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EVALUATION OF KIDNEY INJURY MOLECULE-1, CYSTATIN C, AND SERUM ELECTROLYTES IN STREPTOZOCIN INDUCED DIABETIC RATS TREATED WITH MORINGA OLEIFERA LEAF POWDER

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ABSTRACT

This work was done to determine the kidney injury markers in strreptozocin-induced diabetic rats treated with *moringa oleifera* leaf. This was conducted using 5 groups of 10 rats each were used. Group1 negative control, group2 positive control (diabetics), groups 3 and 4 treated with 150mg/kg and 300mg/kg of *moringa oleifera* leaf respectively.The parameters analysed includescystatinc C, kidney injury molecule-1 and electrolytes. P values $p<0.05$ were considered statistically significant. The study showed that there was no significant difference in the CYS-C levels of all the rats across the group. Also there was a significant decrease in the KIM-1 values of the rats in group 4 (1.72 ± 0.39) when compared with those in group 1, 2 and 3 (2.30 ±0.38, 2.35 ± 0.30 and 2.76±0.39) respectively, Also, there was no significant difference in the serum electrolyte values of all the rats across the groups, indicting absence of severe kidney injury. From this study, it could be inferred that *moringa oleifera* leaf powder used in treatment of streptozocin-induced diabetes in rats metabolic acidosis and reduced the toxic damage to the kidney and liver.

KEYWORDS: Kidney injury molecule-1, cystatin c, serum electrolytes, streptozocin induced diabetic rats. moringa oleifera leaf powder.

INTRODUCTION

Diabetes mellitus is a disease characterized by hyperglycemia caused by impairment of insulin secretion, transportation, stimulation and insulin action. Extended period of continuous increase in glucose levels may lead to macro/ microvascular complications, such as heart disease, hypertriglyceridemia, nephropathy, and neuropathy. For prevention of the consequences of diabetes mellitus, blood sugar level control through diet is very necessary; this may be achieved using orthodox medication or herbal medication. Many plants are consumed for therapeutic purposes for their nutritional and bioactive compounds constituents. *Moringa oleifera* leaves are one of the plants used for glycermic control due to the nutritional content of its leaves, such as protein, vitamins, and minerals (Misrha *et al.*, 2011).

Moringa oleifera contains soluble fibers that enhance reduction of glucose levels, proliferation of lymphocytes and induced nitric oxide from macrophages. The leaves contains polyphenols such as quercetin-3-glycoside, rutin, kaempferol and glycosides, and has been found to be useful in diabetes conditions because of their possible capacity to decrease blood glucose concentrations after ingestion (Arora *et al.*, 2013; Al-Malki and El-Rabey, 2015).

Complications in diabetes are characterized by inflammation, oxidative stress, and immune failure. These may lead to the loss of intestinal mucosal integrity, and as a result, may decrease the intestinal absorption of essential nutrients and thereby predisposes the individual to increase oxidative stress (Rech *et al.*, 2014). In terminal conditions seen in severely ill patients, the network of antioxidant defense mechanisms (e.g., superoxide dismutase, catalase, and glutathione peroxidase) formed by trace element-dependent enzymes may protect cells from superoxide radicals and nitric oxide (Cander *et al.*, 2011). Trace elements such as zinc (Zn), selenium (Se), and copper (Cu) contributes to the protection of cells from oxidative stress (Rech *et al.*, 2014).

Cystatin – C is a low molecular weight (13 Da) cytoplasmic protein, functioning as an inhibitor of various cystein proteases in the blood stream. Cystatin C has a stable production rate and removed from the blood circulation by Glomerular filtration. In healthy

individuals Cystatin C is completely reabsorbed and degraded in the tubules but in subjects with renal disorders, its level in the blood may be raised as high as 2 to 5 times the normal values. Cystatin C is superior to serum creatinine as a marker of Glomerular filtration Rate (GFR).

Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein that serves as an early marker of acute kidney injury. Acute kidney injury has been defined as a rapid decline in glomerular filtration rate. Blood urea nitrogen and serum creatinine are not specific or sensitive enough for the diagnosis of acute kidney injury because they are affected by many renal and non-renal (age, sex, race, muscle mass, nutritional status, infection) factors that are independent of kidney injury or kidney function. Kidney injury molecule-1 (KIM-1) is a recently discovered biomarker that appears to overcome some of the shortcomings associated with urea (BUN) and serum creatinine. It is undetectable in healthy kidney tissue, but expressed at very high levels in proximal tubule epithelial cells in human kidneys after ischemic or toxic injury.

This work aimed to evaluate biomarkers of kidney injury in strreptozocin-induced diabetic rats treated with pulverised *moringa oleifera* leaf.

MATERIALS AND METHODS

Plant Materials and Preparation

The plant was harvested from garden within Madonna University and was identified in the department of plant science of the University. The leaves were air dried at room temperature for two weeks, after which it was pulverized using electronic blender, the pulverized sample was subjected for extraction using four different solvents namely; ethanol, methanol, ethyl alcohol and water.

Animals

Male wistar albino rats (n=60) six weeks old weighing 150-250g was purchased from the animal farm of Madonna University Elele. Each of the animals was housed in animal cage with wire mesh and saw dust lining, and they were kept in a room inside the animal house, with 12 hours light/dark circle, the animals were allowed to acclimatize for 2 weeks, and were given food and water.

Experimental Design

After two weeks, they were numbered and separated into four groups of 10 rats each, group one were fed with animal feed throughout the experimental period, while other groups were fed with high fat diet (HFD) for seven weeks to increase the body mass index. At the end of the 9th week, 0.5ml of of streptozocin 37mg/kg in citrate buffer was administered intraperitoneally to the rats in groups 2, groups 3 and group 4. The rats in groups 3 and 4 in addition to streptozocin were fed with pulverized *Moringa oleifera* leaf daily with the aid of rats cannular, according to the experimental deisgn below. Fasting blood sugar was measured weekly by cutting the tip of the animals tail, using Easy Touch HealthPro glucose monitoring system.

Group 1 (Negative control): The animals in this group were fed with only animal mesh and water throughout the experiment.

Group 2 (Positive control): The animals in this group were given 0.5ml of 37mg/kg of Streptozotocin intraperitoneally in addition to feed and water.

Group 3: The animals in this group were given 0.5ml of 37mg/kg of streptozotocin and 150mg/kg of *Moringa oleifera* leave powder daily, in addition to food and water throughout the experiment period.

Group 4: The animals in this group were given 0.5ml of 37mg/kg of streptozotocin and 300mg/kg of *Moringa oleifera* leave powder daily, in addition to food and water throughout the experiment period.

Determination of Lethal Dose

This involves two steps; in the first step, nine animals were used grouped into three animals, each group were given different doses of the *Moringa oleifera* leaf powder (50, 100, 150mg/kg). The animals were mornitored for 24 hours. Second step three groups of one animal each were given different higher doses of *Moringa oleifera* leaf powder (200, 300, and 400mg/kg). The animals were mornitored for 24 hours.
LD50 was determined using the formular;
$LD50 = \sqrt{(D_O \times D_{100})}$
Where Do = the highest dose that gave no mortality
D_{100} the lowest dose that produced mortality

Induction of Diabetes Mellitus In Rats

Diabetes mellitus was induced by intraperitoneally injecting the rats with STZ (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 37 mg/kg body weight (b.w.) after two weeks of adaptation and seven weeks of feeding with high fat diet. STZ was freshly prepared as solution in 10 mM sodium citrate buffer (pH 4.5) and injected to after overnight fasting. Fasting blood glucose was measured before injection. On the third day after the STZ injection, the blood was sample was collected from the tail of STZ-injected animals, and glucose levels were measured using glucometer (Easy Touch HealthPro glucose mornitoring system).

Samples Collection

At the end of the experimental period, the animals were euthanized by exposure to chloroform; blood sample was collected via cardiac puncture. Blood was collected into test tubes labelled accordingly, Serum samples were separated and used for determination of different biochemical parameters. Liver and kidney were surgically removed. Liver and kidney were washed with ice cold (4^0C) phosphate buffer saline (immediately after

removal) to remove blood, tissue homogenate was prepared by homogenization of 1g of liver/ kidney using BeadBug 6 position tissue homogenizer, the remaining part of the tissue was preserved using formalin for histological studies.

Laboratory Assays Procedures
All reagents were commercially purchased, / prepared and the manufacturers' SOP was strictly followed.

Determination of Rat Kidney Injury Molecule-1 by Elisa (Bioassay, 2017)
Procedure
The microplate wells were numbered and arranged accordingly in the rack; 50ul of standard was added to standard microplate wells and 40ul of sample was added to sample miroplate well and 10ul of anti-KIM-1 antibody was also added to sample microplate wells. This was followed by addition of 50ul of streptavidin (Horse radish peroxidase) to sample wells and standard wells. This was gently mixed. The plates were covered with a seal and incubated for 60mins at 37^0C.

At the end of incubation period, each plate was washed 5times using 0.35ml wash buffer), and was blotted onto an absorbent paper. Then 50ul of the substrate solution A and B was added into each well. The plate was covered with a seal and incubated for 10minutes at 37^0C. This was followed by addition of 50ul of the stop solution to each well. Then the absorbance was read at 450nm using microplate reader.

Estimation of Cystatin C By Elisa (Bioassay, 2017)
Procedure
The microplate wells were numbered and arranged accordingly in the rack; 50ul of standard was added to standard microplate wells and 40ul of sample was added to sample miroplate well and 10ul of anti-Csytatin C antibody was also added to sample microplate wells. This was followed by addition of 50ul of streptavidin (Horse radish peroxidase) to sample wells and standard wells. This was gently mixed. The plates were covered with a seal and incubated for 60mins at 37^0C.

At the end of incubation period, each plate was washed 5times using 0.35ml wash buffer), and was blotted onto an absorbent paper. Then 50ul of the substrate solution A and B was added into each well. The plate was covered with a seal and incubated for 10minutes at 37^0C. This was followed by addition of 50ul of the stop solution to each well. Then the absorbance was read at 450nm using microplate reader.

Estimation of Electrolytes By Ise (Sfri 2000) And Anion Gap By Calculation
Procedure
Serum was introduced into the analyser through the probe. And the result was printed out from the analyzer within a few minutes.

Statistical Analysis
Data obtained from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 for windows 7. The results were expressed as mean ± Standard deviation. Independent sample t-test which was used to compare means and values at 95% confidence limit. P. values $p<0.05$ were considered statistically significant. Pos-Hoc comparison was carried out using Turkey LSD. The results are presented in tables, and figures.

RESULTS

Table 1: Shows The Mean ± SD Values of The Kidney Injury Molecule -1(KIM-1) (nm/l) AND Cystatin C (CYS-C) (nm/l) of All The Rats In The Study.

Groups	KIM – 1	CYS-C
Group 1	2.30± 0.38	26.23 ± 3.26
Group 2	2.35 ± 0.30	26.89 ± 4.32
Group 3	2.76 ± 0.39	26.82 ± 2.77
Group 4	1.72 ± 0.38	26.11 ± 4.34
P values	0.000	0.077

Table 1: shows that there, is no significant difference in the CYS-C levels of all the rats across the groups. Also, there is no significant difference in KIM-1 values of the rats in group 2 and 3(2.35±0.30 and 2.76 ± 0.39) when compared with these in group 1(2.30± 0.38). But there is a significant decrease in the KIM-1 values of the rats in group 4 (1.72 ± 0.39) when compared with those in group 1, 2, and 3 (2.30 ±0.38, 2.35 ± 0.30, and 2.76±0.39) respectively.

Table 2: Shows the mean ± sd values of the electrolytes; sodium, potassium, calcium, chloride, bicarbonate and anion gap of all the rats in the study.

Groups	K^+(mmol/l)	Na^+(mmol/l)	CL^+(mmol/l)	Ca^+(mmol/l)	HCO_3^-(mmol/l)	AG
Group 1	5.22 ± 0.72	139.95 ± 2.69	105.02 ± 3.08	2.60 ± 0.08	17.00 ± 3.25	17.95 ± 4.87
Group 2	5.95 ± 1.89	154.15±30.98	104.45 ± 0.74	2.87 ± 0.69	14.08 ± 2.99	35.55 ±32.59
Group 3	5.59 ± 1.57	142.00 ± 1.60	105.48 ± 2.56	2.73 ± 0.16	17.07 ± 3.34	19.43 ± 2.64
Group 4	5.24 ± 0.94	142.21 ± 0.93	109.97 ± 6.04	2.52 ± 0.29	14.80 ± 4.00	17.45 ± 2.87
P value	0.484	0.443	0.072	0.322	0.117	0.024

Table 2: shows that there is no significant difference ($P>0.05$) in the serum electrolyte values of all the rats across the groups, indicting absence of severe kidney injury. But there is a significant increase ($P<0.05$) in the anion gap of the rats in the group 2 (35.55±32.59) when compared with the rats in the groups 1, 3, and 4, (17.95±4.87, 19.43±2.64, and 17.45±2.87) respectively.

DISCUSSION

The result from this study also showed no significant difference in the CYS-C levels of all the rats across the groups. Also, we observe no significant difference in KIM-1 values of the untreated diabetic rats and diabetic diabetic rats treated with 150mg of *Moringa oleifera* (2.35±0.30 and 2.76 ± 0.39) when compared with those in non diabetic rats (2.30± 0.38). But there is a significant decrease in the KIM-1 values of the rats in the diabetic rats treated with 300mg/kg *moringa oleifera* (1.72 ± 0.39) when compared with those in non diabetic rats, untreated diabetic rats and diabetic rats treated with 150mg of *Moringa oleifera* (2.30 ±0.38, 2.35 ± 0.30,and 2.76±0.39) respectively. The Cystatin-C result contradicted the study of Mussap *et al.* (2000), who stated that there was a statistically significant increased level of Cystatin-C in type 2 Diabetic patients. The result of KIM-1 agrees with the findings of Mori, *et al.* (2007) who stated that there are reasons to consider that KIM-1 maybe released into the circulation after kidney proximal tubule injury. With injury, tubular cell polarity is lost, such that KIM-1 may be released directly into the interstitium. From this study also we found no significant difference ($P>0.05$) in the serum electrolyte values of all the rats across the groups. The histological studies also show no damage to the kidney. But there is a significant increase ($P<0.05$) in the anion gap values in the untreated diabetic rats (35.55±32.59) when compared with the non diabetic rats, diabetic rats treated with 150mg of *Moringa oleifera* and diabetic rats treated with 300mg of *Moringa oleifera* (17.95±4.87, 19.43±2.64, and 17.45±2.87) respectively. The results of the Cystatin C, KIM-1, Electrolyte values and histological studies, showed no damage to the kidney in all the rats. The rise in the Anion gap value in the untreated rats may be as a result of oxidative stress/metabolic acidosis induced by the streptozocin used in inducing diabetics.

CONCLUSION

From this study, it could be inferred that *moringa oleifera* leaf powder used in treatment of streptozocin-induced diabetes in rats metabolic acidosis and reduced the toxic damage to the kidney and liver.

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