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Behaviours of liver and kidney function markers in diabetic rats treated with *Calotropis procera* leaf aqueous extract

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Abstract

Long-term high blood glucose causes diabetes mellitus; this metabolic disorder is accompanied by both short – and long terms complications. In the present study, behaviours of liver and kidney function markers in diabetic rats treated with different doses of Calotropis procera leaf aqueous extract were studied. At p>0.05, the blood glucose, hepatic GOT, GPT, albumin, total bilirubin, GOT/GPT ratio, serum urea and creatinine were significantly elevated in diabetic groups (B, C, D, E and F), while hepatic glycogen was significantly reduced with induction of alloxan. Treatment with the extract at different doses of administration (25, 50 100mg/kg b.w.) particularly higher doses restored the markers back to their normal levels when compared to mean values of non-diabetic group (A). Mean values of markers in group administered 100mg/kg b.w. dose of extract, non-diabetic and metformin treated groups (A and B) were in the same range. In the present study, it can be deduced that aqueous extract of Calotropis procera leaf at 100 mg/kg b.w dose showed significant potency in amelioration of alloxan disturbances on hepatic and renal function markers.

Keywords: Behaviours, hepatic, renal, markers, extract, potency.

Introduction

One of the household diseases that is globally recognised is diabetes mellitus. In as much as its aetiology is medical, clinical, physiological and biochemical related, its associated complications are well recognised by literate and illiterate. These complications include excess urine (polyuria), excess hunger (polyphagia), excess thirst (polydipsia), retinopathy, neuropathy and nephropathy[1].

From documented history of diabetes, the term "diabetes" was first coined by Araetus of Cappadocia. After which the word mellitus (honey sweet) was added by Thomas Willis of Britain upon the discovery of sweetness of urine. Although ancient Egyptians described clinical features similar to diabetes about 3000 years ago. Establishment of role of liver in glycogenesis is an important milestone in the history of diabetes, this discovery constituted the basis of insulin isolation and clinical use[2].

However, an estimate for diabetics' population (age 20–79 years) in Nigeria for 2010 was put at 2,819,000 and was projected to rise up to 5,316,000 in 2030 while the calculated mean annual increment was put at 125,000[3].

According to documented report, indices for the determination of liver function include transaminases, phosphatase, glycogen, albumin and bilirubin[4]. The roles of kidney include regulation of water and salts in the body through the filtering of blood via the nephrons resulting in secretion of certain waste products from the body. Urea, creatinine and dissolved salts are the routine tests for the determination of kidney function[5].

Higher incidence of liver and kidney function tests abnormalities have been reported in diabetic than non-diabetic individuals[6]. Function tests abnormalities resulting from diabetes mellitus was also reported as the cause of liver and kidney dysfunctions[6]. The prevalence of diabetes mellitus in Nigeria is high and virtually every home has at least an individual who suffers the consequences of diabetes related liver and kidney dysfunctions.

In this study, behaviours of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, total bilirubin, glycogen, urea and creatinine in alloxanized rats treated with different doses of *Calotropis procera* leaf aqueous extract were studied.

Materials and methods

Materials: Fresh matured leaves (2kg) of *Calotropis procera* were collected from the parent plant in August 2018 when rainfall was at the peak from Doko town, Niger State; the plant was identified by a botanist at the botanical garden of the Federal Polytechnic Bida, Niger State. After identification, plant sample with No. 95002 was deposited at plant record unit.

Aqueous Extraction: Dirts and other foreign substances were handpicked from harvested leaves, followed by subjecting the leaves to air drying at 28°C for 14 days. After drying, the prepared sample was subjected to aqueous extraction.

Animals: Adult laboratory animal (*Ratus novergicus* both sexes, mean weight 90.05±0.91g, n=30) were obtained from the

animal breeding section of Federal Polytechnic Bida. The rats were kept in animal house, fed *ad libitum* on standard rat pellet (Vital mix) and allowed to acclimatize for 2 weeks.

Drug and Chemicals: Metformin (Springville, USA), alloxan (Pune, India) and chemicals (Poole–England) were used. Distilled water was used to prepare chemicals unless otherwise stated.

Induction of diabetes: Diabetes was induced through intraperitoneal administration of 150mg of alloxan monohydrate in 10ml physiological saline solution; calculated amount based on body weight was administered to the animals.

Experimental design: Animal grouping: After confirmation of diabetes, 25 diabetic rats and 5 non – diabetic rats (not induced) were used, five rats per group given a total of 30 rats in groups A to F. A= Not induced rats +distilled water, B= Induced rats + distilled water, C= Induced rats + Metformin, D= Induced rats + 25mg dose of extract. E= Induced rats + 50mg dose of extract. F= Induced rats + 100 mg dose of extract.

Distilled water was used to constitute the required doses of 25, 50 and 100mg. Doses used were obtained from ethno-botanical survey on *Calotropis procera* plant in Bida town. Treatment (0.5ml) was administered orally with feeding bottle to respective groups; this experiment was terminated on the 10th day. European convention guidelines for the protection of vertebrate animals were used in handling of the rats[7].

Blood glucose: 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 Contour TM AG, Postfash, Basel, Switzerland) on days 0, 5 and 10.

Preparation of serum and liver homogenate: At the end of 10th day, under an aesthesia (diethyl ether, 50mg/ml), blood was collected through the neck areas of the rats into tube, this was allowed to clot. The clot blood was centrifuged using model SM 800B centrifuge, Suygifriend. Thereafter, sera was aspirated and preserved for analysis.

After dissection, liver was removed, cut into tiny pieces and homogenised in sucrose solution (0.25M, 1:5 w/v) with hand-held homogenizer (D1000 Asteria, USA). Homogenates obtained were kept frozen for 24 hours before analysis.

Other Analysis: These markers were determined using standard procedures. Glutamate oxaloacetate transaminase[8], glutamate pyruvate transaminase[8], albumin[8], total bilirubin[8], creatinine[9], urea[10] and glycogen[11].

Statistical analysis: Mean values of five replicates were graphically presented using bar chart. Test for significance was analysed at 95% level of confidence using ANOVA.

Results and discussion

Figure-1 showed mean value of blood glucose level of diabetic rats treated with *Calotropis procera* leaf aqueous extract. At p>0.05, blood glucose level of alloxan administered animals in groups B to F significantly increased. The non-diabetic group A animals showed a negligible increase of 2.3% in blood glucose throughout the experimental period. Continuous increase in blood glucose was observed in diabetic group B animals treated with distilled water only. On day 10, blood glucose had elevated by 33.6%. However, treatment with metformin and doses of extract gives 80.9%, 42.2%, 61.3% and 81.3% decrease in blood glucose. The hypoglycaemic performance exhibited by the extract was observed to be dose-dependent.

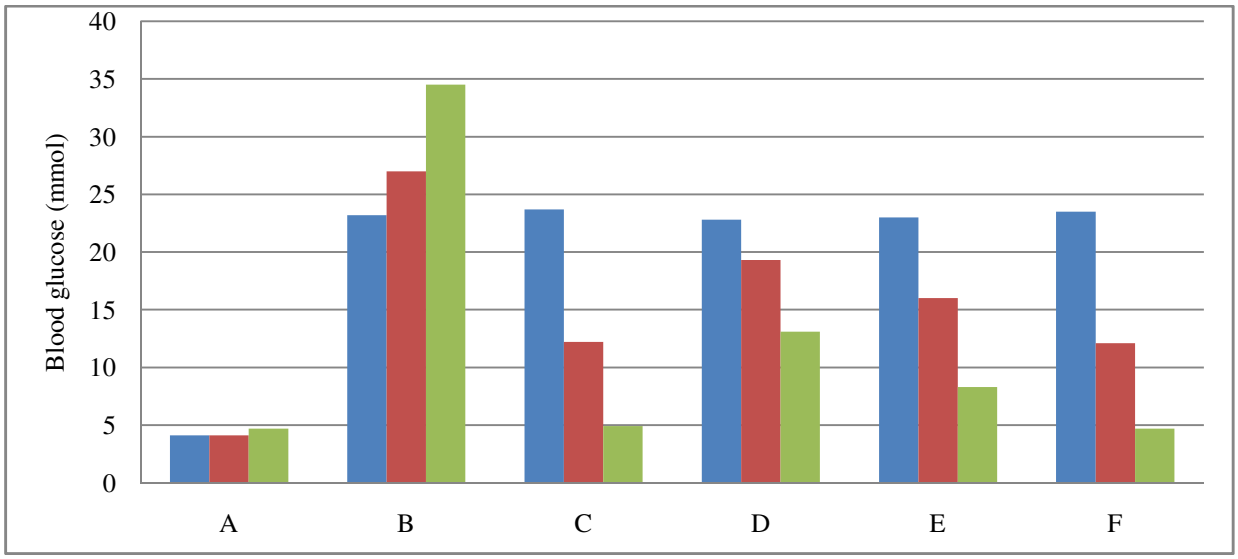

Figure-1: Mean value of blood glucose level of diabetic rats treated with *Calotropis procera* leaf aqueous extract [Day: 0= Blue; 5=Brown; 10= Lemon Green].

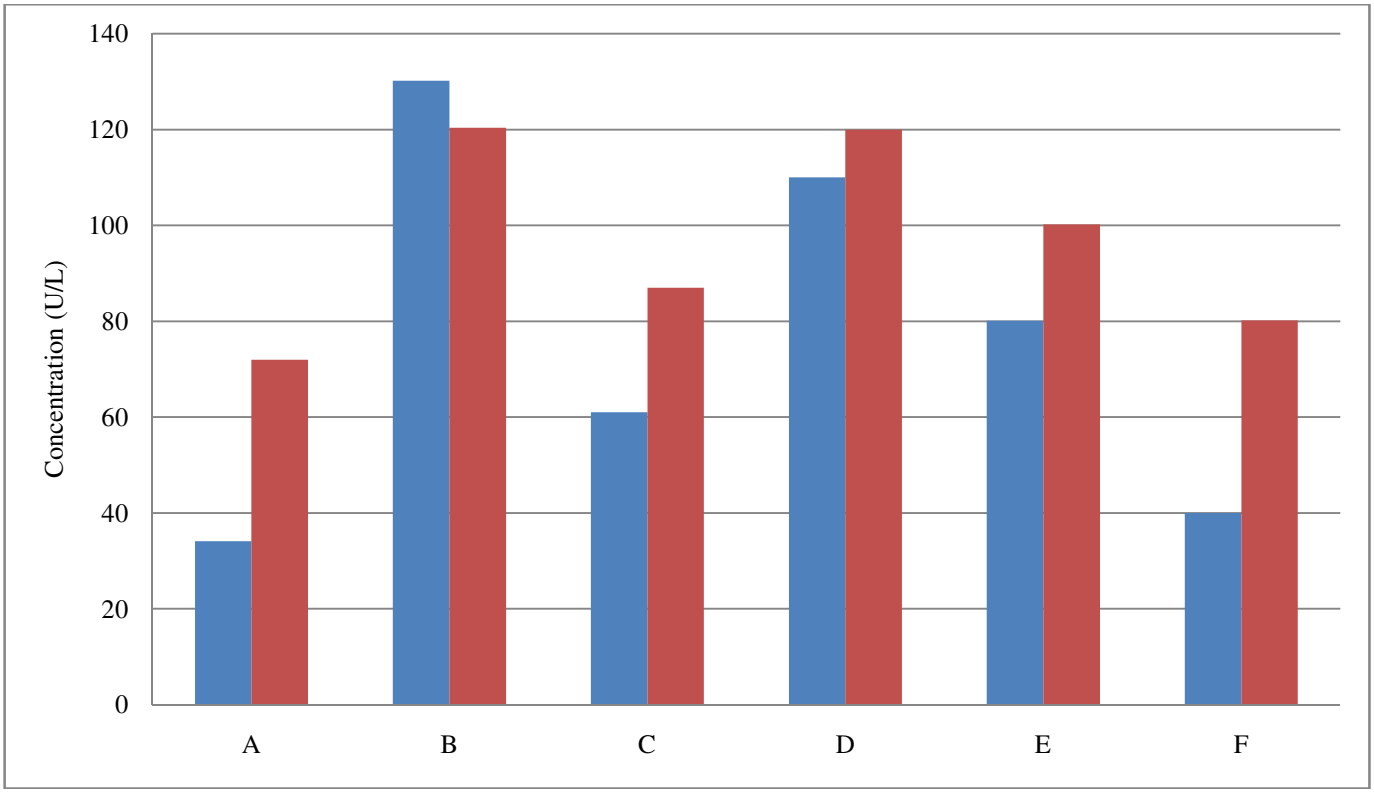

Figure-2: Mean value of GOT and GPT activity of diabetic rats treated with *Calotropis procera* leaf aqueous extract [Column type: GOT = Blue; GPT = Brown].

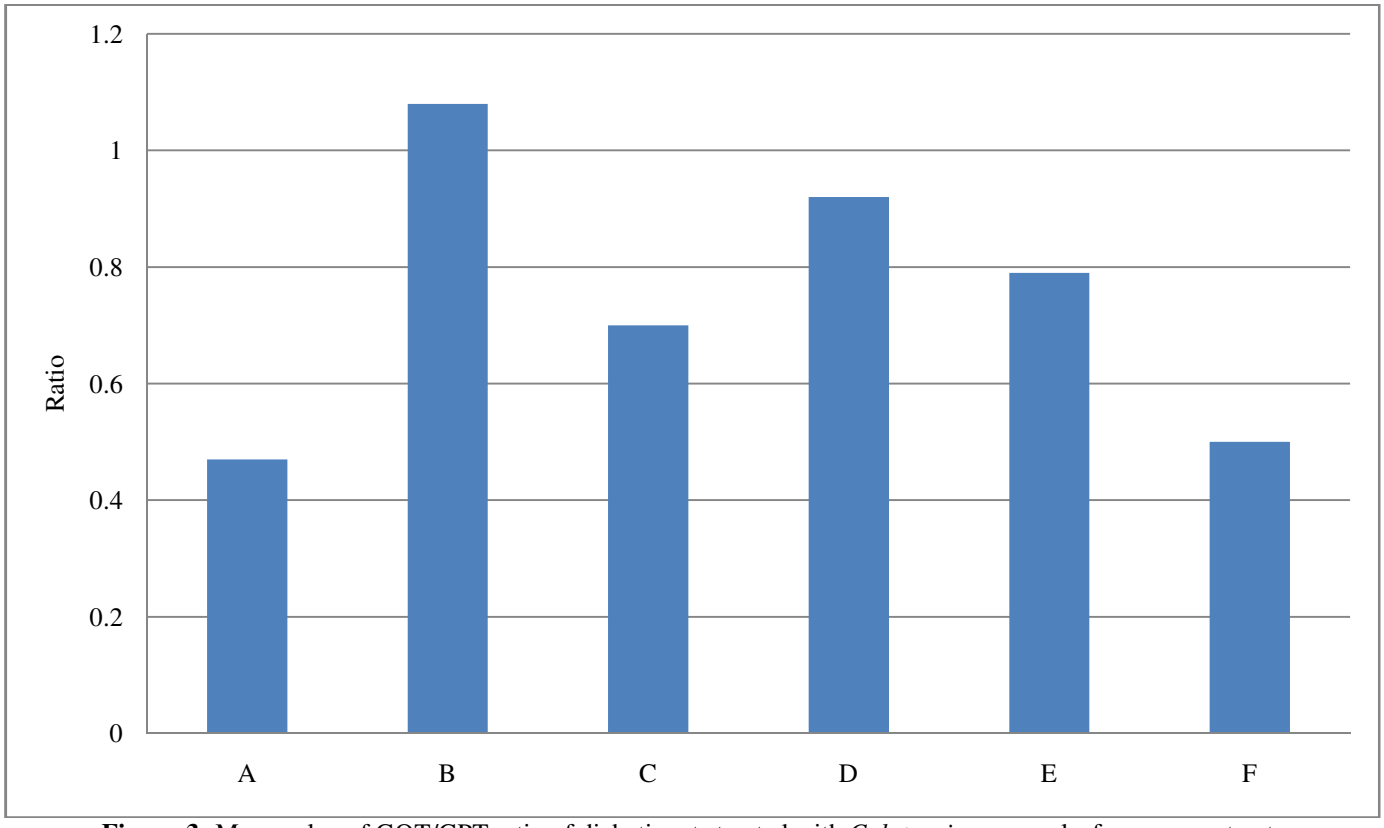

Figure-3: Mean value of GOT/GPT ratio of diabetic rats treated with *Calotropis procera* leaf aqueous extract.

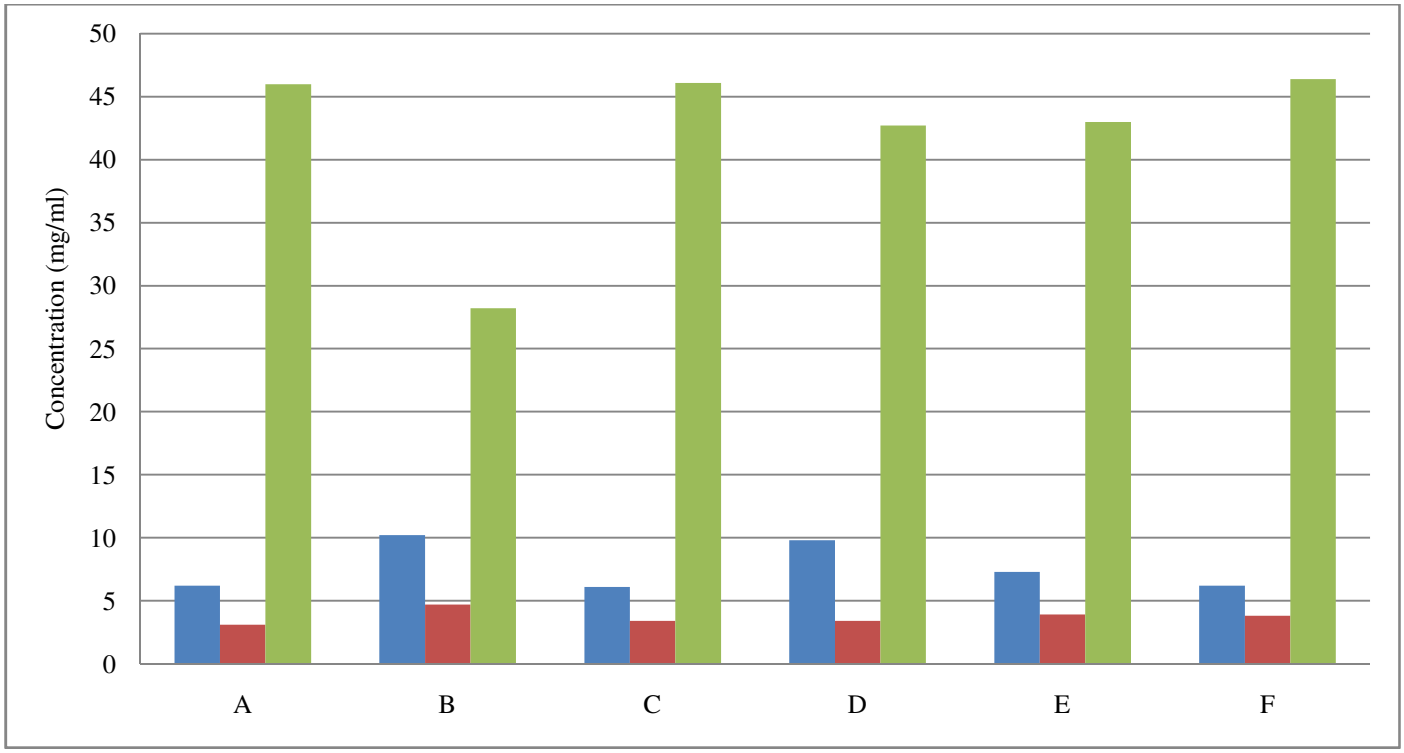

Figure-4: Mean values of levels of liver function markers of diabetic rats treated with *Calotropis procera* leaf aqueous extract [Column type: Albumin = Blue; Total Bilirubin = Brown; Glycogen = Lemon Green].

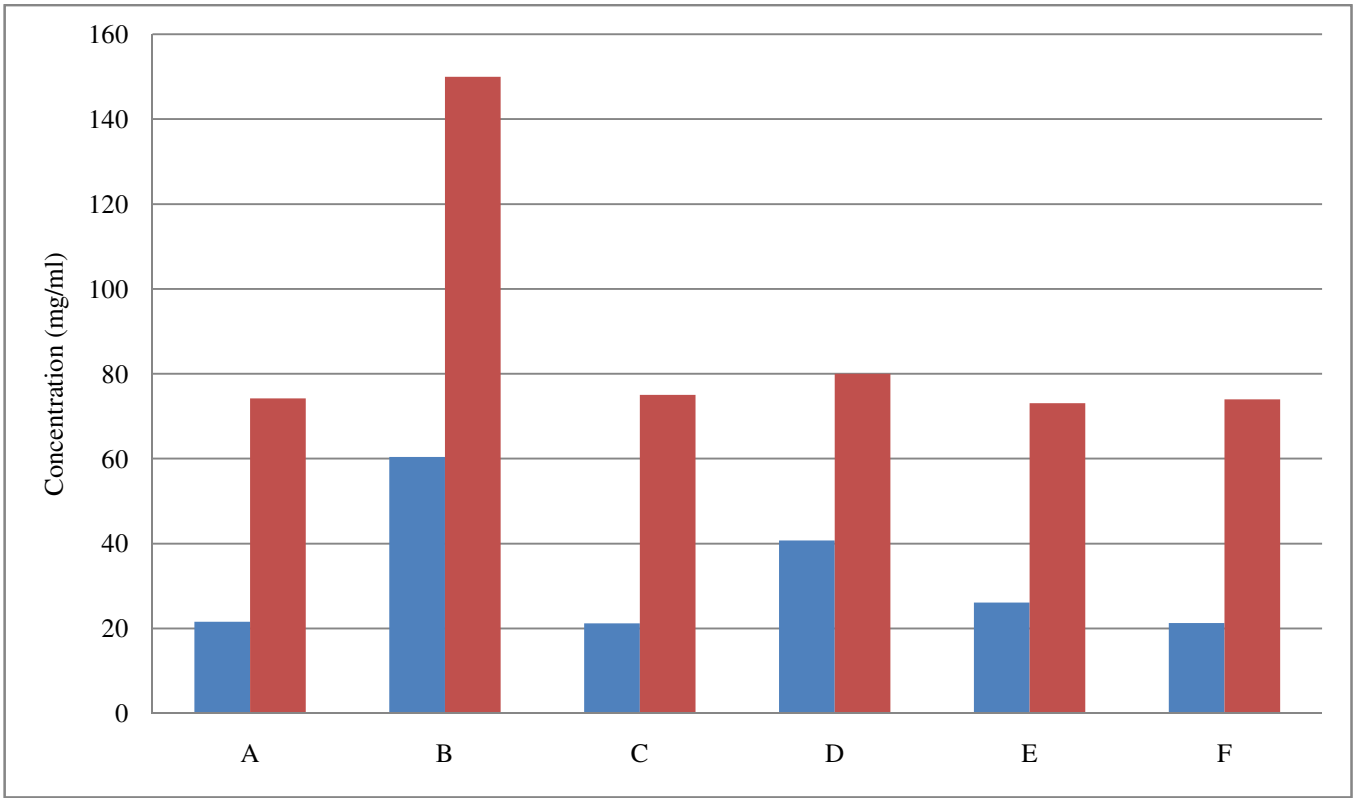

Figure-5: Mean values of levels of kidney function markers of diabetic rats treated with *Calotropis procera* leaf aqueous extract [Column type: Urea = Blue; Creatinine = Brown].

Figures-2, 3 and 4 showed mean values of levels of liver function markers of diabetic rats treated with *Calotropis procera* leaf aqueous extract. The liver function markers generally increased while liver glycogen content decreased in distilled water – treated diabetic rats (group B). However, extract (100mg/kg dose – group F) ameliorated the values of the parameters in a manner similar to metformin (group C).

Figure-5 showed mean values of levels of kidney function markers of diabetic rats treated with *Calotropis procera* leaf aqueous extract. Kidney function markers significantly increased in distilled water – treated group B compared to non-diabetic (group A). Treatment with extract decreased the levels of these markers. The values obtained for group F treated with 100 mg/kg dose and metformin treated group C were similar.

Discussion: The hypoglycaemic potency and efficacy of the extract agree with earlier investigation and report[12]. The positive correlation between the ameliorative property of the extract on blood glucose and higher dosage observed in the present study has earlier been reported[13].

Studies by several scientists have shown that adequate chemical composition of body fluids is maintained through removal of urea, uric acid, creatinine and ions by the kidney. Renal dysfunctions are evaluated through blood urea and creatinine determination[14]. Similar investigation on uncontrolled diabetes mellitus also show increase in concentrations of these metabolites in blood during renal diseases or renal damage. Increased levels of creatinine and urea in untreated diabetic rats may be attributed to renal damage due to uncontrolled diabetes mellitus. Report has shown that glucose via pentose phosphate pathway brings about formation of ribose-5-phosphate, phosphoribosyl pyrophosphate (PRPP) and increased blood uric acid concentration[14]. The decrease in creatinine and urea of extract administered groups may be attributed to the secretion and control of PRPP formation by the aqueous extract of *Calotropis procera* leaf.

According to reviewed literature[15], functions of liver include metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. Liver toxicity has been reported in experimental animals through the measurements of markers such as glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, albumin and bilirubin[16]. Abnormal elevation of these markers in diabetes has been linked to necrosis of liver[17].

Research reveals that hyperalbuminemia occurs in alloxanised rats and GOT/GPT value that is greater than 1.0 is an indication of liver damage[18], liver damage is evident in the distilled water – treated diabetic rats in the present study due to GOT/GPT ratio that is greater than 1.0. The reduction in the elevated liver function markers upon administration of aqueous extract of *Calotropis procera* leaf may be due to the protection offered by the extract to the liver.

Glycogen, synthesized by glycogen synthase is the primary intracellular form in which glucose is stored, the amounts in tissues, particularly liver, correlates with activity of insulin[19]. Decreased hepatic glycogen in diabetic subjects has been linked to destruction of β-cells, reduction in level of insulin, and dependence of enzymes on insulin for glucose influx[19]. This accounts for depletion of liver glycogen observed in distilled water treated diabetic rats in the present study. Improvement of the hepatic glycogen content in extract administered groups may be attributed to insulinotropic property offered by the extract in administered groups[19].

Conclusion

From the present study, aqueous extract of *Calotropis procera* leaf at 100mg/kg b.w dose showed significant potency in amelioration of alloxan disturbance on hepatic and renal function markers.

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