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Protective Potentials of Aqueous Bark Extracts of Unripe Lemon (Citrus limon) on Plasma Glucose and Triacylglycerol in Healthy Albino Rats

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***Abstract*— Background- Citruslimon is one of the major fruits that grow throughout the world with refreshing juice and health benefits. Numerous therapeutic properties have been ascribed to its use such as anti- ulcer, anti-tumor, anti-inflammatory as well as its ability to control blood glucose.**

Objective

The aim of this study was to determine the effects of aqueous bark extracts of unripe citrus limon on plasma glucose, triacylglycerol concentrations and weight of normal albino rats as well as histopathological examination of the liver, hearts and kidney sections of the rats.

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, triacylglycerol and weight in the test group when compared to the control. The histological report showed that the aqueous extract of citrus limon was neither cardiotoxic, hepatotoxic nor nephrotoxic.

Conclusion

Conclusively the aqueous bark extract of Citruslimon could have protective and therapeutic effects in the reduction of high blood glucose and triacylglycerol levels in a number of metabolic syndromes including diabetes mellitus in humans and may also be effective for weight loss as may be necessary for stable health conditions.

***Index Terms*— Citruslimon, Plasma glucose, Plasma Triacylglycerol, Weight, Albino Rats.**

I. 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. [1]

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The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals. These active substances that contain the healing properties are known as active principles and differ from plant to plant. [2] Generally, the active principles found in plants can be extracted and used in different forms which include infusions, syrups, concoctions, decoctions, infusion and essential oils, ointments and creams in the treatment, management and prevention of a number of diseases. [3] Herbal medicines have been shown to have utility and about 80 percent of rural human population depends on it as primary health care. Most fruits have been found to contain high fibre and vitamin c contents which are very essential in reducing blood glucose.[4] Lemon (*Citruslimon*) in various countries of the world has long history of folk remedy for treatment of diabetes. *Citruslimon* belongs to the Rutaceae family and believed to have first grown in Asia, in the North East India.[5] Its genetic origin reported it to be a hybrid between bitter orange (Sour orange) and citron with up to 21 species.[6] It is widely grown in Nigeria for its medicinal values. It has potential of improving nutrition and boosting food security. Lemons are rich in vitamin C, the juice is about 5-6% citric acid with a pH of 2.2 giving it a sour taste which makes it a key ingredients in drinks and foods like lemonade.[7] Lemon is used mainly for its alkanoid properties with a broad spectrum of biological activities including anti bacteria, anti fungal, anti diabetes, anti cancer and anti viral activities.[8] Citrus peels are also used in treatments of scurvy, indigestion, respiratory disorders, peptic ulcer, eye infections, gout, skin infections, piles, urinary disorders and may promote weight loss. The strong aroma emitted by lemon is found to be effective in reducing nausea and vomiting during pregnancy. It has also been proven that the use of lemon juice on daily basis lowers systemic blood pressure.[9] It has been shown that lemon is beneficial in treatment of rheumatism, internal bleeding, cholera, kidney stones, stroke, hair growth and strengthening of the immune system.[10] It is of great economic value to industries that produce lemonades, lemon beverages, toothpaste, syrups (for cough, catarrh, cold and sore throat), soap,, body sprays, deodorants, insect repellants and wood polishes.[11] It also gives appreciable income to farmers who trade on it and dehydrated lemon peel is also marketed as cattle feed.

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.[12] Normal blood glucose is approximately 4gs in the blood at all times and tightly regulated by metabolic homeostasis[12] 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. [13] it is used as fuel and stored as fat 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. Sources include shell fish, butter and dairy products. It is a secondary energy source released during carbohydrate metabolism which helps in the absorption of fat soluble vitamins A, D,E and K.[14]

The American diabetes association includes lemon 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, promote weight loss and lower the risk of heart disease by reducing blood pressure and cholesterol.[4] Therefore it is expedient to determine the effects of the aqueous bark extract of lemon on blood glucose, triacyglycerol and weight as this will enhance better management of a number of metabolic disease conditions including diabetes mellitus and cardiovascular disorders even in humans.

Figure1: **Unripe*Citruslimon***

II. MATERIALS AND METHODS

A. 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.

B. 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 throughout 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.

C. Preparation of Extract:

The unripe lemons were procured from Opolo local market in Yenagoa, Bayelsa State. The unripe lemon 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^{o}C (35^{o}F) and used via oral administration within 24hr.

D. Administration of Extract:

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

E. 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 specimens 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.

F. 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 lemon peel orally twice daily and fed on pelleted grower's feed and clean water throughout the experiment (28 days).

G.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^{o}C 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:

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

Where A = absorbance

H. ***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^{o}C 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:

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

Where A = absorbance

I. ***Histological Assessment of Tissues:***

The animals were sacrificed on the 28th 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.

J. 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 $\pm$ SEM) and the comparisons in group values were done using the One Way Analysis of Variance (ANOVA) followed by Post-Hoc LSD. Box and Whisker plots showed graphical representation of values. P-values<0.05 were considered statistically significant in all analyses.

III. RESULTS

Tables 1 and 2 showed plasma concentration of glucose and triacylglycerol while table 3 showed the mean weights of albino rats in both test and control groups.

The results showed that the plasma glucose, triacylglycerol and weight in the test groups were significantly ($p<0.05$) lower compared to the control groups.

Table 1: Mean plasma Glucose concentration of Control and Test Rats

group	day 0	day 1	day 6	day 12	day 18	day 24	day 28
Control	55.7±2.72	55.7±3.24	57.76±3.33	61.60*± 2.60	67.32*± 2.90	74.62*±0.55	74.62*±2.05
Test	56.69±2.61	56.40± 1.80	55.36± 0.72	42.38*± 2.38	39.48*±2.81	36.78*± 0.55	32.86*±2.06

Each value represents the mean ± SEM. *= test values are statistically different from control at p< 0.05 using the one way analysis of variance (ANOVA) followed by post-hoc LSD.

Table1 showed significant reduction ($p<0.05$) in the glucose concentration of test groups when compared with the control from day 12 through day 28.

Table 2: Mean plasma Triacylglycerol concentration of Control and Test Rats

group	day 0	day 1	day 6	day 12	day 18	day 24	day 28
Control	99.80± 4.71	98.44±7.01	100.73±5.68	105.37*±0.56	107.34*±7.57	109.8*±3.70	109.20*±1.60
Test	99.16± 9.69	99.99± 1.58	94.52±6.00	89.63*± 9.56	82.78*±4.57	77.23*±2.14	71.47*±4.91

Each value represents the mean ± SEM.*= test values are statistically different from control at p< 0.05 using the one way analysis of variance (ANOVA) followed by post-hoc LSD.

In table 2, the test group administered 2ml/kg of the aqueous bark extract of unripe *Citruslimon* showed significant ($p< 0.05$) decrease in the triacylglycerol concentration when compared to the control group from day 12 to day 28.

Table 3: Mean weight (g) of rats in both Control and Test groups

Each value represents the mean ± SEM. Mean in the same column with different superscript letters are statistically significant, P<0.05(One Way Analysis of Variance (ANOVA) followed by post-hoc LSD).

	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
Control	160±0.01[a]	160±0.00[a]	180±0.02[a]	190±1.02[a]	200±2.04[a]	220±0.82[a]	220±0.98[a]
Test	160±0.02[a]	160±0.01[a]	150±0.21[a]	140±0.04[b]	140±1.00[b]	130±0.05[b]	130±0.79[b]

In table 3, it was observed that the rats in the control progressively increased in weight in days; 6, 12,18 and 24 but the weight of rats in the test group decreased ($p<0.05$) in day 12 through day 28.

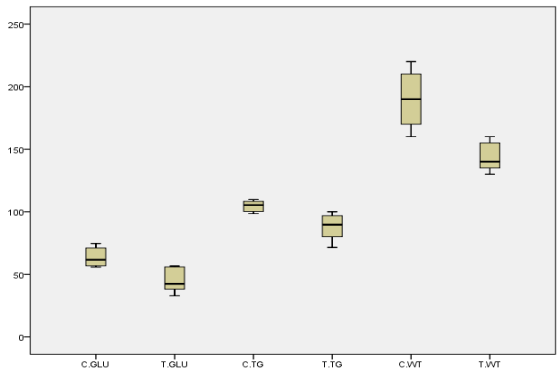

Figure 2: Control and Test group mean values for Plasma Glucose, Triacylglycerol and weight respectively of Albino Rats.

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 *Citruslimon* all had lower levels of plasma glucose and Triacylglycerol levels and reduced weight levels when compared with the control groups.

Figure 3 shows the histological report of the heart, liver and kidneys of both test and control groups. Both the test and control groups have normal histology.

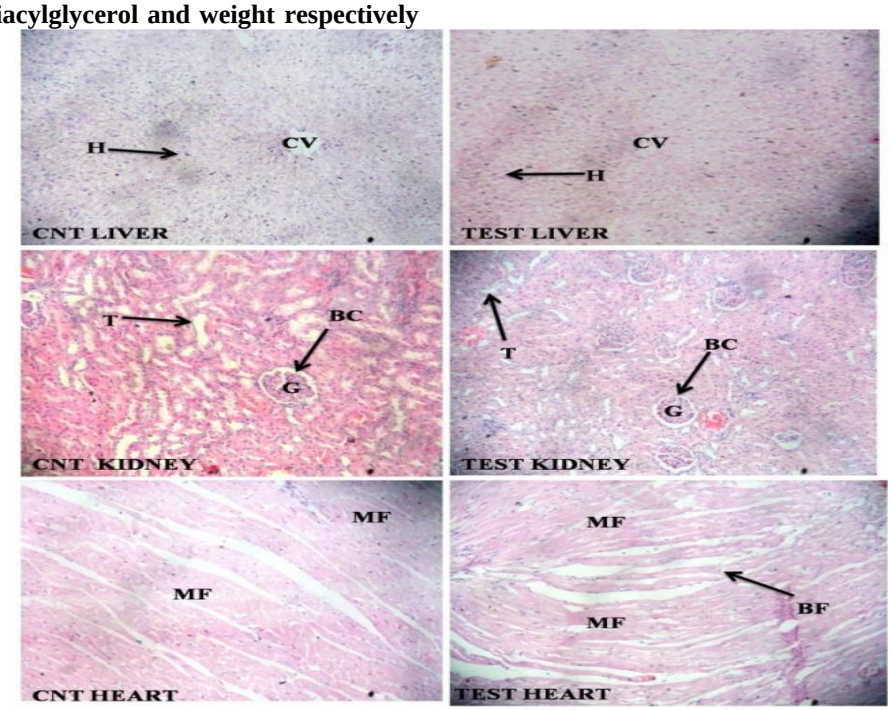

Figure 3 shows Photomicrographs (histology) of the heart, liver and kidneys of both test and control groups.

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.

IV. DISCUSSION

Citruslimon have nutritional values as seen in its vitamins, minerals and amino acid contents. Its naturally active components comprise dietary fibre 19%, vitamin C 28%, vitamn B6 5%, vitamin A 20%, vitamin E, folate, niacin, thiamine (3%), riboflavin, pantothenic acid, copper (13%) calcium (6%), iron (4%), magnesium, potassium, zinc, phosphorus and protein (2%). It is an invaluable source of nutrients for all age groups.[15] Its phytochemical characteristics makes it useful as an antioxidant and modulates gene expression and signal transduction pathways

[16] It also contains sterols which are integral components of membrane lipid bilayer where they play important role in the regulation of membrane fluidity and permeability. [17]

Medicinal plants are of great importance to health of individuals and communities. Their values lie in the inherent characteristic chemical components necessary for some physiological actions even in humans.[13,18] The phytochemical constituents of plants are responsible for their biologic, pharmacological and medicinal potential which remain largely unassessed by scientific research.[18] Medicinal plants are widely used in non-industrialized societies mainly because they are readily available and cheaper than modern medicines. The annual global export of 50,000 to 70,000 plants with suspected medicinal value was estimated to be 2.2 billion US Dollars in 2012 and keep increasing to several billions in recent years (Medicinal and Aromatic Plants Trade 2017).[18] In many developing countries like Nigeria, there is little regulation of traditional medicine but the WHO coordinates a network to encourage safe and rational usage. These plants are also subject to general threats like climate change, habitat destruction and over use to meet market demand.

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 is persistently exposed to high levels of glucose. To monitor body response to glucose lowering therapy, continuous 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.[19]
In this study, the concentration of glucose and triacylglycerol which decreased in the test groups with administered unripe lemon extract, demonstrated dose and time-dependent relationship with the tested rat. The initial mean glucose and triacylglycerol concentrations and mean weight were 56.6mg/dl, 99.1mg/dl and 160g respectively but with continuous administration of extract over time to the test groups resulted in significant reduction to 39mg/dl, 77mg/dl and 130g respectively. The mechanism of reduction in blood glucose by *Citruslimon* is not clear however, a constituent(Resistant starch) has been shown to directly increase insulin sensitivity in healthy people and in type 2 Diabetes Mellitus patients [20] who are insulin resistant thereby possibly reducing the risk of hyperglycemia in them. This is also in keeping with studies on high fiber dietary content of lemon which confers hypoglycemic effects and weight loss on consumption.[21]

The weight loss could be induced due to soluble pectin fibres in them which expands in the stomach thereby giving a feeling of fullness and a reduction in calorie intake. However, no study has confirmed the weight loss effect of lemon compounds in humans. The high content of alkaloid is also responsible for its lipid lowering mechanism by reducing cholesterol levels thereby lowering the risk of cardiovascular diseases.

The histological report which revealed that the extract was neither toxic to the liver, heart nor kidneys is in keeping with the study[22] on detoxification function of *Citruslimon* which due to its bitterness activates bile flow thereby emulsifying and removing fat soluble toxins, thereby acting as liver health enhancing purifier. Normal renal histological findings in this study is in keeping with conducted urology findings[22] on rats where it was revealed that rats fed on lemon juice had reduced calcium levels thereby preventing renal stones like calcium oxalate urolithiasis. It is believed to do this by increasing urine volume and pH thereby creating a less favorable environment for kidney stone formation. Normal cardiac histology was also seen in this study. This protective effect may be ascribed to the short duration (28days) of the study. However, contrary to this, prolonged usage could be toxic when taken in excess of body requirements with clinical effects like heart burn, ulcers, frequent micturition, nausea and vomiting etc. Generally, daily requirement and maintenance dosage for its use is advised.

V. CONCLUSION

To summarize, this study infers that aqueous bark extract of unripe *Citruslimon* has the potential of reducing high levels of plasma glucose, triacylglycerol and may promote weight loss with no significant toxicity to the heart, liver and kidneys. This may confer its protective role against a number of metabolic disorders including diabetes mellitus, cardiovascular disorders and may promote weight loss as may be necessary for stable health conditions in humans.

Therefore, more well conducted studies needs to be done to prove the usefulness or not.

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