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EFFECT OF AQUEOUS LEAF EXTRACT OF *Murraya Koenigii* ON SOME BIOCHEMICAL AND HAEMATOLOGICAL INDICES OF NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The effect of aqueous leaf extract of *murraya koenigii* on glycemic and some biochemical and haematological indices of normal and alloxan-induced diabetic rats was evaluated. Twenty albino rats were randomized into four groups (A-D), such that group A (non-diabetic) received 0.5 ml distilled water, group B (diabetic untreated) received 0.5 ml distilled water, group C (diabetic treated) received 200 mg/kg bwt of extract while group D (control treated) received 200 mg/kg bwt of extract. The blood glucose of alloxanized rats significantly and progressively reduced in extract treated animals. The significant ($p<0.05$) increase in the levels of the creatinine, protein and urea in the untreated diabetic rats was reduced significantly in the diabetic treated rats with the protein and urea values below the control group. The significant ($p<0.05$) decrease in the levels of Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cells (RBC) and White blood cells (WBC) of diabetic untreated rats were significantly ($p<0.05$) elevated in diabetic treated rats. With the exception of ALP, the elevations in the activities of ALT and AST were reverted back to their respective control in the extract treated animals. The significant ($p<0.05$) increase in the levels of total cholesterol and triacylglycerol observed in the diabetic animals was drastically decreased to near normal level after administration of extract. The results obtained indicated that oral administration of aqueous leaf extract of *Murraya koenigii* at the dosage and duration used resulted in hypoglycemia as well as reversal of the adverse complications associated with diabetics.

Key Words: Alloxan, Curry, Diabetes, Haematological, *Murraya koenigii,*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance [1]. It is the most common endocrine disorder and World's fastest growing metabolic disorder characterized with

chronic high blood glucose with an average annual growth of 1-2% [2]. It is a disease of worldwide significance and increasing prevalence. The number of people suffering from diabetes worldwide is increasing at an alarming rate. Currently there are over 150 million people living with diabetes mellitus and it is predicted that numbers is likely 366 million people or more are likely to be diabetic by the year 2030 [3,4]. Chronic hyperglycemia of diabetes mellitus results to long-term tissue damages and complications, as well as dysfunction associated with liver, kidney, eyes, sexual inadequacies, nerve damage and loss of weight among others [5,6,7]. It is imperative to explore options in herbal medicine for management of diabetes. In Africa, particularly in Nigeria, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus but unfortunately, only a few of such medicinal plants have been scientifically validated [8]. A wide range of medicinal plants has been used by various cultures to treat diabetes mellitus because of their hypoglycemic properties [9]. Phytotherapy has a promising future in the management of diabetes, the continued search for new effective drug for diabetes from herbs is still attractive because they are less expensive and safer [6]. *Murraya koenigii* (L.) family Rutaceae is an aromatic more or less deciduous shrub or a small tree up to 6m in height found throughout Nigeria and is commonly known as Curry, is used traditionally as antiemetic, antidiarrhoeal, febrifuge and blood purifier. The whole plant is considered to be a tonic and stomachic. The leaves are used extensively as a flavoring agent in curries and chutneys. The people use the leaves of this plant as a spice in different curry preparations [10]. *Murraya koenigii* (L) is one of the most widely acclaimed remedies for the treatment of diabetes [11]. Similarly, several studies have demonstrated the antidiabetic property of the *Murraya koenigii* leaves on diabetic animal models [12,13,14]. However, the safety of hypoglycemic due to *Murraya koenigii* leaf treatment has not been established. The aim of the present study was to examine the effect of aqueous leaf extract of *Murraya Koenigii* on the levels of blood glucose and some biochemical and hematological parameters in alloxan-induced diabetic rats in order to complement the findings into its antidiabetic activities.

MATERIALS AND METHODS

Plant Material

The leaves of *Murraya Koenigii* used for this study were obtained from the Federal College of Forestry, Jos, Nigeria and were authenticated at the Department of Plant Science and Technology, University of Jos, Jos, Nigeria.

Chemicals

Alloxan monohydrate was obtained from Sigma-Aldrich Chemical Company, St Louis, U.S.A. All the other chemicals used were obtained commercially and were of analytical grade.

Experimental Animals

Adult Wistar rats of both sexes weighing between 180-200g were obtained from the National Veterinary Research Institute, Vom, Jos, Nigeria. The animals were housed in aluminum cages under standard conditions. They were maintained on standard animal pellets (purchased from Grand Cereal and Oil Mills Limited Jos, Nigeria) and water *ad libitum*. The animals were acclimatized for two weeks prior to commencement of the experiment.

Preparation of Plant Extract

Fresh leaves of *Murraya koenigii* (L.) were procured from a local vegetable farm along Miango road Jos, Nigeria. The leaves were shade dried to a constant weight. The dried leaves were then pulverized using electric blender (model MS-223, Taipei, Taiwan). The powdered form was stored in airtight plastic container until required for use. 100g of the fine powder was dissoved in one 500 ml of distilled water for 48 hours at 37°C (to ensure maximum extractions of phytochemicals). This was then filtered with Whatman No. 1 and then concentrated on a steam bath and reconstituted in distilled water to give the required dose of 200 mg/kg body weight as used in this study. The reconstituted aqueous extract was administered orally to normal and diabetic rats using cannula.

Induction of Diabetes and determination of Blood Glucose

The animals were fasted overnight. Thereafter diabetes was induced by a single intraperitoneal injection of a freshly prepared Alloxan monohydrate (150 mg/kg body weight) in ice cold 0.9% NaCl solution. The animals were allowed 5% glucose solution overnight *ad libitum* to overcome the drug-induced hypoglycemia. Control (normal) rats were not injected with alloxan and were placed on normal saline alone. Blood samples were drawn from the tail vein and glucose levels were determined to confirm the induction of diabetes using the Bayer Contour™ test strips and Glucometer according to the instructions outlined in the User Guide. Only rats with blood glucose level higher than 7.0 mmol/L were considered diabetic and used for the experiment. Feeding was stopped 12 hours before blood sampling.

Experimental Design

The experimental period was 14 days (extract given orally per day using cannula). After randomizing into various groups and before initiation of the experiment, the rats were acclimatized to the animal house conditions. Twenty albino of both sexes were randomized into four groups (A-D) such that;

GROUP A: Control rats on 0.5 ml normal distilled water per day.
GROUP B: Diabetic untreated rats on 0.5 ml normal distilled water per day.
GROUP C: Diabetic treated rats on 0.5 ml of extract (equivalent to 200mg/kg aqueous *Curcuma longa* rhizome extract).
GROUP D: Control treated rats on 0.5 ml of extract (equivalent to 200mg/kg aqueous *Curcuma longa* rhizome extract).

The study was carried out after approval from the Departmental Animal Science Ethical Committee on the Use and Care of Experimental Animals.

Collection of Blood Sample and Preparation of Serum

The methods described by Yakubu *et al.* [15] were used for the collection of blood samples and preparation of serum. In brief, with the animal under diethyl ether anaesthesia, the neck area was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade. Blood was collected into EDTA sample bottles for haematological assay and also collected into clean, sterile sample bottles which were allowed to clot for 30 minutes. This was then centrifuged at 33.5 g for 15 minutes using a Uniscope Laboratory Centrifuge. The sera were aspirated with Pasteur pipettes and stored frozen until required for the biochemical analyses.

Determination of Haematological Parameters

Red Blood Cells (RBC), Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Platelet (**PLT)**, White Blood Cell count (WBC) and its differential counts (neutrophil (NEUT) and lymphocyte (LYM) were assayed using the prepared plasma by the method of Dacie and Lewis [16].

Biochemical Assays

The biochemical parameters were determined the serum using standard methods described for activities of Aspartate transaminase (AST) (E.C.2.6.1.1 EC 2.6.1.2) and Alanine transaminase (ALT) (E.C.2.6.1.2 EC 2.6.1.1) [17], activity of

Alkaline phosphatase (ALP) (E.C.3.1.3.1) [18], Creatinine [19] and Urea [20]. All measurements were done using Spectronic 21 spectrophotometer (Bausch and Lomb, NY). Jenway Clinical PFP7 Flame Photometer was used to determine the levels of serum sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Comparison of the data from test control groups of animals were analyzed by One Way Analysis of Variance (ANOVA) at the confidence limit of 95% and where applicable, Least Significant Difference (LSD) was used to determine significant results, differences between groups were considered statistically significant at $p<0.05$.

RESULTS

The results revealed that all alloxan-induced rats became diabetic after 48 hours with blood glucose of 9.35 and 9.50 mmol/L (Table 1). No statistical difference was observed among the diabetic groups before the treatment. The blood glucose were however reduced significantly ($P<0.05$) and progressively in the diabetic rats treated with extract. After 7^{th} and 14^{th} day the blood glucose in diabetic treated group was restored to the basal level (with values below control group) while the diabetic control still recorded an elevated value (Table 1).There was no statistical difference observed between the control group and the control treated with extract throughout the period of experiment. The effect of aqueous leaf extract of *Murraya koenigii* on creatinine, protein and urea of both normal and alloxanized diabetic rats is as presented in Table 2. There was a significant ($p<0.05$) increase in the levels of the creatinine, protein and urea in the untreated diabetic rats when compared with the control and the treated rats. The administration of the aqueous leaf extract of *Murraya koenigii* significantly ($p<0.05$) decrease the level of this parameters in the diabetic treated rats with the protein and urea values below the normal control group. Meanwhile, in the haematological parameters, a significant ($p<0.05$) decrease was recorded in the levels of PCV, Hb, RBC and WBC of both diabetic untreated and diabetic treated rats compared to control group (Table 3). However, the values of all the parameter were significantly ($p<0.05$) higher in diabetic treated rats in comparison to diabetic untreated rats. Compared with the control, the activities of ALT, AST and ALP in the serum were significantly ($p<0.05$) elevated in the untreated diabetic rats (Table 4). With the exception of ALP which was higher in diabetic treated rats, other elevations were reverted back that can be compared favourably ($P>0.05$) with their respective control in the extract treated animals. The significant ($p<0.05$) increase in the

levels of total cholesterol and triacylglyrol observed in the diabetic animals was drastically decreased to near normal level after administration of extract (Table 5). There was slight decrease in HDL in both diabetic untreated and diabetic treated rats compared to control.

Table 6 shows the effect of aqueous leaf extract of *Murraya koengii* on electrolytes (Na^+, K^+ and Cl^-) of both normal and alloxan induced diabetic rats. There was a significant ($p<0.05$) increase in the serum level Cl^- with a significant ($p<0.05$) decrease in the serum level of Na^+ and K^+ untreated diabetic rats when compared with the control and the treated groups. Aqueous leaf extract *Murraya koengii* improves the levels of K^+, Na^+ and Cl^- in diabetic treated rats by reversing the values.

Table 1: Effect of Administration of Aqueous Leaf extract of *Murraya koenigii* (L.) on Blood Glucose (mmol/L), of Alloxanized Diabetic Rat Serum

	Days after Administration of Alloxan		
Treatment groups	**Day 0**	**Day 7**	**Day 14**
Control	5.40±0.14	5.50±0.14	5.41±0.14
Diabetic rats + Distilled water	9.35±0.22[a]	12.50±0.53[a]	15.15±0.35[a]
Diabetic rats + 200mg/kg bwt extract	9.50±0.22[b]	5.00±0.65[b]	3.45±0.07[ab]
Control + 200mg/kg bwt extract	5.55±0.01[b]	5.00±0.10[b]	5.05±0.07[b]

Values are expressed as mean ± SD, n= 4 for each group

[a] values are significantly different from normal control ($p<0.05$)

[b] values are significantly different from the diabetic control group ($p<0.05$)

Table 2: Effect of Administration of Aqueous Leaf extract of *Murraya koenigii* (L.) on Creatinine, Protein and Albumin of Alloxanized Diabetic Rat Serum

Treatment groups	Creatinine (mmol/L)	Protein (g/L)	Urea (mmol/L)
Control	95.40±0.14	73.50±4.60	3.80±0.14
Diabetic rat + Distilled water	208.35±0.22[a]	70.50±3.53[a]	5.15±0.35[a]
Diabetic rats + 200mg/kg bwt extract	109.50±0.22[ab]	61.00±5.65[ab]	3.45±0.07[ab]
Control + 200mg/kg bwt extract	84.95±0.01[ab]	73.90±4.10[b]	4.35±0.07[ab]

Values are expressed as mean ± SD, n= 4 for each group
[a] values are significantly different from normal control (p<0.05)
[b] values are significantly different from the diabetic control group (p<0.05)

Table 3: Effect administration of aqueous leaf extract of *Murraya koenigii* (L.) on some haematological parameters of alloxanized diabetic rat serum

	Parameters			
Treatment groups	PCV (%)	HB (g/dL)	WBC(µ/L)	RBC (10^6/µL^3)
Control	55.00±2.82	15.80±1.07	5525.00±247.49	5.70±0.30
Diabetic rats + Distilled water	34.50±0.71[a]	8.17±0.23[a]	4675.00±106.10[a]	2.50±0.10[a]
Diabetic rats + 200mg/kg bwt extract	46.50±0.70[ab]	10.67±0.47[ab]	4975.00±106.10[ab]	6.90±0.23[ab]
Control + 200mg/kg bwt extract	53.50±2.12[b]	12.80±0.12[ab]	5525.00±247.49[b]	5.90±0.20[b]

Values are expressed as mean ± SD, n= 4 for each group
[a] values are significantly different from normal control (p<0.05)
[b] values are significantly different from the diabetic control group (p<0.05)

Table 4: Effect of administration of aqueous leaf extract of *Murraya koenigii* on liver marker enzymes, of alloxanized diabetic rat serum

	Marker Enzymes (IU/L)		
Treatment groups	**ALT**	**AST**	**ALP**
Control	20.50±2.12	50.00±4.07	103.00±2.16
Diabetic rats + Distilled water	23.50±2.53[a]	58.50±2.16[a]	112.00±2.94[a]
Diabetic rats + 200mg/kg bwt extract	20.00±1.16[b]	49.00±2.82[b]	118.00±4.94[ab]
Control + 200mg/kg bwt extract	21.00±2.82[ab]	40.50±2.16[b]	108.00±2.16[ab]

Values are expressed as mean ± SD, n= 4 for each group
[a] values are significantly different from normal control (p<0.05)
[b] values are significantly different from the diabetic control group (p<0.05)

Table 5: Effect of administration of aqueous leaf extract of *Murraya koenigii* (L.) on some lipid profile of alloxanized diabetic rat serum

Treatment groups	Total Cholesterol (TC) (mmol/L)	Triglycerol (TG) (mmol/L)	High Density Lipoprotein (HDL) (mmol/L)
Control	2.00±0.14	0.35±0.07	0.35±0.07
Diabetic rats + Distilled water	3.15±0.78[a]	0.95±0.67[a]	0.30±0.01
Diabetic rats+200mg/kg bwt extract	2.45±0.35[b]	0.45±0.07[b]	0.30±0.07
Control+200mg/kg bwt extract	2.00±0.14[b]	0.35±0.07[b]	0.5±0.14[ab]

Values are expressed as mean ± SD, n= 4 for each group
[a] values are significantly different from normal control (p<0.05)
[b] values are significantly different from the diabetic control group (p<0.05)

Table 6: Effect of Administration of Aqueous Leaf extract of *Murraya koenigii* (L.) on serum Electrolyte in Alloxanized Diabetic Rat Serum

	Electrolytes		
Treatment groups	**Na^+**	**K^+**	**Cl^-**
Control	126.00±0.71	3.95±0.21	89.8±2.07
Diabetic rats + Distilled water	117.00±2.12[a]	2.30±0.21[a]	94.30±2.01[a]
Diabetic rats+200mg/kg bwt extract	124.00±1.35[ab]	4.70±0.14[ab]	85.30±2.07[ab]
Control+200mg/kg bwt extract	125.00±1.41[b]	3.75±0.35[b]	88.2±1.14[b]

Values are expressed as mean ± SD, n= 4 for each group
[a] values are significantly different from normal control (p<0.05)
[b] values are significantly different from the diabetic control group (p<0.05)

DISCUSSION

Plants have always been a good source of drugs. Numerous studies have revealed that a wide variety of plant extracts are effective in lowering glucose level in alloxan-induced diabetic animals [21,22]. In the present study, aqueous leaf extract of *Murraya koengii* significantly decreased the blood glucose level in alloxanized diabetic rats and effectively attenuated other biochemical and haematological parameters relating to diabetes. Diabetes mellitus (DM) is a metabolic disorder which occurs when the pancreas produces insufficient amounts of insulin, or when the individual's system fails to respond appropriately to insulin due to defects in reactive oxygen species scavenging enzymes and high oxidative stress impairing pancreatic beta cells [23]. It is characterized by increases in glucose levels build up in the blood and urine, causing excessive urination, thirst, hunger and problems with carbohydrate, fat and protein metabolism [24,25,26]. Alloxan induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell of the islets of Langherhans, leading to reduced synthesis and release of endogenous insulin characteristically similar to type 1 diabetes in humans [27,6]. The cytotoxic action of alloxan is mediated via reactive oxygen species, with simultaneous massive increase in cytosolic calcium concentration, resulting to rapid destruction of the β-cell [27,6]. Therefore, the determination of bood glucose concentration among others is a useful quantitative parameter for diabetes. Therefore, the increase in the blood glucose levels of the rats to a chronic state and its subsequent reduction by the administration of aqueous leaf extract of *Murraya koengii* in this study suggests anti-hypoglycemic effect of the plant extract. The probable mechanisms of action of the plant extract could be via several mechanisms which are linked to either potentiation of insulin from beta cells, increasing peripheral glucose uptake, slowing down the absorption of sugar from the intestinal gut or by decreasing the release of glucose from the liver [28,6]. The reduction in the blood glucose of the rats by aqueous leaf extract of *Murraya koengii* in this study is similar to the previous work of Vinuthan *et al.* [29] that the aqueous extract of *Murraya koengii* leaf at a dose of 600 mg/kg body weight and methanolic leaf extract of *Murraya koengii* at a dose of 200 mg/kg body weight reduced the blood glucose of alloxan-induced diabetes.

Renal disease is one of the most common complications of diabetes. Insulin plays critical role in the maintenance of protein balance, since in addition to stimulating the uptake of amino acids and protein synthesis, it also inhibits protein degradation [30]. In this present study, the significant increase in the levels of serum creatinine and urea (renal function markers) in untreated

diabetic rats compared with the control, indicate signs of kidney dysfunctions in the diabetic disease [31] compared to control. These results were also similar to previous report by Uladimir [32], who state that hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, like the kidneys. There was significant reduction in the plasma protein in both diabetic treated and untreated animals. Therefore, the increased urea and creatinine in diabetic rats may be attributed to enhanced catabolism of both liver and plasma proteins that accompany glyconeogenesis [33]. Administration of plant extract to the diabetic rats reverses the renal function indices to near normal. The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs [34]. The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins [35]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [36]. In this study, the levels of PCV, Hb and RCB were drastically reduced. Some of these abnormalities might be due to destruction of mature red blood cells, leading to the low Hb counts accompanied by the fall in the RBC and PCV [37]. This observation agrees with report of Baskar *et al.* [38] who reported antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin-induced diabetic rats. The alterations of PCV and Hb are well known to cause anaemic condition in man [39]. Following plant extract administration, the levels of PCV, Hb and RBC were appreciably improved. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. WBC counts and related indices are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes has been rare due to an increase in all the types of leukocytes [40]. The reduction of these WBC in diabetic untreated rats could be linked to suppression of leukocytosis from the bone marrow which may account for poor defensive mechanisms against infection [41]. White blood counts were significantly restored to near normal after plant extract administration.

Hyperglycemia leads to long-term tissue damages and complications, such as liver-kidney dysfunctions, often associated with serious diseases [7,5]. The

activities of AST, ALT and ALP have been reported to increase in alloxan-induced diabetic rats [42,43]. In this study, we reported significant ($p<0.05$) increase in the activities serum AST, ALT and ALP alloxanized diabetic untreated rats when compared with the control (Table 4). Measurement of the activities of "marker" enzymes or biomarkers in body fluids can be used to assess the degree of assault and the toxicity of a chemical compound on organs/tissues [44,45]. Such measurements can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques [46]. With the exception of ALP which was higher than the diabetic untreated, the extract was effective in restoring the activities of AST and ALT back to normal. Several studies have shown that diabetes is associated with increases in serum lipids such as total cholesterol and triacylglycerol, which is related to significant changes in lipid metabolism and structure in the diseased state [47,48,6]. The abnormalities in cellular cholesterol metabolism could be partly responsible for the changes in the serum cholesterol levels in diabetes which have been reported to increase the accumulation of lipids in cells [48]. Therefore, the normalization of serum total cholesterol and triacylglycerol by aqueous leaf extract of *Murraya koengii* suggest that the extract is effective in reversing the abnormalities associated with lipid metabolism in diabetes. The extract had no significant effect on the HDL. Electrolytes play an essential role in many biological processes, such as controlling body fluid levels, acid-base balance (pH), nerve conduction and blood clotting and muscle contraction. It has been suggested that one of the contributing factors in the complications of diabetes and other endocrine disorder is electrolytes imbalance resulting from kidney failure, dehydration and fever and vomiting [49]. The increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds give rise to imbalance in homeostasis with respect to electrolytes [50]. It imperative that the increased electrolytes and water levels usually observed in diabetes could lead to depletion of the extracellular fluid electrolyte and thus lead to the excretion of electrolyte by parietal and non-parietal cells [51], which may account for the observed significant decrease in the serum Na^+ and K^+ of diabetic untreated rat when compared with control (Table 6). On the other hand, the significant increase in serum Cl^- may be due to renal tabular acidosis or metabolic acidosis. However, the reversal of the electrolytes following oral administration of extract suggests that the extract could effectively attenuate the altered extracellular fluid electrolytes levels of the diabetic treated rats.

CONCLUSION

Conclusively, the significant reduction of the elevated blood glucose in the diabetic to values comparable with the control by the aqueous leaf extract of *Murraya koengii* in this study shows hypoglycemic activity of the extract. This study has also revealed that the aqueous leaf extract of *Murraya koengii* effectively ameliorates some complications and metabolic imbalance associated with diabetes. The study also provides scientific evidence to complement the early findings on its antidiabetic activities and justify its safe use traditionally as a herbal supplement in the management of diabetes mellitus. Further work is needed to isolate and characterize the antidiabetic bioactive principle(s) in the extract, elucidation of its possible mode of action(s) and the toxic implications in the rats.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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