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Research Article

Aqueous Extract of Unripe Golden Apple (*Spondias Dulcris*) Is Effective in the Reduction of Plasma Glucose and Triacylglycerol Levels In Healthy Albino Rats

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Abstract: ***Background:*** Spondiasdulcris has diverse traditional medicinal uses in its fruits, leaves and bark in different parts of the world. It is useful for burns and wound healing, remedy for diarrhea, anti-diabetic, antihypertensive and the inner bark is used to treat cough and fever. ***Objective:*** The aim of this study was to determine the effects of aqueous bark extracts of unripe Spondias dulcris on plasma glucose, triacylglycerol concentrations and weight in normal albino rats as well as histopathological examination of the liver, hearts and kidney sections of the rats. This was done with a view of understanding the possible role of this plant in the treatment of diabetes mellitus. ***Materials and Methods*:** The test groups were administered a dose of the extract (2ml/kg body weight) twice daily in addition to growers mash while the control group was exposed to only grower's mash and distilled water. The plasma glucose and triacylglycerol concentrations were measured by spectrophotometry. Both groups were weighed on the weighing scale on test days up to the 28th day after which the animals were sacrificed and histological examination was done. ***Results*:** The result showed significant ($p<0.05$) reduction in plasma glucose and triacylglycerol in the test group when compared to the control. The histological report showed that the aqueous extract of Spondias dulcris was neither cardiotoxic, hepatotoxic nor nephrotoxic.
***Conclusion*:** Conclusively the aqueous bark extract of Spondias dulcris could have therapeutic effects by its anti-diabetic and hypolipaemic effects in a number of metabolic syndromes including diabetes mellitus in humans.

Keywords: Spondias dulcris,Plasma glucose and triacylglycerol ,weight,Wistar Albino Rats.

INTRODUCTION:

Medicinal plants have been used since ancient times for the treatments and management of diabetes mellitus in traditional medicine systems of many cultures throughout the world (Lev, E. 2006). They are commonly used in treating and preventing specific ailments and diseases, and are generally considered to play a beneficial role in health care. They are very important to the global economy in that their steadily increasing demand occurs not only in developing countries but also in the industrialized nations (Lev, E. 2006).

The genus *Spondias* consist of seventeen described species, seven of which are native to the neotropics and about ten are native to tropical Asia. *Spondiasdulcis*, a native of Melanesia and Polynesia belongs to the family *Anacardiaseae (*Mitchell, J. D., & Daly, D. C. 1998*). Spondiasdulcis* is a deciduous perennial fast growing tree that can reach up to twenty meters, with pinnate leaves, twenty to sixty centimeters in length, composed of nine to twenty-five glossy, elliptic or obviate-oblong leaflets nine to ten centimeters long which are finely toothed toward the apex. The tree produces small, inconspicuous white flowers in terminal panicles. Its oval fruits, 6-9cm long, are borne in bunches of twelve or more on a long stalk (Mitchell, J. D., & Daly, D. C. 1998). Over several weeks, the Fruit fall to the ground while green and hard, then turn golden yellow as they ripen.

The plant grows best in the sub-humid and frost free tropics, it is found from sea level up to 700m.

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It grows best in areas where annual daytime temperatures are within the range 22-27^{o}C, but can tolerate 12-32^{o}C. *Spondiasdulcis* has the potential to be used as a specialty plant for medicinal purposes. *Spondiasdulcis* is used as a medicinal plant for several illnesses such as coughs, fever and stomach aches (Gurib-Fakim, A. 2006). The shoots of the plants are used to treat hemorrhaging after child birth. The fruits, leaves and bark are used in treatment of wounds, sores and burns (van de Venter, M. *et al*., 2008). A few drops of the pressed bark fluid are applied to the eyes as a remedy for cataracts.

Figure 1: *Fruit of Spondiasdulcis* .

The grated fruit, mixed with water, is used to treat high blood pressure (Schmidt, B. *et al*., 20008). In the American tropics, *Spondiasdulcis* is used as juice and as a flavoring for ice creams and sorbets due to its high vitamin C content and to flavor yogurts in the Caribbean. The plant is also grown as a living fence thereby increasing its economic value. Most fruits including *Spondiasdulcis* have been found to contain high fibre and vitamin C contents which are very essential in reducing blood glucose (Sofowora, A. 1996). Glucose is a simple sugar with a molecular formular of $C_6H_{12}O_6$. It is the most important source of energy for cellular respiration. It is stored as a polymer in plants as starch and in animals as glycogen. Glucose is on the WHO list of essential medicines and most important medication in a basic health system.[7]Normal blood glucose is approximately 4gs in the blood at all times and tightly regulated by metabolic homeostasis (Wasserman, D. H. 2009) Glucose is stored in skeletal muscle and liver cells in form of glycogen. Normal values by Randox Laboratories = 4.2-6.4mmol/l or 75-115mg/dl.

Triacyglycerol is an ester from glycerol and three fatty acids (Busch, S. 2017). they are used as fuel and stores fats in adipose tissues. Extremely high levels of > 500mg/dl are major risks of heart disease and pancreatitis. Normal value is < 150mg/dl. Its major function is to contribute to membrane lipid layer thereby protecting against shock and acting as thermal insulator and adds flavor to palatability of food. Sources include shell fish, dairy products, butter and margarine which are high in unhealthy saturated fat and trans fats, will raise triacylglycerol and cholesterol levels (Berglund, L. *et al*., 2012). It is a secondary energy source released during carbohydrate metabolism, it helps in absorption of fat soluble vitamins A, D,E and K (Dhanavade, M. J. *et al*., 2011).Triacylglycerols are non-polar, hydrophobic and insoluble in water (Wedro, B. 2016).The American diabetes association includes *Spondiasdulcis*, on the list of super foods due to its soluble fiber and vitamin C contents. High fiber diets have been shown to reduce blood glucose and lower the risk of heart disease by reducing blood pressure and cholesterol (Dhanavade, M. J. *et al*., 2011). Therefore it is expedient to determine the effects of the aqueous bark extract of *Spondiasdulcis*, on blood glucose and triacyglycerol as this will enhance better management of a number of metabolic disease conditions including diabetes mellitus and cardiovascular disorders in humans.

MATERIALS AND METHODS

Chemical Reagents:

All reagents used were of analytical grade. They were produced by Randox laboratories, England and were obtained from a commercial supplier here in Nigeria.

Experimental Animals:

Out-bred matured six (6) healthy male albino rats of Wistar strain aged eight (8) to ten (10) weeks old with average weight of 160-200grms were used. These were obtained from the animal house of Department of Biochemistry, College of Health Sciences, Niger Delta University, Bayelsa State. On arrival the animals were maintained under standard animal house condition. The animals were allowed to acclimatize for 2 weeks and fed with pelletized feeds through the experiment (14 days) with water. The entire experimental protocol was performed in accordance with the Institutional Animal Ethical Committee (IAEC), in line with the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Preparation of Extract:

The unripe *Spondiasdulcis* were procured from Opolo local market in Yenagoa, Bayelsa State. The unripe *Spondiasdulcis* fruits were then thoroughly washed with water before debarking. 10g of the peel was cut into tiny bits and homogenized in 100ml of distilled water after which it was sieved with the aid of a sterile cheese cloth. The extract was stored in a bottle and kept in the refrigerator at 4^oC (35^oF) and used via oral administration within 24hr.

Administration of Extract:

The aqueous extract of the unripe *Spondiasdulcis* peel was administered orally to the experimental animals using gavage at 2ml/kg body weight of rat twice daily at 12hr interval.

Blood specimen collection:

Rats were immobilized before blood collection usually early in the morning. The tail of the rat was sterilized with a swab dabbed in methylated spirit and lubricated with a brand of petroleum jelly to reduce friction. A gentle and persistent massage was applied until the tip of the tail became reddish (indicating blood accumulation). A sterile blade was used to make an incision at the tip of the tail. The tail was continuously massaged until the required amount of blood needed was collected into the specimen bottles (lithium Heparin and fluoride oxalate) for plasma triacyglycerol and glucose respectively. Blood was collected from both test and control groups on days 0, 1, 6, 12, 18, 24 and 28^{th} day. After the blood collection the tail was cleaned with swab dabbed in methylated spirit to prevent infection and then a dry sterile swab to stop the bleeding. Blood specimen were immediately subjected to centrifugation at 3,000rpm for 20mins to obtain plasma samples. Analysis was carried out immediately after centrifugation .A standard weighing balance was used to weigh the rats in both groups in every experimental day.

Experimental Design:

The healthy male albino rats of Wister strain after acclimatization for a period of 2 weeks were randomly distributed into 2 groups with 3 rats in each group.

Group 1: Served as controls and fed on pelleted growers feed and distilled water throughout the experiment (28 days).
Group 2: Served as Test groups that were administered extract of unripe *Spondiasdulcis* peel orally twice daily and fed on pelleted grower's feed and clean water throughout the experiment (28 days).

Glucose Assay:

Glucose assay was carried out by enzymatic oxidation in presence of glucose oxidase as described by Randox laboratories, England. Exactly 10 micro litre of plasma collected in fluoride oxalate bottle was transferred into the test tubes containing 1000 micro litre of reagents. Samples were thoroughly mixed and incubated at 37^oC for 10 mins. The absorbance values were read at 540nm using distilled water and glucose reagent as blank. The concentration of the sample was calculated as follows:

$$\text{Glucose concentration(mg/dl)} = \frac{\text{Asample}}{\text{A standard}} \times \text{Standard concentration(mg/dl)}$$

Where
A = absorbance

Triacyglycerol Assay:

This was determined after enzymatic hydrolysis and condensation as described by Randox laboratories ltd. England. Exactly 10 micro litre of plasma sample collected in lithium heparin bottles were transferred to test tubes containing 1000 micro litre of triacylglycerol reagent. Samples were mixed thoroughly and incubated at 37^oC in the water bath for 5mins. The absorbance value was read at 546nm using distilled water and triacylglycerol reagent as blank. The concentration of triacylglycerol was calculated as follows:

$$\text{Triacyglyride(mg/dl)} = \frac{\text{A Sample}}{\text{A standard}} \times \text{Standard concentration(mg/dl)}$$

Where
A = absorbance

Histological Assessment of tissues:

The animals were sacrificed on the 28^{th} day and dissected to collect the heart, kidney and liver for histological studies. The tissue samples were immediately immersed into 10% formalin. The fixed

tissue sample were embedded in paraffin wax,cleared in xylene and sections cut using 5 micron in a rotatory microtome. The sections were examined for general tissue structure using light microscopy after staining with hematoxylin and eosin dye for general tissue structure and interpreted by a specialist.

Statistical Analysis:

Statistical Analysis was done using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc. Chicago, Illinois, USA). Data were analyzed using tables and graphical representations. Results were expressed as (means ± SEM) and the comparisons in group values were done using the One Way Analysis of Variance (ANOVA) followed by Post-Hoc LSD. Box plots showed graphical representation of values. P-values<0.05 were considered statistically significant in all analyses.

RESULTS:

The result obtained from the study are represented in tables 1,2 and 3 below, showing the mean ± SEM of plasma glucose, plasma triacylglycerol and the weight of rats in both test and control groups within 28 days. From the result obtained, it was observed that the aqueous extract of *Spondiasdulcis* caused a significant decrease in plasma glucose and triacylglycerol concentrations in days 6, 12, 18, 24 and 28, when compared with the control group. Both study groups had observable weight gain. However, this was not significant statistically.

Table 1: Mean plasma glucose concentration in male albino rats administered aqueous bark extract of *Spondiasdulcis* for 28 days.

Group	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
Control	67.23± 13.67	68.75± 3.37	70.83*± 3.52	68.14*±2.54	68.17*±1.78	68.14*± 2.50	67.18*±1.12
Test	70.30± 9.83	33.35± 1.02	38.83*± 0.87	31.20*±0.90	31.32*±0.86	30.69*±0.64	30.09*±0.12

Each value represents the mean ± SEM of three separate determinations. Values are statistically different from control at p<0.05* One Way Analysis of Variance (ANOVA) followed by post-hoc LSD.

Table 2: Mean plasma Triacylglycerol concentration in male albino rats administered aqueous bark extract of *Spondiasdulcis* for 28 days**.**

Group	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
Control	141.48± 12.31	130.29± 13.21	119.20*± 9.03	127.91* ±9.37	112.78*±3.78	113.27*± 4.87	112.97* ±5.70
Test	138.62± 10.5	112± 2.93	73.91*± 1.07	71.65*± 3.01	66.44*± 3.16	65.62* ± 2.79	64.11* ± 2.91

Each value represents the mean ± SEM of three separate determinations. Values are statistically different from control at p<0.05* One Way Analysis of Variance (ANOVA) followed by post-hoc LSD.

Table 3: Mean weight of normal rats administered aqueous bark extract of *Spondiasdulcis* for 28 days.

Group	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
Control	181± 2.30	182± 2.08	184± 1.00	185± 4.16	187± 4.16	188± 3.60	194± 6.00
Test	181±1.00	183±0.57	185±1.15	185±4.72	187±4.72	188±4.50	190±7.00

Each value represents the mean ± SEM of three separate determinations. Values are not statistically different from control at p<0.05. One Way Analysis of Variance (ANOVA) followed by post-hoc LSD

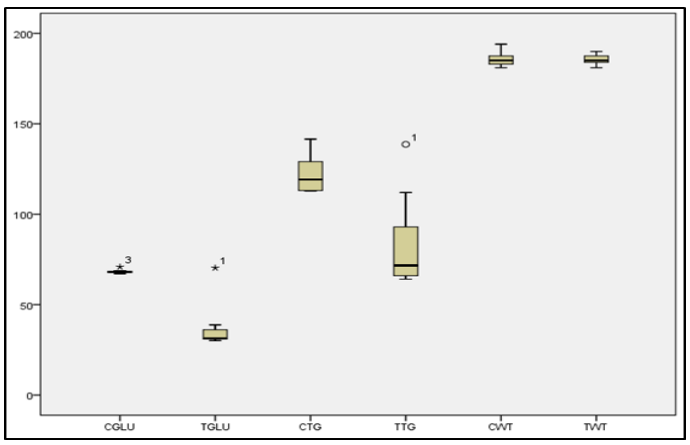

Figure 2: Control and Test group mean values for Plasma Glucose, Triacylglycerol and weight respectively of Albino Rats

Key:

CGLU=Control Glucose
TGLU=Test Glucose,
CTG=Control Triacylglycerol,
TTG= Test Triacylglycerol
CWT = Control Weight
TWT= Test Weight

Figure 2 is a box and whisker plot which showed that the test groups that were administered ***Spondiasdulcis*** all had lower levels of plasma glucose and Triacylglycerol levels. Both groups had weight gain with no statistically significant difference.

Figure 2 shows relatively normal histology of the heart, liver and kidneys in both groups.

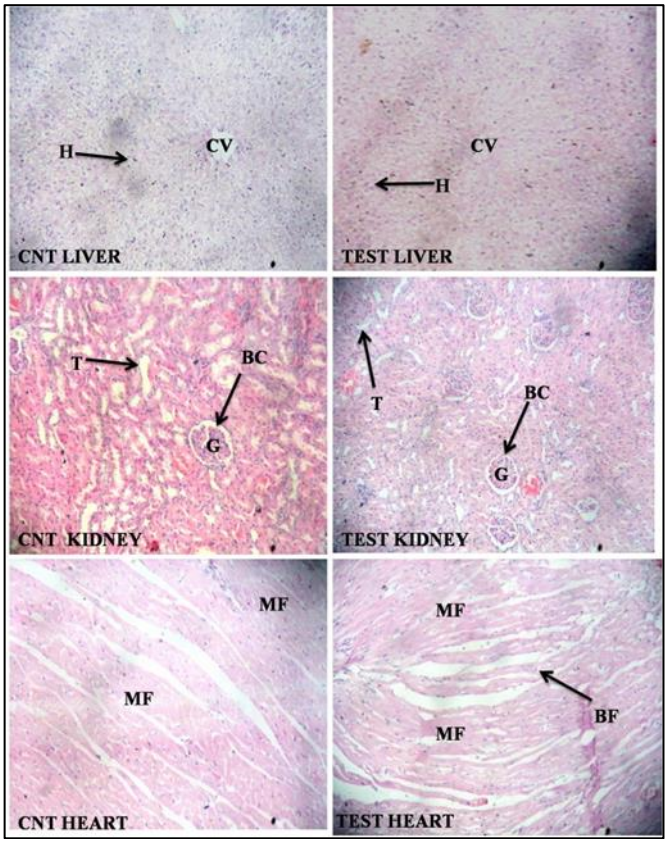

Figure 3 shows Photomicrographs (histology) of the heart, liver and kidneys of both test and control groups.

Key:

Control (CNT) Liver shows normal central vein (CV) with Hepatocytes (H). Test group liver is not different from the control as there are no degenerative or inflammatory changes. Similarly, Control and Test kidney show normal glomeruli (G), Bowman's capsules (BC) and Tubules in their stroma. The Histology of the Control and Test Heart also shows normal cardiac muscle fibers (MF) and their branching feature (BF). H&E x100**.**

DISCUSSION:

In the last few decades, there has been an increase in the study of Medicinal plants and their traditional use in treating and preventing specific ailments and diseases in different parts of the world (Wedro, B. 2016). Spondias dulcris has the potential to be used as a specialty plant for medicinal purposes for treatment of special illnesses and are generally considered to play a beneficial role in health care. The biochemical parameters monitored in the albino rats are useful markers for assessment of disease. The measurement of parameters in the albino rats plays a significant role in disease investigation, diagnosis, assault on the organs/tissues and to a reasonable extent the toxicity of the drug (Elujoba, A. A. *et al*., 2005; Malomo, S. O. 2000).

Diabetes mellitus is a chronic metabolic hyper glycaemic disorder characterized by polyuria, polydipsia, polyphagia and weight loss due to relative or absolute lack of insulin. Insulin resistance occurs when the pancreatic islet cells are persistently exposed to high levels of glucose. To monitor body response to glucose lowering therapy, continous measurement of glucose is necessary. The goal of management of diabetes mellitus is Non drug method (losing some

weight, healthy diet, avoiding alcohol and continuous exercise) and Drug method (Yakubu, M. T. *et al*., 2003).

Very low levels of plasma glucose concentration (hypoglycemia) is when blood sugar decreases to below normal reference levels. This may result in a variety of symptoms including clumsiness, trouble talking, confusion, loss of consciousness, seizures, or death (Geijselaers, S. L. *et al*., 2017).

Elevated levels of triacylglycerol are associated with atherosclerosis, and predispose to cardiovascular disease. Very high triglyceride levels also increase the risk of acute pancreatitis (Yanai, H. *et al*., 2015). In this study, it was observed that the intake of aqueous bark of Spondias dulcris led to reduction in both the plasma glucose and triacylglycerol levels in the test groups which is in keeping with the anti-hyperglycaemic and hypolipaemic potency of the ethanolic leaf extract of *Gongronemalatifolium* according to the study by Ugochukwu *et al*. (2003) This is thought to be mediated through the activation of hepatic hexokinase (HK), phosphofructokinase (PFK) and glucose- 6-phosphate dehydrogenase (G6PDH) and inhibition of glucokinase (GK) activity in the liver.This is also comparable to studies on Alcoholic leaf extract of *Ocimumsanctum (*Chattopadhyay, R. R. 1998*)* and Allium sativa(garlic)[19] which ameliorates hyperglycemia in normal-glucose fed hyperglycemic and streptozotocin-induced diabetic rats by potentiating the action of exogenous insulin in the rats. The anti-diabetic action of alcoholic leaf extract of *O. sanctum* was comparable with that of the standard anti-diabetic drug-tolbutamide. Rao *et al*. (1999) also observed antidiabetic and hypolipaemic effects of Momordicacymbalaria hook fruit powder in alloxan-diabetic rats.

Weight gain was observed in both groups with no significant difference in the test and control groups. This showed that Spondiasdulcris has no effect on weight rather the weight gain in both groups was probably due to proper feeding with the growers mash.

According to the histological analysis, the aqueous bark extract of *Spondias*dulcis had minimal or no toxic effect on the heart ,liver nor kidneys. This is in keeping with Idowu *et al*. (2018) whose report on aqueous extract of *Brachylaena elliptica caused a significant increase in glucose uptake in HEPG2 in liver cells with resultant hypoglycaemic effect.This was used by tradomedical healers in eastern Capetown Province of South Africa which* gave the best overall results, with all plant parts exhibiting high activity scores and negligible toxicity at all doses. This is also comparable to the aqueous leaf extract of *Cissampeloscapensis* (De Wet, H. *et al*., 2011) where a tincture of the rhizome in alcohol or brandy or a decoction is taken as blood purifier to treat boils, glandular swellings, syphilis, cholera, colic. diarrhea, diabetes and several cancers with minimal toxic effect. Contrary to this however, is the in vitro toxicity effects from the study on *Momordicabalsamina* (African pumpkin) (Thakur, G. S. *et al*., 2009) extracts which raised concerns for chronic use despite its beneficial effects.. That is both aqueous and organic extracts of *Momordicabalsamina* were toxic to the heart and liver following prolonged use. This underscores the need for dose-related medication for intended use.

CONCLUSION

In the course of this study, there was significant reduction in the plasma glucose and triacylglycerol concentration in the rats administered with aqueous bark extract of *Spondiasdulcis* as against the control group and negligible weight gain was observed in both groups...Histological results showed that after 28 days of administration, there was no toxic effect on the heart, no nephrotoxic nor hepatotoxic activity. The fruit could be further investigated for the treatment of diabetes mellitus and or cardiovascular diseases in humans.

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