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Evaluation of Aqueous Leaf Extract of *Solanun Melogena* on Some Plasma Electrolytes and Liver Enzymes Markers of Diabetic Mice

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Abstract
The recent study investigated the effect of aqueous leaf extract of *Solanun melongena* (Eggplant) on some plasma electrolytes and liver enzyme markers of diabetic mice. Twenty five male mice (body weight 40 to 45g) were randomly divided into five groups. First group served as control while 4 groups were induced diabetes by administration of alloxan (120 mg/kg i.p). Second group served as diabetic control, third, fourth and fifth group were adminstered 200, 400 and 600 mg/kg *S. melongena* extract for 28 days. Daily fasting blood sugar was also monitored. Electrolyte and liver function tests were performed. The result showed that serum sodium levels of untreated diabetic mice were higher than control, though the mean difference was not statistically significant ($p>0.05$). Diabetic Mice treated with 200 and 400 mg/Kg *S. melongena* extract had higher potassium levels compared to untreated diabetic. However, *S. melongena* extract resulted in increased bicarbonate levels, however, the mean difference were not statistically significant ($p>0.05$). Mean serum AST levels of untreated diabetic Mice was significantly higher ($p<0.05$) than control. Diabetic Mice treated with mg/kg *S. melongena* extract had lower serum AST compared to diabetic untreated, however the mean difference was not statistically significant ($p>0.05$). Serum ALT levels of untreated diabetic Mice were lower than control Mice. However, there was no significant difference ($p>0.05$) in serum ALT levels of extract treated mice compared to diabetic untreated. These findings suggest that garden egg leaf extract could be used in management of liver and kidney complications associated with diabetes.

Keywords: Diabetes mellitus, Liver enzymes, Plasma Electrolytes, Solanun melongena, Alloxan

Introduction
Solanun melongena (eggplant) is a common and popular vegetable crop grown in the subtropics and tropics. It is locally known as anyara or igba in the eastern and western part of Nigeria, brinjal in india and aubergine in Europe [6]. *Solanum melongena* is a large leaved woody perennial shrub and also cultivated for its ovoid fruit [6]. The fruit varies in size and may be black, purple, white, or striped. It has an erect busy stem, large ovate, slightly lobed leaves and pendant violet solitary flowers [3]. Research has shown that *S. Melongena* may serve both for treatment of weight loss, asthma, anti-ulcer, constipation and diabetes [4], reduce glaucoma [10].

The liver is a vital inner organ that performs numerous functions.[8] They include secretion of bile, excretory function, synthetic function, metabolic function, hemopoietic function, hemolitic function, heat production, defence and detoxification function[8], clotting factors and albumin synthesis.[13] The human liver consists of numerous enzymes that aids necessitate chemical processes in the body.[12, 13] The most commonly used indicators of liver functions are the Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) [7] and alkaline phosphatase concentration. Variations in the concentrations of these enzymes have many implications. Increased levels of ALT and AST are indications of hepatocellular disease, active cirrhosis, metastatic liver tumor, toxic hepatitis, severe, pancreatitis, myocardial infarction (heart attack), trauma, severe burns, acute hemolytic anemia, crushing injuries and shock. [12] Alkaline phosphatase is commonly used to access

obstruction in the biliary system. Its increase is majorly detected in biliary tumor, gallstone disease, alcohol abuse and drug-induce hepatitis. [15] Induction of diabetes is normally done on experimental animals using Alloxan and streptozotocin [1]. The use of these agents in inducing diabetes in rats involves destruction of beta cells of pancreas by autoimmune [1]. The treatment of diabetes involves administration of insulin [8].

Diabetes mellitus type 1 known blood insulin dependent diabetes mellitus [IDDM], causes increased glucose concentration. Thus increase blood glucose causes dehydration due to excess excretion of water and resulting into decrease in plasma osmolality, depletion of extracellular fluid HCO_3^-(bicarbonate ion), a fall in PH level and decrease in salt concentration [19].

Materials and Methods

Aqueous preparation of *solanun melongena* leaf extract

Fresh leaves of *Solanun melongena* was gotten from a garden in Orlu, Imo state, Nigeria and was sent to a Botanist in Pharmacology Department Madonna University Nigeria for identification. It was dried in an oven under temperature of about 90^{0c} and ground to powder and was dissolved in 500mls of distilled water for 48 hours then filtered with a filter paper and evaporated to dryness in water bath (B-Bran Scientific and Instrument Company, England) at 60^0C. The brownish residue were weighed and kept in an air tight bottle in the refrigerator until use. This method was recently used by, [11].

Laboratory animals

Twenty five (25) male Wistar Mice aged 6 weeks and weighing 40–45g were used for this study. The animals were housed in the Department of Physiology Animal house, Madonna University, Elele, Rivers State, Nigeria. Standard animal cages with wood dust as bedding were used in keeping the animals. They were allowed *ad libitum* access to mice specific feed and clean water, and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days. The animals were kept in line with laid down principles for animal care as prescribed in Helsinki's 1964 declaration. The animal ethics committee of Madonna University approved our study protocol graciously.

Induction of Experimental Diabetes

Diabetes was induced via intra-peritoneal injection of alloxan at a dose of 120mg/kg b.w., reconstituted in normal saline. Prior diabetes induction, the animals were fasted for 12 hours. Confirmation of diabetes was done after 12hours of alloxan injection through determination of Fasting Blood Sugar, using a glucometer (B-Bran Scientific and Instrument Company, England). Blood sample for the FBS determination was obtained from tail puncture of the mice, and animals with FBS ≥ 200mg/dl were considered diabetic and included in the study as diabetic animals, method used by Udosen, et al., (2012) [21]

Experimental design and *Solanun melongena* extract administration

The animals were randomly assigned into five (5) groups of five animals each. First group serves as the control; second, third, fourth and fifth groups were induced diabetes with alloxan. The second group serves as diabetic untreated, whereas third, fourth and fifth groups were fed with 200, 400 and 600 mg/kg *S. melongena* extract for 28 days. Administration of aqueous extract of *S. melongena* was done via orogastric feeding once daily for the 28 days while the control group received normal saline as vehicle after which the animals were sacrificed under chloroform anaesthesia and blood sample was collected for electrolyte and liver enzyme assay.

Determination of Serum Electrolytes

Determination of Sodium Ion (Na^+) and Serum Potassium (K^+) Concentration were measured using Centronic Gmbh kit via turbidimetric determination method by Hillmann et al., (1967) and Tietz (1976), [9, 20].

Determination of Serum Chloride Ion Concentration was done based on the method of Schoenfield and Loewell, (1964), [16].

Determination of Serum Bicarbonate Concentration was done via Flame Photometry method, as used by Sidney and Simpson, (1955), [17].

Determination of liver enzymes

ALP was measured according to standard procedure.[5] P-nitrophenyl phosphate was hydrolysed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01ml in a test tube was mixed with reagent (0.5ml) containing the substrate p nitrophenyl phosphate and kept at room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was allowed to stand for 3 minutes and the absorbance read again at 405nm. Alkaline phosphate activity was calculated from.

UL = 2760 x ΔA nm/minute micro

Where UL = Unit of alkaline phosphatase affinity

ΔA = Change in absorbance

Serum AST and ALT levels were determined using endpoint colorimetric diagnostic kit (Randox Labouratories, UK) based on Reitman and Frankel's method.[14] The pyruvate produced by transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a coloured hydrazone, and was used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine was used to measure aspartate aminotransfarase (AST). Both ALT and AST were read at 540nm wavelength. [14]

Statistical Analysis

All results are presented as mean ±standard error of mean (SEM). One way analysis of variance (ANOVA) was utilized in comparing the difference within groups, followed by post hoc multiple comparisons. The level of significance was placed at $p<0.05$.

Results

Table 1: Serum Electrolyte Levels of Control, Diabetic Untreated and *S. melongena* Treated Mice after 28 days.

Electrolyte Parameters (mEq/L)	Control	Diabetic untreated	200mg/Kg of extract	400mg/Kg of extract	600mg/Kg of extract
Na^+	136.06±13.20	163.72±10.73	152.66±9.58	170.93±12.58*	182.42±8.21*
K^+	4.07±0.45	3.92±0.25	4.29±0.23	5.77±1.36	3.31±0.14
Hco_3^-	17.76±3.43	17.68±1.82	19.02±0.22	23.49±2.41	18.11±3.92
Cl^-	144.17±2.85	128.20±10.14	124.17±6.01	109.31±12.13	150.05±15.64*

Values are expressed in mean ± SEM, n = 5.

Table 2: Serum Liver Enzyme Levels of Control, Diabetic Untreated and *S. melongena* Treated Mice after 28 days.

Liver Enzymes (U/L)	Control	Diabetic untreated	200mg/Kg Extract Treated	400mg/Kg Extract Treated	600mg/Kg Extract Treated
AST	45.33±6.98	73.33±7.88*	59.67±7.31	65.00±12.49	63.00±14.00
ALT	8.00±2.31	6.67±1.33	8.67±0.67	9.33±1.33	9.33±1.33
ALP	41.35±10.27	52.36±0.82	45.70±3.27	46.49±5.39	46.39±5.65

Values are expressed in mean ± SEM, n = 5.

Discussion

Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including increased risk of non-alcoholic fatty liver disease, liver inflammation or cirrhosis and kidney problems. The role of the kidneys is to control endocrine processes such as Vitamin D secretion, RBC production and regulation of blood pressure. The kidney plays an important role in the regulation of electrolyte/fluid balance, the PH buffer system and removal of waste products. Decline in the function of the kidneys result to the impairment of these processes. Oftentimes, people with diabetes may develop complication like diabetic nephropathy which causes sclerotic changes in the structure of the kidney. The eventual result of these structural changes is development of proteinuria and reduction in renal glomerular filtration rates. Liver enzyme markers and electrolyte levels are employed in the assessment of liver and kidney functions, [8].

The present study investigated the effect of aqueous leaf extract of *Solanun melongena* (Eggplant) on some plasma electrolytes and liver enzyme markers of diabetic mice using the alloxan induced diabetes model. Alloxan monohydrate has been documented to cause destruction of beta cells of pancreas which result to hyperglycemia. The hyperglycemia induced by alloxan mimics insulin dependent diabetes mellitus (IDDM) in experimental animals and also induces a variety of metabolic abnormalities, [18].

The plasma electrolytes parameters assessed in this study were plasma concentration of sodium ion, potassium ion, Bicarbonate ion and Chloride ion and liver enzyme markers include the plasma levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP). The aforementioned parameters were assessed in both the control, diabetic untreated and *S. melongena* leaf extract treated groups.

The result from this study showed that the serum sodium ion levels of the untreated diabetic mice were higher than that of the normal control, though the mean difference was not statistically significant ($p>0.05$). However, mice treated with 400 and 600 mg/kg *S. melongena (*garden egg*)* extracts had significantly higher ($p<0.05$) plasma sodium levels compared to the normal control. The significant increase in plasma Na^+ concentration in *S. melongena* treated mice correspond to the study done by Atangwho, *et al.*, (2007) [2]. It may also be attributed to increased production of aldosterone and other mineralocorticoids which may have increase the reabsorption of Na^+, [8].

The plasma potassium levels of the untreated diabetic mice were lower compared to non-diabetic Mice. Diabetic Mice treated with 200 and 400 mg/Kg *S. melongena (*garden egg*)* leaf extract had higher potassium levels compared to the untreated diabetic, however the mean difference was not statistically significant ($p>0.05$). The plasma bicarbonate levels showed no significant difference ($p>0.05$) in untreated diabetic Mice compared to the non-diabetic Mice. Treatment with 200, 400, and 600 mg/kg *S. melongena (*garden egg*)* leaf extract resulted in increased bicarbonate levels, however, the mean difference were not statistically significant ($p>0.05$). This result correspond to the report of Atangwho *et al*., 2007 [2], who reported no significant difference on the plasma electrolyte levels of *Vernonia amygdalina* treated diabetic animals and was also supported by Udosen et al., 2012, [21].

The plasma chloride levels were lower in the untreated diabetic mice compared to the normal control, although the mean difference was not statistically significant ($p>0.05$). Diabetic Mice treated with 600 mg/kg *S. melongena (garden egg) leaf* extract however, had significantly higher serum chloride compared to the control. The significant increase in plasma Cl^- level in 600 mg/kg *S. melongena* treated diabetic mice may likely be due to increased levels of Na^+ in this group since most sodium ion reabsorption is coupled with chloride ion reabsorption, [8].

The mean serum AST levels of the untreated diabetic Mice was significantly higher ($p<0.05$) than that of the non-diabetic Mice. Diabetic Mice treated with 200, 400 and 600 mg/kg *Solanun melongena (garden egg)* leaf extract had lower serum AST compared to the diabetic untreated, however the mean difference was not statistically significant ($p>0.05$). The serum ALT levels of the untreated diabetic Mice were lower than that of the non-diabetic Mice. However, there was no significant difference ($p>0.05$) in the serum ALT levels of the extract treated mice compared to the diabetic untreated. The serum ALP levels of the untreated diabetic Mice were higher than that of the non-diabetic Mice, although the mean difference was not statistically significant ($p>0.05$). Treatment with different doses of *Solanun melongena (garden egg)* leaf

extract decreased the ALP levels in diabetic rats, however, the mean difference was not statistically significant ($p>0.05$) compared to the diabetic untreated. The significant increased plasma levels of AST in diabetic untreated mice may likely be due to the hepatotoxic effect of alloxan monohydrate which correspond to the report of *Adesokan, et al., 2009*, [1]

Conclusion

The findings of this study suggest that garden egg leaf extract could be used in the management of liver and kidney complications associated with diabetes.

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