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RESPONSE OF SOME HEPATIC CARBOHYDRATES METABOLIZING ENZYMES TO AQUEOUS EXTRACT OF *CALOTROPIS PROCERA* LEAF IN DIABETIC RAT

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ABSTRACT

Diabetes mellitus is a global disease affecting mankind regardless of age, race, gender, economic status and geographical location. Diabetes mellitus is associated with biochemical complications such as alteration and fluctuation in enzyme activity. The response of some carbohydrates metabolizing enzymes to aqueous extract of *Calotropis procera* leaf in alloxanised rat was studied under standard conditions. In the present study, there was significant increase ($p>0.05$) in blood glucose level (21.80 – 22.70mmol/L) and activities of hexokinase (5.70unit/g tissue) and phosphofructokinase (17.00unit/g tissue) while activities of glucose-6-phosphatase (56.80unit/g tissue) and fructose-1,6-biphosphatase (6.60unit/g tissue) were significantly decreased ($p>0.05$) in alloxanised groups when compared to non-diabetic group. Administration of aqueous extract of *Calotropis procera* leaf lowered the high blood glucose level and reverted back the values of the enzymes back to normal in extract administered groups. The extract performance in terms of hypoglycaemic activity and reversion of enzyme activity back to normal was dose – dependent, with highest performance exhibited by 100 mg/kg dose of extract, this dosage (100 mg/kg) compared favourably in performance with metformin – a standard antidiabetic drug. From the present study, the 100 mg/kg dose of the extract can be used to correct diabetic complications and biochemical disorders associated with these enzymes.

KEYWORDS: diabetes, extract, carbohydrates, metabolism, enzymes, complications.

INTRODUCTION

Diabetes mellitus is a metabolic disorder which is majorly caused by deficiency in the production of insulin by the pancreas. According to June *et al.*, (2012), diabetes is a major public health problem affecting 285 million or 6.4% of the world population for the year 2010. Patients with type 2 diabetes or non-insulin dependent diabetes mellitus are approximately 90–95% (Arya *et al.*, 2012). By implication, type 1 or insulin dependent diabetes mellitus may account for the remaining 5 – 10% diabetes cases.

Diabetes mellitus is associated with complications such as nephropathy, retinopathy, neuropathy, morbidity, reduced life expectancy, increased risk of macrovascular complications, diminished quality of life and death (Piero *et al*., 2015). Due to unavailable antidiabetic drugs without side effects, there is a dire need for alternative sources of treatment from medicinal plants. According to Sanogo (2011), African traditional medicine is a very old practice and it is commonly encountered in both urban and rural regions.

Medicinal plants with hypoglycaemic properties are on high demand among Nigerian populace; the use of these plants is also common in folklore traditional medicine of Nigeria in the treatment of several diseases and ailments including diabetes mellitus, one of such plants is *Calotropis procera*. Some of the ethno medicinal uses of *Calotropis procera* include treatment of fever, rheumatism, indigestion, cold, eczema and diarrhoea, elephantiasis, dysentery, cholera, guinea worms and indigestion (Chopra *et al.*, 1956; Jain *et al.*, 1985; Kew, 1985).

Hepatic carbohydrates enzymes are tightly regulated system of enzymes and kinases regulating either glucose breakdown or synthesis in hepatocytes. Braithwaite *et al.*, (1995) has reported abnormal expression of enzyme activity in insulin resistance diabetic mice; this abnormality was found to exacerbate hyperglycemia.

Therefore, the objective of the present work was to study the response of glucose-6-phosphatase, fructose-1,6-biphosp hatase, hexokinase and phosphofructokinase to aqueous extract of *Calotropis procera* leaf in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

Plant sample (fresh leaves of *Calotropis procera)* was collected in June, 2017 from Departmental garden, Department of Pharmaceutical Science, Bingham University, Karu Nasarawa State, Nigeria.

Aqueous Extraction

The method described by Yakubu *et al.,* (2010) was used to prepare the extract.

Fresh leaves of *Calotropis procera* were cut into very thin slices, air dried at room temperature for 72 hours to a constant weight. The dried materials were pulverized using an electric blender (Phillips Comfort, model HR 1727, Holland). A known weight of the powder (280g) was extracted in 500ml of distilled water for 12 hours with intermittent shaking. The extra ct was filtered with Whatman No. 1 filter paper and thereafter evaporated on a lyophilizer at 55^{o}C for 30 minutes to give a yield of 7.98g.

Animals

Male and female adult rats *(Rattus novergicus*) of mean weight 90.00±0.05g obtained from animal house of Bingham University, Karu Nasarawa State, Nigeria were used for the study. The animals were fed on rat standard diet (Basal), throughout the period of the experiment.

Drug and Chemicals

Alloxan monohydrate, a product of Explicit Chemicals PVT, Ltd., Pune, India and Metformin, a product of NWP Springville, Illinois, USA were used.

All other chemicals were products of Sigma-Aldrich CHEME Gmbh, Steinhelm Germany. The chemicals were prepared in glass distilled water unless otherwise stated.

Induction of Diabetes in Experimental Animals

The method described by Yakubu *et al.,* (2010) was used to induce diabetes. The blood glucose levels of the rats were determined before the administration of alloxan. Only animals with blood glucose level higher than 11.1 mmol/L were used for the study.

Experimental Design

Animal grouping and extract administration were done according to the procedures described by Ajiboso *et al.,* (2016).

30 rats (5 normal, 25 alloxan induced-diabetic rats) were distributed into six groups (A-F) of five rats each after diabetes had been confirmed.

A= Non-diabetic rats given 0.5 ml of distilled water

B= Diabetic untreated rats administered 0.5 ml of distilled water

C= Diabetic rats administered 0.5 ml of 2.5 mg / kg body weight of Metformin

D= Diabetic rats administered 0.5 ml of 25 mg / kg body weight of extract.

E= Diabetic rats administered 0.5 ml of 50 mg / kg body weight of extract.

F= Diabetic rats administered 0.5 ml of 100 mg/ kg body weight of extract.

Calculated amount of the residue was weighed and constituted in distilled water to give the required doses of 25, 50 and 100 mg/kg body weight. The doses used in this study were as obtained from the ethno-botanical survey carried out on the plant within our locality. Treatment was administered orally with feeding bottle to respective groups. Preliminary studies conducted by Yakubu *et al.* (2010) revealed that the diabetic untreated rats could survive up till the 12th day; therefore this experiment was terminated on the 10th day. The rats were handled in accordance with the guidelines of European Convention for the protection of vertebrate animals and other scientific purposes -ETS-123 (ETS, 2005).

Collection of Blood Sample and Blood Glucose Determination

Blood glucose level of each rat was collected through cutting of tail of the rat and was determined directly with the aid of glucometer (Bayer ContourTM AG, Postfash, Basel, Switzerland) on days 0, 5 and 10.

Preparation of Liver Homogenate

The rats were quickly dissected and the liver removed. The liver was cut into tiny pieces and homogenised 0.25M sucrose solution (1:5 w/v) using hand-held homogenizer (model D1000 Asteria Inc. New Jersey, USA). The homogenates were immediately transferred into specimen bottles and kept frozen for 24 hours before analysis.

Determination of Activities of Hepatic Carbohydrate Enzymes

Some hepatic carbohydrate enzymes activities in diabetic rats were determined according to the following procedures: hexokinase (Brandstrup *et al.,* 1969); phosphofructokinase (Castano *et al.,* 1979); glucose-6-phosphotase (Koide and Oda, 1959) and fructose-1,6-biphosphatase (Gancedo and Gancedo, 1971).

Statistical Analysis

Data were presented as Means SEM of five replicates and were statistically analysed using one way analysis of variance (ANOVA) and Duncan Multiple Test Range.

RESULTS

Table 1: Effect of administration of aqueous extract of *Calotropis procera* leaf on blood glucose level of diabetic rats Blood glucose (mmol/L).

Group / Day	0	5	10
A	4.10±0.50	4.25±0.00 (3.7%)	4.40±0.01 (3.5%)
B	22.00±0.00	25.20±0.10 (14.6%)	28.90±4.02 (14.7%)
C	21.90±0.03	10.20±0.05 (53.4%)	4.70±0.01 (53.9%)
D	22.30±1.17	20.90±3.12 (6.3%)	17.40±1.10 (16.7%)
E	21.80±3.10	17.10±0.00 (21.6%)	11.20±1.05 (34.5%)
F	22.70±1.55	12.10±0.82. (46.7%)	4.90±1.00 (59.5%)

Values are Means + SEM of 5 determinations; Values down each column carrying different superscript are significantly different ($p<0.05$) from non-diabetic control

A=Non-diabetic + distilled water; B=Diabetic rats + distilled water; C=Diabetic rats + Metformin; D=Diabetic rats + 25mg/kg body weight of the extract; E=Diabetic rats + 50mg/kg body weight of the extract; F=Diabetic rats + 100mg/kg body weight of the extract

The effect of administration of aqueous extract of *Calotropis procera* leaf on blood glucose level of diabetic rats is presented in Table 1.

Administration of alloxan resulted in more than 5-fold increase in blood glucose concentrations of administered groups (B to F) when compared to non-diabetic group (A) within 36 hours of administration.

There was slight increase of 3.5-3.7% in blood glucose level of non-diabetic rat while the untreated diabetic rat in group B showed spontaneous and significant increase of 14.6-14.7% at $p > 0.05$ in blood glucose level throughout the period of the experiment.

The groups administered aqueous extract of *Calotropis procera* leaf at different dosages showed significant reduction ($p > 0.05$) of the elevated blood glucose. The level of reduction as shown with percentage in parenthesis in Table 1 was dose - dependent, highest hypoglycaemic activity was observed in groups F administered 100mg/kg b.w dose of extract. The blood glucose concentration lowering performance of 100mg dose of extract compared favourably with that of metformin - standard antidiabetic drug administered to group C. the percentages of reduction of blood glucose of groups administered metformin and 100 mg dosage were 53.9% and 59.9% respectively.

Table 2: Effect of administration of aqueous extracts of *Calotropis procera* leaf on hepatic carbohydrate – metabolizing enzymes of diabetic rats.

Group	Hexokinase (unit/g tissue)	Phosphofructokinase (unit/g tissue)	Glucose-6-Phosphatase (unit/g tissue)	Fructose-1,6-biphosphatase (unit/g tissue)
A	7.00 ± 0.00^{a}	19.70 ± 1.00^{a}	46.00 ± 2.40^{a}	5.20 ± 0.00^{a}
B	5.70 ± 0.10^{c}	17.00 ± 1.50^{c}	56.80 ± 0.30^{b}	6.60 ± 0.10^{b}
C	7.00 ± 0.10^{a}	19.50 ± 0.30^{a}	53.20 ± 3.00^{a}	5.00 ± 0.00^{a}
D	5.90 ± 0.00^{c}	20.20 ± 1.00^{b}	50.10 ± 0.10^{c}	4.70 ± 0.10^{c}
E	6.40 ± 0.20^{b}	20.40 ± 0.70^{b}	40.20 ± 4.70^{d}	5.00 ± 0.00^{a}
F	7.10 ± 0.10^{a}	19.10 ± 0.30^{a}	45.10 ± 2.10^{a}	5.10 ± 0.00^{a}

Values are Means SEM of 5 determinations
Values down each column carrying different superscript are significantly different
($p < 0.05$) from non – diabetic control

A=Non-diabetic + distilled water; B=Diabetic rats + distilled water; C=Diabetic rats + Metformin; D=Diabetic rats + 25mg/kg body weight of the extract; E=Diabetic rats + 50mg/kg body weight of the extract; F=Diabetic rats + 100mg/kg body weight of the extract

The effect of administration of aqueous extracts of *Calotropis procera* leaf on hepatic carbohydrate – metabolizing enzymes of diabetic rats are presented in Table 2. The results showed significant decrease ($p > 0.05$) in activities of hexokinase and phosphofructokinase and significant increase ($p > 0.05$) in glucose-6-phosphatase and fructose-1,6-biphosphatase activities in distilled water – treated diabetic rats (group B) when compared to those of non-diabetic rats (group A). Treatment with extract reduced the values in the treated rats in a manner similar to metformin (group C) and non-diabetic groups. There was no significant difference ($p>0.05$) in phosphofructokinase, glucose-6-phosphatase and fructose-1,6-biphosphatase activities in rats treated with 100 mg/kg body weight of the extract (group F) – and metformin – treated rats when compared to non – diabetic rats. Moreover, hexokinase activity did

not differ significantly in rats treated with 100 mg/kg b.w of the extract – and metformin – treated rats.

DISCUSSION

One of the most potent methods to induce experimental diabetes mellitus is chemical induction by alloxan (Etuk, 2010). Alloxan is a well – known diabetogenic agent that is used to induce Type 1 diabetes in experimental animals (Viana, *et al.*, 2004). In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used (Etuk, 2010).

The 5-fold increased in blood glucose concentrations of administered groups within 36 hours of administration in the present study agreed with the report of Lenzen (2008). According to Lenzen (2008), alloxan injection has been noted to induce diabetic hyperglycaemic phase during which complete degranulation and loss of the integrity of the beta cells within 24 – 48 h after administration of the alloxan takes place.

Final permanent diabetic hyperglycaemic phase was also observed in untreated diabetic group which showed spontaneous increased in blood glucose concentration throughout the period of the experiment. However, integrity of beta cells of treated groups was reverted back to normal due to decreased blood glucose concentrations after administration of aqueous extract of *Calotropis procera* leaf. The hypoglycaemic performance of the extract in the present study at the experimented doses mostly 100 mg/kg dose was in accordance with previous studies of Ajiboso *et al.*, (2016) and Ajiboso and Tarfa (2018).

The liver is an important organ that plays a vital role in glycolysis and gluconeogenesis (Wolfert *et al.*, 2013). A partial or total deficiency of insulin causes derangement in carbohydrate metabolism that alters activity of several key enzymes including hexokinase, phosphofructokinase, glucose-6-phosphatase and fructose-1,6-biphosphatase resulting in impaired peripheral glucose utilization and augmented hepatic glucose production (Chen *et al.*, 2000). The decreased activities of glycolytic enzymes (hexokinase and phosphofructokinase) in the liver of distilled water treated diabetic rats suggest slowing down of glycolysis in the rats and implies that metabolism of glucose will be impaired. This makes the rats to be continuously suffering from hyperglycemia. Therefore, the increased activities of liver phosphofructokinase and hexokinase following the administration of the extract may lead to enhanced glycolysis and increased utilization of glucose for energy production (Susztak *et al.*, 2006).

Glucose-6-phosphatase is a crucial enzyme for the final step of gluconeogenesis where it catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and phosphate (Chen *et al.*, 2000). Insulin inhibits the hepatic glucose production by suppressing glucose-6-phosphatase and fructose-1,6-biphosphatase (Chen *et al.*, 2000). Normally, the increased activities of the two gluconeogenic enzymes from the liver may be due to the activation or increased synthesis of the enzymes contributing to the increased glucose production during diabetes by the liver. However, normalization of the activities of these enzymes by aqueous extract of *Calotropis procera* leaf may possibly be primarily achieved by modulating and regulating the activities of the two gluconeogenic enzymes, through the regulation by cyclic AMP (Susztak *et al.*, 2006).

CONCLUSION

Aqueous extract of *Calotropis procera* leaf at the experimented doses mostly 100 mg/kg dose repeatedly lowered blood glucose level and also corrected alterations in activity of carbohydrates metabolizing enzymes in the present study. Thus, the 100 mg/kg dose of the extract can be used to correct diabetic complications and biochemical disorders associated with carbohydrates metabolizing enzymes.

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