evergreen tree that grows up to 18 to 40 meters in height in the forest but not higher than 12 meters in plantations [4]. The root, bark, and leaves are used for a variety of therapeutic uses, while the fruit is palatable [5]. The plant enhances soil quality and makes a significant contribution to conventional medications [6]. Researchers have found that *Dacryodes edulis* contains anti-microbial, anti-oxidant, and liver-protective characteristics [7-10].

Psidium guajava (L.) is a member of the Myrtaceae family and is cultivated for its therapeutic and dietary benefits. In many nations, the leaf, stem, bark, roots, and fruits of the guava tree have been used to treat various medical ailments. *Psidium guajava* (L.) is abundant in minerals, vitamins (A, B, and C), iron, phosphorus, calcium, and other nutrients that support healthy blood circulation, nerve relaxing, and stimulation of cognitive function. Additionally, it contains a lot of organic and inorganic secondary metabolites that have the potential to be anti-inflammatory, anti-oxidant, antiviral, and anti-diabetic.

There is a need to focus more research on potential management solutions utilizing medicinal

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plants like *Dacryodes edulis* and *Psidium guajava* because diabetes is one of the leading causes of death and causes renal failure, neuropathy, cardiovascular disease, stroke, and even blindness. Therefore, the purpose of this study was to evaluate the anti-diabetic and synergistic effects of combined ethanolic leaf extract of *Dacryodes edulis* and *Psidium guajava* on alloxan-induced diabetic Wistar rats.

II. METHODS

Plant collection

The leaves of the plants *Dacryodes edulis* and *Psidium guajava* were collected from Choba Campus of the University of Port Harcourt in Obio/Akpor Local Government Area of Rivers State and identified in the herbarium of the Department of Plant Science and Biotechnology in the University of Port Harcourt by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology.

Plant Preparation and Extraction

The leaves of the plants *Dacryodes edulis* and *Psidium guajava* were pulverized and air tried for three weeks after which the leaves were ground into fine powder using an Electric Blender. 10g of *Dacryodes edulis* and 10g of *Psidium guajava* were combined in 200ml of ethanol and shaken vigorously for 15 minutes and then sealed in a maceration jar and left to stand for 48 hours at room temperature. After the 48 hours period, the mixture was filtered using a clean handkerchief and then a clean Whatman (No.24) filter paper. After filtration process, a gel-like extract was obtained. The ethanolic extract was then made into different concentrations using different volumes of ethanol in different plastic bottles as follows;

- 10ml of extract was diluted in 90ml of ethanol to give 10% concentration of extract
- 15ml of extract was diluted in 85ml of ethanol to give 15% concentration of extract
- 20ml of extract was diluted in 80ml of ethanol to give 20% concentration of extract

Acute toxicity test on combined Ethanolic Leaf extract of *D. edulis* and *P. guajava*

Acute toxicity was determined using the methods of Lorke [11].

Procurement of Animal

Twenty (25) Wistar rats weighting 130 to 200g were used for this study and were obtained from Animal House of Department of Pharmacology, University of Port Harcourt, River State, Nigeria and acclimatized for 2 weeks. The animals were maintained before and throughout the experiment period in standard cages with access to clean water and food (pellets) ad libitum under standard environmental conditions (temperature: 27.0±1.0°, relative humidity: 55-65% and 12 h light/12 h dark cycle). At the start of the experiment, the animals were randomly distributed into 5 groups of 5 animals each.

Induction of Diabetes

In order to induce diabetes in rats, freshly made alloxan monohydrate was diluted to a dosage of 150mg/kg body weight and injected intraperitoneally. Alloxan-induced rats were found to have diabetes three days later when their Random Blood Glucose (RBG) levels were below 200mg/dL. Blood samples were drawn from the tail vein, and glucose levels were checked using a hand-held glucometer (Accu-CHEK).

Experimental Design

Twenty (25) adult Wistar rats of both sexes were used and were randomly selected into five groups of 5 rats each. Group A served as the Normal control, they were not induced with diabetes and were fed with rat pellets and water only, Group B served as ethanol control and were administered 2ml of ethanol containing 10% volume/volume of ethanol, Group C served as diabetes control and did not receive any treatment, Group D was treated with 10% concentration of *Dacryodes edulis* and *Psidium guajava* combined ethanolic leaf extract, Group E was treated with 15% concentration of *Dacryodes edulis* and *Psidium guajava* combined ethanolic leaf extract and Group F was treated with 20% concentration of *Dacryodes edulis* and *Psidium guajava* combined ethanolic leaf extract. The experimental animals were treated with the extract for 14 days.

Collection of Blood samples and Organs

After receiving treatment for 14 days, the rats were fasted for 12 hours, given a chloroform anesthetic, and then sacrificed. Direct cardiac puncture was used to obtain blood samples into lithium heparin vials. The University of Port Harcourt Teaching Hospital (UPTH), Rivers State Chemical Pathology Laboratory received the blood samples for analysis.

Biochemical Assay

For the ALT and AST assay, the Reitman and Frankel [12] method was used. ALP was calculated using the King and King [13] method

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that was modified by Cheesbrough [14]. As modified by Tietz [15], Malloy and Evelyn's [16] methods were used to estimate glucose, while Tietz's [17] method was used to determine serum lipids such as total cholesterol, total protein, and triacylglycerol.

Ethical Clearance

The Research Ethics Committee of the University of Port Harcourt and the National Institutes of Health's guidelines for the care and use of laboratory animals were both used to guide all techniques used throughout this research.

Method of Data Analysis

SPSS version 23.0 was used to analyze the data. Each piece of data was reported as Mean ±SEM. The means of the groups were compared using one-way analysis of variance (ANOVA), with a p-value of less than 0.05 deemed significant. To determine whether there were significant differences between the groups, a Tuckey's post-hoc test was used.

III. RESULT

Acute toxicity test on Combined Ethanolic leaf Extract of *P. guajava* and *D. edulis*

Even at the greatest dose tested (6000mg/kg body weight), no mortality was seen over a period of 24 hours using Lorke's [11] methodology. However, at dosages of 4000 and 6000mg/kg body weight, certain symptoms of listlessness, shivering, and mouth scratching were seen in experimental rats. As a result, it was evident that the extracts were acutely non-toxic to experimental animals.

Table 1: Effect of combined ethanolic leaf extract of *Dacryodes edulis* and *Psidium guajava* on some lipid profile parameters of alloxan-induced Wistar rats

Groups	Glucose (mmol/L)	Total Protein (g/L)	T. Cholesterol (mmol/L)	Triglyceride (mmol/L)
Normal Control	4.26±0.60	66.00±4.47	5.25±1.95	1.44±0.51
Diabetes Control	13.22±2.53*	48.40±7.40*	7.06±1.05*	3.10±0.83*
Ethanol Control	9.12±1.20*#	57.42±4.08*#	5.09±0.82#	1.89±0.54*#
10% Extract	11.74±1.93*#	53.60±6.54*#	6.38±0.59*#	2.72±0.80*#
15% Extract	8.86±1.06*#	58.80±3.63*#	5.38±0.40*#	2.10±0.60*#
20% Extract	4.75±0.64#	61.00±1.41*#	4.00±0.28*#	1.50±0.28*#

Each value represents mean ± SEM, values marked with (*) differ significantly from normal control group (*p<0.05) while those marked with (#) differ significantly from diabetes control group (#p<0.05).

Table 2: Effect of combined ethanolic leaf extract of *Dacryodes edulis* and *Psidium guajava* on some Liver enzyme parameters of alloxan-induced Wistar rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Control	7.80±1.48	9.20±2.28	46.26±3.51
Diabetes Control	20.60±4.56*	26.00±4.95*	80.45±5.83*
Ethanol Control	9.12±1.65*#	12.37±2.18*#	52.50±4.60*#
10% Extract	14.80±3.03*#	19.00±3.16*#	60.56±5.80*#
15% Extract	13.20±2.28*#	11.60±1.67*#	52.10±4.72*#
20% Extract	7.00±1.41*#	9.01±1.42*#	45.50±3.84*#

Each value represents mean ± SEM, values marked with (*) differ significantly from normal control group (*p<0.05) while those marked with (#) differ significantly from diabetes control group (#p<0.05).

IV. DISCUSSION

Increased oxidative stress is linked to diabetes progression [18]. Experimental animals were given the pancreatic islets of Langerhans oxidative cytotoxin, alloxan, which induced severe glucose intolerance, hyperglycemia, and a reduction in endogenous insulin secretion and release. These signs and symptoms come before impaired peripheral tissue glucose uptake [19-21]. Therefore, treating diabetes with plants high in antioxidants as well as diets supplemented with eatable plants may be a creative way to lower complications brought on by diabetes's characteristically abnormal production of free radicals [22,23].

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Although there is paucity of information regarding the combination of the leaf extracts of *P. guajava* and *D. edulis* as possible antidiabetic agent, but there are some evidences that the plants *P. guajava* [24,25] and *D. edulis* [26,27] can prevent pancreatic beta cell apoptosis and preserve function. *P. guajava* leaf extracts were subjected to phytochemical screening, which revealed the presence of bioactive components including tannins, saponins, steroids, flavonoids, alkaloids, and anthraquinones [25,28], whereas *D. edulis* leaf extracts revealed the presence of bioactive components including alkaloids, phenols, flavonoids, triterpenoids, tannins, and saponin [29]. Uahomo *et al*. [30] stated that plant-derived phytochemicals key in the treatment of chronic diseases such as diabetes, breast cancer, and coronary artery disease.

The findings of this study showed that the treatment with combined ethanolic leaf extract of *P. guajava* and *D. edulis* against rats with alloxan-induced diabetes exhibited antihyperglycaemic and antihyperlipidemic effects. As shown in Table 1, when compared to diabetic rats that were not treated; the treatment with combined ethanolic leaf extract of *P. guajava* and *D. edulis* considerably lowered blood glucose levels. Hence, the combined plants' ethanolic leaf extract may have antihyperglycemic properties and work synergistically in inhibiting diabetic effects in Wistar rats. This is consistent with the findings of Ofoha and Nimenibo-Uadia [24], Ononamadu *et al*. [27], Parker *et al*. [25] and Akoko *et al*. [31]. Blood glucose levels were significantly higher in diabetic control rats, but group D (treated with 10% extract combination), E (treated with 15% extract combination) and F (treated with 20% extract combination) experimental animals' blood glucose levels were significantly reduced as a result of the intervention with the combined ethanolic leaf extract. Serum lipid levels, such as cholesterol, free fatty acids, and phospholipids, are typically higher during diabetes. In this study, treated experimental rats had significantly lower total cholesterol and triglyceride levels and significantly higher levels of total protein as compared to untreated diabetic animals. Alkaloids and tannins, in addition to insulin, have been linked to the regulation of hyperglycemia [32].

In this study, a significant ($p<0.05$) rise in the serum AST and ALT activity was seen in the diabetes control group compared to the normal control group and the treatment groups (Table 2). This might signify hepatic tissue injury that causes these cellular enzymes to leak from the cytosol into the circulation [33,34]. Additionally, a comparable rise in serum ALP levels was seen in diabetic controls, indicating that alloxan administration to rats affected the plasma membrane's structural integrity. This is due to the fact that in diabetic states, hyperglycemia is a root cause for the non-enzymatic glycation of proteins, amino groups of phospholipids, and DNA via pro-inflammatory cytokines that activate cyclo-oxygenase. ROS production is the primary cause of most diabetes mellitus complications [35]. The activation of the transcription factor NF-kb by ROS also causes an increase in the mRNA levels of the cytokines IL-12 and tumor necrosis factor (TNF) alpha, which damage liver cells. Additionally, superoxides function as cellular messengers and cause inflammation, which activates the production of inflammatory proteins (such as collagenases and elastases-type proteinases) and damages tissue [36].

When compared to the mean values recorded for untreated diabetic animals, treatment with the combined ethanolic leaf extract of *P. guajava* and *D. edulis* resulted in a significant ($p<0.05$) decrease in serum AST, ALT, and ALP activities in groups D (treated with 10% extract combination), E (treated with 15% extract combination), and F (treated with 20% extract combination). Both the observed decrease in serum ALP and AST activities in group B animals (ethanolic control) treated with 10% ethanolic and the decrease in serum ALP and AST activities in the groups treated with combined ethanolic leaf extract of *P. guajava* and *D. edulis* were slightly similar. These results support those of Parker *et al*. [25].

Since elevated AST and ALT enzyme activities are linked to both heart and liver diseases, decreased serum levels of these enzymes in diabetic animals treated with combined ethanolic leaf extract of *P. guajava* and *D. edulis* suggested that eating fresh *P. guajava* and *D. edulis* leaves may reduce the risk of liver and heart diseases in diabetic patients. Increased ALP levels are a sign of liver disease, bile system obstruction, or bone disease [37]. As a result, the combined ethanolic leaf extract of *P. guajava* and *D. edulis* showed a reduction in ALP level while improving the effectiveness of liver function.

The presence of phytochemicals in the extract with known antidiabetic effects may account for the observed drops in blood glucose and cholesterol levels as well as the hepatoprotective effect in diabetic rats treated with the combined ethanolic leaf extract of *P. guajava* and *D. edulis*.

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V. CONCLUSION

The study has shown that the combined ethanolic leaf extract of *P. guajava* and *D. edulis* has synergistic anti-diabetic properties and protects diabetic rats after alloxan induction. The combined ethanolic leaf extract of *P. guajava* and *D. edulis* significantly reduced hepatic enzyme markers and blood glucose levels. As a result, it is suggested to utilize *P. guajava* and *D. edulis* leaves together as a treatment for the management of diabetic disorders.

Conflict of Interest

The authors declare no conflict of interest.

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