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Research Article

Amelioration of Hematological and Electrolyte Imbalances in Type 2 Diabetic Rats by Methanolic and Flavonoid-Rich Leaf Extracts of *Synsepalum dulcificum*

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ABSTRACT
The aim of the present study was to evaluate the effect of methanolic extract (MSD) and flavonoid-rich extract (FSD) of *Synsepalum dulcificum* on alterations of hematological and electrolyte parameters in type 2 diabetic rats. A total of 63 animals were randomly distributed into nine groups of seven animals per group. Type 2 diabetes was induced in experimental animals by intraperitoneal injection of 40 mg/kg streptozotocin after administration of 10% fructose in their drinking water for 14 days. After confirmation of diabetes, treatment was continued for 21 days. At the end of the study hemoglobin (Hb) level, red blood cell count (RBC), packed cell volume (PCV), white blood cell count (WBC) and neutrophils were evaluated. Furthermore, serum levels of potassium (K^+), calcium (Ca^{2+}) and sodium (Na^+) were also investigated. Results showed that a significantly ($p < 0.05$) higher Hb, PCV and RBC were observed in the MSD and FSD treated groups. However, a significantly ($p < 0.05$) lower WBC was observed in the extract treated groups when compared with the diabetic control. Furthermore, treatment with extracts also showed a significantly ($p < 0.05$) lower electrolyte levels than the diabetic control. It was evident that both MSD and FSD have the potential to improve the imbalances in hematological and electrolyte levels associated with type 2 diabetes as observed in this study. Therefore, the extracts could be considered relevant in the management of the disease.

Keywords: Hematological, extract, fructose, experimental, streptozotocin

INTRODUCTION

Diabetes is a disease that has become a major concern the world over. The prevalence of diabetes in the age groups between 20 to 70 years worldwide was 8.3% in 2013 and estimated to be 10.1% in 2035[1]. The main features of diabetes mellitus include absolute or relative deficiency in insulin and/or action, persistent hyperglycemia and dysregulation in the metabolism of carbohydrate, lipid and protein[2]. Diabetes is a metabolic disorder that is also characterized by alterations in hematologic and electrolyte parameters.

Metal ions are known to play an essential role in living systems, both in growth and in metabolism[2], and disturbances in electrolytes metabolism is a common phenomenon in a wide range of diseases. Macroelements including sodium (Na^+), potassium (K^+) and calcium(Ca^{2+}) are natural elements the body needs in more quantity than some other elements, and are often more important than many other minerals[3]. Diabetes mellitus is among the diseases characterized by increased frequency of electrolyte abnormalities as a result of impaired renal function, malabsorption syndromes, acid-base disorders, and multidrug regimens[4].

Furthermore, a number of studies have reported significant independent associations of routinely measured hematological parameters, including hematocrit (Hct) and white blood cell count (WBC) with incident type 2 diabetes mellitus in different populations[5,6,7]. About 27% of diabetic patients are anaemic[8]. Moreover, the mean values of RBC, Hb and PCV for the diabetic patients were found to be lower than the values of control group[9]. Considering the independent association between these hematological indices and cardiovascular diseases (CVD), derangements in their status could be a contributing factor to the incidence of diabetes and CVDs[10].

Synsepalum dulcificum Daniell (Sapotaceae) is native to tropical West Africa. It is otherwise known as miracle fruit, with a characteristic orange coloured fruits[11]. The fruit is reputed to have the ability to turn sour taste into sweet. Preliminary studies on the leaves of the plant show that the leaves are very rich source of phytosterol[12]. It was also reported that the fruit extract has the ability to improve insulin resistance induced by fructose-rich chow in rats[13].

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Table 1: Effect of MSD and FSD treatment on electrolytes level in diabetic rats.

Groups	Ca^{2+}	Na^{+}	K^{+}
Normal control	5.83±0.17*	122.06±2.22*	1.80±0.02*
Diabetic control	14.76±0.20	192.59±2.71	3.85±0.01
Diabetic + 30 mg/kg MSD	7.28±0.06**	141.05±1.98**	2.72±0.04**
Diabetic + 60 mg/kg MSD	7.01±0.05**	133.45±0.90**	2.34±0.04**
Diabetic + 30 mg/kg FSD	6.54±0.04**	125.05±0.97*	2.21±0.02**
Diabetic + 60 mg/kg FSD	6.36±0.06**	115.82±0.93*	1.93±0.04**
Diabetic + 5 mg/kg glib.	6.92±0.05**	144.30±2.55**	1.94±0.02**
60 mg/kg MSD only	5.95±0.03**	125.32±2.62*	1.86±0.01*
60 mg/kg FSD only	5.46±0.24*	127.49±2.85*	1.84±0.02*

Each value is a mean of seven determinations ± SEM. Values with * in the same column are significantly ($p<0.05$) different from the positive control while values with ** in the same column are significantly ($p<0.05$) different from both positive and Negative control.
MSD = Methanolic extract of *Synsepalum dulcificum* leaves, FSD = Flavonoid-rich extract of S*ynsepalum dulcificum* leaves.

There is at present paucity of information on the effect of methanol and flavonoid-rich extracts on alterations in hematological and electrolytes levels associated with type 2 diabetes in experimental animals, hence this study

MATERIALS AND METHODS

Chemicals

Glibenclamide, sodium citrate, citric acid and streptozotocin were purchased from Sigma-Alrich (St-Louis, MO, USA). All other reagents used were analytical grade.

Plant material and extraction

Synsepalum dulcificum leaves were obtained from Olode Village, Osun State, Nigeria, and authenticated by Mr Donatus at the Botany Department, University of Ibadan, Ibadan, Nigeria. A voucher number UIH-22457 was obtained for the leaf. The leaves were air-dried for three weeks and pulverized. 700g of the pulverized sample was extracted in 80% methanol by maceration for 72 hours. The methanolic extract was concentrated in a rotary evaporator, lyophilized and preserved for further use.

Extraction of flavonoids

A known gram of the methanol extract was dissolved in 20 ml of 10% H_2SO_4 and hydrolysed by heating in the water bath for 30 mins at 100ºC. The mixture was placed on ice for 15 mins for precipitation of the flavonoid aglycones. The flavonoid aglycones were then dissolved in 50 ml of warm 95% ethanol, filtered and concentrated by rotary evaporation.

Experimental design

Diabetes was induced in rats through interperitoneal injection of 40 mg/kg body weight streptozotocin dissolved in ice-cold 0.1 M citrate buffer (pH 4.5), after administration of 10% fructose in their drinking water for 14 days[14]. Diabetes was confirmed after 72 h and animals with blood glucose ≥ 250 mg/dl were considered diabetic and used for the study. The animals were randomly distributed into groups as follows:

Group 1 Normal control
Group 2 Diabetic control
Group 3 Diabetic + 30 mg/kg MSD
Group 4 Diabetic + 60 mg/kg MSD
Group 5 Diabetic + 30 mg/kg FSD
Group 6 Diabetic + 60 mg/kg FSD
Group 7 Diabetic + 5 mg/kg glib.
Group 8 60 mg/kg MSD only
Group 9 60 mg/kg FSD only

The study lasted for 21 days and adhered to the Principles of Laboratory Animal Care (NIH publication 85−23, revised in 1985). Ethical clearance was obtained for the study from the university's ethical committee.

Electrolytes analyses

At the end of the experiment, rats were euthanized and blood was collected through cardiac puncture. Serum was thereafter obtained and used for evaluation of serum electrolytes. Serum levels of Na^{+}, Ca^{2+} and K^{+} were evaluated using Teco kits (Lakeview Avenue, Anaheim CA, USA) according to instructions provided by kit manufacturer.

Hematological analysis

Blood samples were collected into heparinized tubes and were immediately used for determination of haematological parameters. Total red blood cell count (RBC) and white blood cell count (WBC) counts were estimated according to the visual method of Dacie and Lewis[15]. The percentage packed cell volume (PCV) was determined according to the hematocrit method, while the blood haemoglobin (Hb) concentration in all samples was estimated according to the cyanomethaemoglobin method using Drabkin's reagent[16]. Neutrophils levels was estimated using the method of Osim *et al*, (2004)[17].

Statistical analysis

Results were expressed as mean value ± standard error of mean (SEM). Data analysis was done using SPSS software version 16 by one-way analysis of variance (ANOVA) followed by Duncan-test. $p < 0.05$ (95% confidence limit) was considered to be statistically significant.

RESULTS AND DISCUSSION

Electrolytes play an important role in maintaining acid-base balance, blood clotting, control body fluid and muscle contractions. The derangement of electrolyte balance may affect the course of diabetes and its management[18].

In our study as shown in table 1 we observed a significantly

Table 2: Effect of MSD and FSD treatment on some hematological parameters in diabetic rats.

Groups	WBC $X10^9$/L	PCV %	Hemoglobin g/d	RBC $X10^{12}$/L	Neutrophils %
Normal control	2171.40±47.38*	40.57±0.57*	13.52±0.19*	4.33±0.05*	36.29±0.92*
Diabetic control	3014.30±63.35	27.86±1.22	9.29±0.41	3.60±0.07	49.14±0.51
Diabetic + 30 mg/kg MSD	2200.00±48.80*	39.57±1.65*	13.19±0.55*	4.20±0.02*	40.14±0.63**
Diabetic + 60 mg/kg MSD	2700.00±53.45**	31.00±0.72**	10.33±0.24**	3.96±0.04**	43.14±1.10**
Diabetic + 30 mg/kg FSD	2485.70±56.22**	34.14±1.35**	11.38±0.45**	4.01±0.08**	46.00±0.31**
Diabetic + 60 mg/kg FSD	2500.00±37.80**	31.24±1.41**	11.67±0.47**	3.80±0.08**	39.71±1.60**
Diabetic + 5 mg/kg glib.	2400.00±21.82**	35.57±0.20**	11.86±0.07**	4.21±0.03*	38.86±0.77**
60 mg/kg MSD only	2300.00±65.47*	33.00±0.87**	11.00±0.29**	3.89±0.06**	40.57±1.15**
60 mg/kg FSD only	2314.30±67.00*	33.71±0.94**	11.24±0.32**	4.21±0.05*	40.57±0.37**

Each value is a mean of seven determinations ± SEM. Values with * in the same column are significantly ($p<0.05$) different from the positive control while values with ** in the same column are significantly ($p<0.05$) different from both positive and Negative control.

MSD = Methanolic extract of *Synsepalum dulcificum* leaves, FSD = Flavonoid-rich extract of S*ynsepalum dulcificum* leaves, WBC = white blood cell count, RBC = red blood cell count, PCV = packed cell volume.

($p < 0.05$) higher serum Na^{2+} level in the diabetic control group when compared with the normal control, glibenclamide, MSD and FSD treated groups. However, the Na^{2+} levels in the MSD treated diabetic animals even though significantly ($p < 0.05$) lower than the diabetic control was higher than the normal control. Serum levels of sodium in animals administered extract only were also not significantly ($p < 0.05$) different from the normal control. Our result is in consonance with an earlier study in which poorly controlled diabetes in 113 patients was implicated in the development of hypernatremia in about 34.5% of the cases in the study[19]. Previous researchers have reported high sodium level in hyperosmolarity of diabetes mellitus[20, 21], including in glucose intolerant rats[22]. Hyperosmolar hyperglycemia (HH), an endocrine emergency linked with mortality ranging between 10 – 50% has been observed in all age groups suffering from type 2 diabetes, but predominant in older people [23, 24]. Its main feature is both extremely high hyperglycemia and serum osmolarity. It is often characterized by hypernatremia[25]. We propose that the high serum sodium level in diabetic control animals might be as a result of hyperosmolar hyperglycemia, a condition that was ameliorated with treatment with both MSD and FSD.

Studies have reported that serum total calcium levels are higher in individuals with diabetes than in those without it[26,27]. Fasting serum glucose and insulin resistance were positively correlated with serum calcium levels in women and men. The significant correlations are independent of age, sex, percent trunk fat, phosphorus, magnesium, medications, 25-OH vitamin D, and PTH[28]. Cross-sectional and prospective studies have shown a direct association between serum calcium levels and risk of diabetes[29,30]. Also in another study, participants with albumin-unadjusted higher serum calcium concentrations had a 49% greater risk of diabetes than the reference group after adjustment for age, sex, BMI, and smoking[30]. Our result presented in table 1 showed a significantly ($p < 0.05$) lower serum calcium level in the normal control, glibenclamide and all the extract treated groups when compared with the diabetic control animals. However, the serum calcium levels in extract and glibenclamide treated groups are significantly ($p < 0.05$) higher than the normal control group. It was reported that increased calcium levels can decrease the expression of GLUT4 transporters and, consequently, decrease glucose uptake and, as a result, increase glucose serum concentrations[31]. We suppose that the effect of our extracts on the serum calcium level might be as a result of its potential to increase glucose uptake and thereby reduce serum glucose concentration.

The incidence of hyperkalemia is higher in diabetic patients than in the general populations[32,33]. Our result in table 1 shows the effect of treatment with MSD and FSD on serum potassium level in diabetic rats. A significantly ($p < 0.05$) higher potassium level was observed in diabetic control group in comparison with the other groups in this study. Furthermore, the k^+ level in the extract treated diabetic animals was significantly higher than that of normal control, although the groups treated with extract only did not show a significantly ($p < 0.05$) different K^+ level from the normal control group. Diabetic ketoacidosis (DKA) is an acute, life-threatening metabolic complication of diabetes mellitus. Among others, the features of DKA include hyperglycemia and concurrent electrolyte imbalance especially abnormalities of potassium homeostasis[34,35]. In DKA, a combination of insulin deficiency and hyperglycemia-induced hyperosmolality frequently lead to hyperkalemia. Insulin promotes potassium entry into cells, and when circulating insulin is lacking as in DKA, potassium moves out of cells thus raising plasma levels of potassium even in the presence of total body potassium deficiency[35,36]. Another significant causal factor of chronic hyperkalemia in diabetics is the reduced tubular secretion of K^+ due to the syndrome of hyporeninemic hypoaldosteronism[37]. The ability of MSD and FSD to ameliorate the imbalance observed in potassium homeostasis in diabetic rats as observed in this study might be due to its potential to improve insulin resistance and hyperglycemia that are important features of type 2 diabetes.

Total white blood cell count (WBC) is significantly higher among diabetics compared with non-diabetics. Chronic

inflammation is involved in the pathogenesis of type 2 diabetes and evidence from epidemiological studies suggests an association between total WBC or leukocyte count, a non-specific marker of inflammation, and diabetes risk[38]. It was reported that for every increase in 1,000 cells/mm^3 within the normal range of the WBC count, the risk for diabetes increases by 7.6%[39]. Our result presented in table 2 shows that the normal control and both extract and glibenclamide treated groups have a significantly ($p < 0.05$) lower WBC than the diabetic control group. However a significantly ($p < 0.05$) higher WBC was observed in most of the extract treated diabetic animals when compared with the normal control, while the values observed in the groups treated with extract only were not significantly ($p < 0.05$) different from the normal control group. It is established that more than 90% of granulocytes are neutrophils, making them the largest fraction of white blood cells. Neutrophils play an essential role in the host inflammatory response against infection[40]. In addition, chronic low grade tissue inflammation is an important cause of systemic insulin resistance, and is a key component of the decreased insulin sensitivity which exists in obesity and type 2 diabetes[41,42]. Neutrophils secrete a proinflammatory proteinase termed neutrophil elastase (NE)[43], which was higher in adipose tissue from high fat fed (HFD) mice compared to lean chow–fed. Furthermore, HFD mice treated with the NE inhibitor for 14 days, showed significantly improved glucose tolerance with no change in body weight[44]. We observed a significantly ($p < 0.05$) higher neutrophil level in the diabetic control animals when compared with the other groups in this study. However, the extract and glibenclamide treated groups have significantly ($p < 0.05$) higher neutrophil levels than the normal control. The potential of MSD and FSD to mitigate diabetes associated inflammation may account for their lowering effect of WBC observed in diabetic animals treated with the extracts. Furthermore, the observed reduction of neutrophils, and by extension neutrophil elastase in animals treated with our extract could indicate the potential of the extracts to improve insulin sensitivity and glucose tolerance in diabetic rats.

It was observed in an earlier study that the mean values of RBC, Hb and PCV for the diabetic patients are lower than the values of control group, indicating the presence of anemia in the diabetic patients[45]. Anemia is a common phenomenon in diabetes and the main factor contributing to its prevalence in diabetes is the inability of the kidney to increase erythropoietin secretion in response to decreased hemoglobin. Moreover, it has been reported that the occurrence of anaemia in diabetes mellitus could also be due to the increased non-enzymatic glycosylation of erythrocyte membrane proteins, which correlates with hyperglycemia[46].

As shown in table 2 we observed a significantly ($p < 0.05$) higher PCV in the normal control and extract treated groups when compared with the diabetic control group. Furthermore, apart from the diabetic animals treated with 30 mg/kg MSD, the PCV in all the extract treated groups were significantly ($p < 0.05$) lower than the normal control group. Table 2 also shows the effect of treatment with MSD and FSD on hemoglobin (Hb) level in type 2 diabetic rats. The diabetic control group showed a significantly lower hemoglobin level than all the other groups in this study. Moreover, the Hb level in the animals treated with extracts only and diabetic animals treated with extracts and glibenclamide showed significantly ($p < 0.05$) lower Hb levels than the normal control apart from the diabetic animals treated with 30 mg/kg MSD whose Hb level was not significantly ($p < 0.05$) different from the normal control group. We also observed that diabetic control animals showed a significant lower RBC than all the other groups in this study. These observations could be linked to either the ability of MSD and FSD to prevent non-enzymic glycosylation of erythrocyte membrane proteins or raise erythropoietin level by the kidney on response to low hemoglobin levels.

CONCLUSION

From the results obtained in this study we opine that both methanolic extract and flavonoid-rich extract of *Synsepalum dulcificum* leaves have the potential to ameliorate the derangements in electrolyte and hematological parameters associated with type 2 diabetic rats. We therefore conclude that the extract might be relevant and as part of treatment regimens for diabetes. However, the active principles in the extracts and their specific mechanism(s) of action should be delineated.

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