



Modulations of Some Carbohydrate Metabolic Enzymes by Aqueous and Ethanol *Buchholzia coriacea* Seed Extract in Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author KOG carried out the bench work, author AON managed and supervised the experimental protocol, author PRCE performed the statistical analysis, author MOO wrote and monitored the first draft of the manuscript, and author MAO managed the literature searches.

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ABSTRACT

Characterised by abnormal increase in blood glucose level, Diabetes is a metabolic disorder that is associated with complications in carbohydrate, protein and fat metabolism. In recent times, medicinal herbs have been implicated in traditional medical practice for the treatment of this ailment. Studies have shown that *Buchholzia coriacea* seed possesses some anti-hyperglycemic properties that may be useful in the management of diabetes. To this point, present study investigated the

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effect(s) of oral administration of aqueous and ethanol extracts of *Buchholzia coriacea* on some carbohydrate metabolism parameters in normal and alloxan-induced diabetic rats. Forty (40) adult rats of both sexes were randomly assigned into two groups (normoglycemic and hyperglycemic). While group 1 (normoglycemic) had normal control, metformin, aqueous extract (250mg/kg) and ethanol extract (250 mg/kg) treated sub-groups respectively, Group 2 (hyperglycemic) contained the diabetic control, metformin, aqueous extract (250 mg/kg), and ethanol extracted (250mg/kg) treated sub-groups dosed daily by oral gavage for 14 days. At the end of the treatment, rats were euthanized via cervical dislocation; blood samples were collected by cardiac puncture for statistical analysis. One-way analysis of variance (ANOVA) revealed that dosing with extracts had insignificant effect(s) on body weight of rats. Fasting Blood Glucose (FBG) levels were elevated before and after extracts administration. Metformin, aqueous and ethanol extracts significantly reduced ($p < 0.05$) FBG levels. Also, compared with control, total carbohydrate, liver glucose, glycosylated haemoglobin, Lactate Dehydrogenase, Isocitrate dehydrogenase, MDH, SDH, 6-phosphogluconate dehydrogenase, G6PD and CcO activities were significantly reduced ($p < 0.05$) in diabetic treated rats. *Buccholzia Coriacea* was therefore seen to pose hypoglycemic and glycolytic effects, regulating activities of carbohydrate metabolic enzymes. Apparently, there is a scientific merit in the use of the extract in the management of diabetes.

Keywords: *Buchholzia coriacea*; alloxan; hyperglycaemia; hypoglycaemia; blood glucose.

1. INTRODUCTION

Diabetes mellitus (DM) is a defiant in carbohydrate, protein and fat metabolic conditions that results in chronic increase in blood sugar levels. It results from either defect in insulin secretion, insulin action, or both. Chronic conditions of diabetes are characterized with chronic damage, dysfunction and disturbances of various organs, especially the heart, kidneys, eyes, nerves, and blood vessels [1]. Diabetes is characterized by increased thirst, hunger and loss of weights, which ultimately complicates in macrovascular, microvascular, and neuropathic disorders [2]. In DM, metabolism of carbohydrates, proteins and fats is altered; primarily because of failure of insulin to aid the efficient uptake and utilization of glucose in most cells of the body, except those of the brain [3-5]. Due to this insufficient uptake of glucose by cells, there is accumulation of glucose in the blood, leading to a significant reduction in cellular utilization of glucose whilst fats and proteins utilization rises significantly.

In recent times, there has been a rapid increase in the prevalence of diabetes globally, with the World Health Organization (WHO) predicting that by 2030 the number of diabetic adults would have almost doubled globally from 177 million in 2000 to 370 million [6]. According to Rowley et al. experts have estimated that the cases of adults with diabetes is set to increase rapidly by 64% by 2025, which means that a shocking 53.1 million citizens will be affected by the disease [7].

In orthodox medicine, Diabetes remains an incurable endocrine disorder with huge cost of management; where found, undesirable and adverse effects are often associated with accepted drugs. Overtime, this has promote the use of suitable minimal side-effect herbs as alternative for the treatment and management of this ailment [2,4].

Found in many tropical countries like Ghana, Gabon, Cameroon, Central African Republic, Congo, Angola, Nigeria, among others, *Buchholzia coriacea* (Wonderful cola) is an evergreen shrub of the *Capparidaceae* family. Previous studies have shown that ethanol extract of *Buchholzia coriacea* elicits hypoglycemic effects, causing it to exhibit synergistic actions with the hypoglycemic agent metformin [8]. Trado-medics and researchers have claimed that wonderful cola is a valuable alternative therapy in the treatment of diarrhea [9], malaria [10], worm infection [11], rheumatism [12], diabetes [13], hypertension, psychiatric disorders, asthma and cough, impotence, among others. This study therefore aimed at investigating the modulatory effect(s) of *Buchholzia coriacea* (wonderful kola) seed extract on some carbohydrate metabolic enzymes, following induction of diabetes mellitus with alloxan in wistar rats. Specifically, study was designed to examine the effect(s) of wonderful kola extract on body weights, blood glucose level, on total carbohydrate level as well as on glycogen level. Study also attempted to establish the effects that wonderful kola may have on selected carbohydrate metabolic enzymes.

2. METHODOLOGY

2.1 Scope of Study

Study was restricted to the role of *Buchholzia coriacea* on blood glucose levels, particularly on carbohydrate metabolism. It was an *in-vivo* study on the effects of *Buchholzia coriacea* on blood glucose level and carbohydrate metabolism, using albino wistar rats as experimental model. Study investigated some biochemical parameters in carbohydrate metabolism.

2.2 Study Design

Fourty (40) rats, weighing an average of 128.6 g were divided into eight (8) groups of five (5) rats each as follows:

2.2.1 Normoglycemic groups

Normal control: neither induced with Alloxan monohydrate, nor administered *Buchholzia coriacea* extract. They received normal feed and water *ad libitum* for the duration of the experiment.

Metformin (Normal rats): administered Metformin 50 mg/kg.

Aqueous extract (Normal rats): administered 250 mg/kg of aqueous *Buchholzia coriacea* seed extract [8].

Ethanol extract (Normal rats): administered 250 mg/kg of ethanol extract from *Buchholzia coriacea* seed.

2.2.2 Diabetic groups

Diabetic Control (Diabetic rats): Induced with DM from administration of 50 mg/kg of Alloxan monohydrate. Neither treated with metformin nor *Buchholzia coriacea* seed extract.

Metformin (Diabetic rats): Induced with DM from administration of 50 mg/kg of Alloxan monohydrate. They were treated with standard oral hypoglycaemic drug and 50 mg/100 g BW of metformin.

Aqueous extract (Diabetic rats): Induced with DM from administration of 50 mg/kg Alloxan monohydrate and treated with 250 mg/kg of *Buchholzia coriacea* seed aqueous extract.

Ethanol extract (Diabetic rats): Induced with DM from administration of 50 mg/kg Alloxan

monohydrate and treated with 250 mg/kg of *Buchholzia coriacea* seed ethanol extract.

2.3 Materials

1. Syringes And Needles
2. Cotton Wool and Hand Gloves
3. Electronic Weighing Balance (Model JA 2003):
4. Sample Containers
5. Glass slides and slide rack
6. Hot plate
7. bucket centrifuge
8. VIS spectrophotometer (model 722N)
9. electrical thermostatic water bath boiler (model DK 420)
10. Rotatory Microtome
11. PH meter
12. Dissecting Board
13. Measuring Cylinder
14. Plastic Specimen Bottle
15. Beaker (20 ml, 50 ml, 500 ml).
16. Graded alcohol (50%, 70%, 95% and absolute alcohol)

2.4 Preparation of Plant's Extract

Fresh seeds of *Buchholzia coriacea* were collected and identified by renown botanists from the Department of Botany, Faculty of Science, Delta State University, Abraka. The seeds were immediately cleaned off debris, peeled, chopped and shade-dried for one (1) week in laboratory trays. The dried seeds were pulverized into powder with sterilized machine and weighed. 500 g of the powder was then divided into two equal parts and macerated in 4500 ml distilled water and 4500 ml of ethanol respectively, following intermittent shaking for 48 hours. Extract was then obtained with rotary evaporator (an electrical evaporator extraction apparatus). The solvent was extracted at temperature of 45°C and pressure of 60 mmhg of water. Paste-like extract was obtained and oven-dried to complete solid, then, grinded to smooth powdered form and stored in a refrigerator till use.

2.5 Procedure

2.5.1 Ethical clearance

Ethical clearance (Ref No: RBC/FBMS/DELSU/14/44, Dated: 19/08/2017) was obtained from the Research and Bio-Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State.

2.6 Acute Toxicity Test

Graded doses (250, 500, 1000, 2000, and 5000) mg/kg of the aqueous and ethanol extracts of wonderful kola was administered to the different groups orally. They were then observed for acute toxicity signs like behavioural changes or death over 24 h. though no deaths were recorded, dizziness was however observed at the dose of 2,000 and 5000 mg/kg body weight.

2.7 Sample Collection

Blood glucose levels were checked every seven (7) days (weekly), using the ACCUCHEK glucometer. To achieve this, blood sample was taken from tail vein of the rats on each occasion. After an overnight fast on the last day of the experiment, a final blood glucose check was done. Then rats were sacrificed by cervical decapitation and each rat was placed on its dorsal surface, and a laparotomy was carried out to expose the internal organs. Blood was collected by cardiac puncture, using 5 ml syringes and 21G needle. Obtained blood samples were centrifuged at a rate of 4000 rpm for 10 minutes, and serum was collected and stored in a refrigerator at 4°C for analysis of the liver enzymes.

2.8 Inducing Diabetes

To induce diabetes, Alloxan monohydrate was dissolved in 0.9% sodium chloride buffer (pH 7) intraperitoneally at a dose of 50mg/kg body weight. Rats in control group were administered with equal volume of 0.9% sodium chloride, pH 7 (that was used to prepare the Alloxan monohydrate solution). Thereafter, rats were fed with normal feed and water. Three days (72 hours) after induction, diabetes was confirmed at a random blood glucose level of ≥ 200 mg/dl, using the ACCUCHEK glucometer.

2.9 Biochemical Assays

2.9.1 Estimation of total carbohydrates

Total carbohydrate was estimated by the method of Carrol et al. 1956, using 10% Trichloro acetic acid (TCA) and anthrone reagents. Tissue glycogen content was however estimated using 5% Trichloro acetic acid (TCA), 95% ethanol, standard glucose and anthrone reagents. Also, Blood glucose was estimated by the method of Marks (1959), using 1% Toluidine, 0.3 N NaOH, 0.15M acetate buffer (pH 5.0), 0.02% peroxidase solution, Glucose oxidase, standard glucose, 5% ZnSO₄.7H₂O and Fermcozyme (a stable liquid

preparation of glucose oxidase containing 750 units l ml). Serum lactate dehydrogenase was estimated by the method of Wroblewski et al. 1955, using 0.1M phosphate buffer (pH 7.4), NADH₂ and Sodium pyruvate. Pyruvate level was estimated by the method of Friedemann and Haugen, 1943, using 10% Trichloro acetic acid (TCA), 0.1% 2, 4 -Dinitrophenyl hydrazine (DNPH), 2.5 N NaOH. Phosphorylase activity was assayed by the method of Cori et al. (1955) in the direction of glycogen synthesis by estimating the inorganic phosphate formed from glucose-1- phosphate, using 0.1M sodium fluoride, 0.037 M ethylenediaminetetracetate (EDTA) (pH 6.5), 0.03 M cysteine, 0.015 M B-glycerophosphate (pH 6.5), 2% glycogen, 0.016 M glucose-1-phosphate, 0.04 M adenosine-5monophosphate (AMP), ammonium molybdate, 1—amino-2-naphthol-4sulphonic acid (ANSA). Tissue activities of enzymes of the Hexose Mono Phosphate Shunt (Pentose Phosphate Pathway) were estimated by the method of Kornberg and Horecker, 1955, using 0.33m sucrose, 0.04M glycyl glycine buffer (pH 7.5), 0.02M glucose-6-phosphate, 1.5 x 10³ NADP⁺ 0.1M Magnesium chloride.

2.9.2 Analytical approach

Results were expressed as Mean \pm SEM (standard error of the mean) and statistical significance of the treatment effect was analyzed using the one way analyses of variance (ANOVA), followed by post Hoc LSD test for multiple comparison, using software social p values < 0.05 were considered to be statistically significant. ANOVA was so chosen as is best suits the analysis of means for three (or more) sets of data as in the case for this study.

3. RESULTS

See chats 1-7 for statistical presentation of results.

4. DISCUSSION

Several beneficial effects of extract of *Buchholzia coriacea* seeds have been described in literature, and it has earned the name “wonderful kola” in folkloric use [13,14]. Chart 1 shows the effect of aqueous and ethanol extracts of *Buchholzia Coriacea* seed on the body weights of rats experimentally induced with diabetes mellitus and normoglycemic rats. Dosing with the extract of *Buchholzia coriacea* seeds showed insignificant effects on body weights of the normoglycemic rats. The body weight of extract

treated rats were insignificantly ($p > 0.05$) was a moderate increase in the body weight of different from those of control rats, but there the rats during experiment.

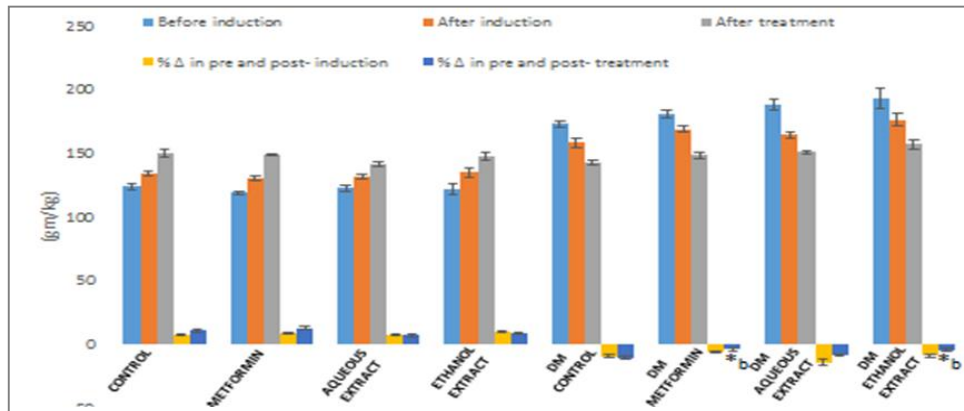


Chart 1. Effect of aqueous and ethanol extract of *Buchholzia coriacea* seeds on body weight of normoglycemic and hyperglycemic rats

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $p < 0.05$

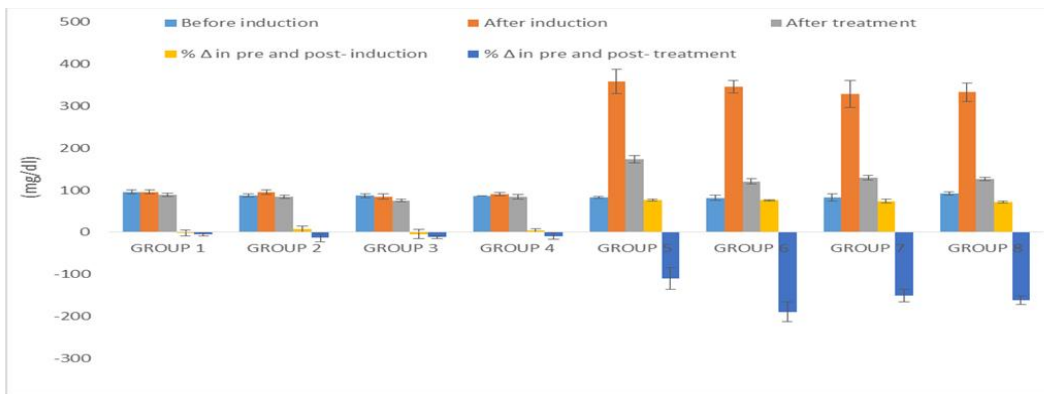


Chart 2. Effect of aqueous and ethanol extract of *Buchholzia coriacea* seeds on fasting blood glucose level of normoglycemic and hyperglycemic rat

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P < 0.05$ (*= $p < 0.001$; a= $p < 0.005$; b= $p < 0.05$)

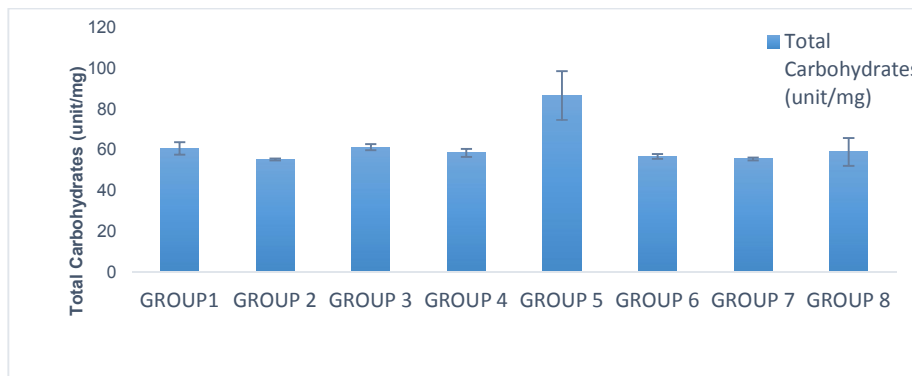


Chart 3. Showing the effect of *Buchholzia Coriacea* extracts on total carbohydrate level in normoglycemic and hyperglycemic rats induced with alloxan

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P < 0.05$ (*= $p < 0.001$)

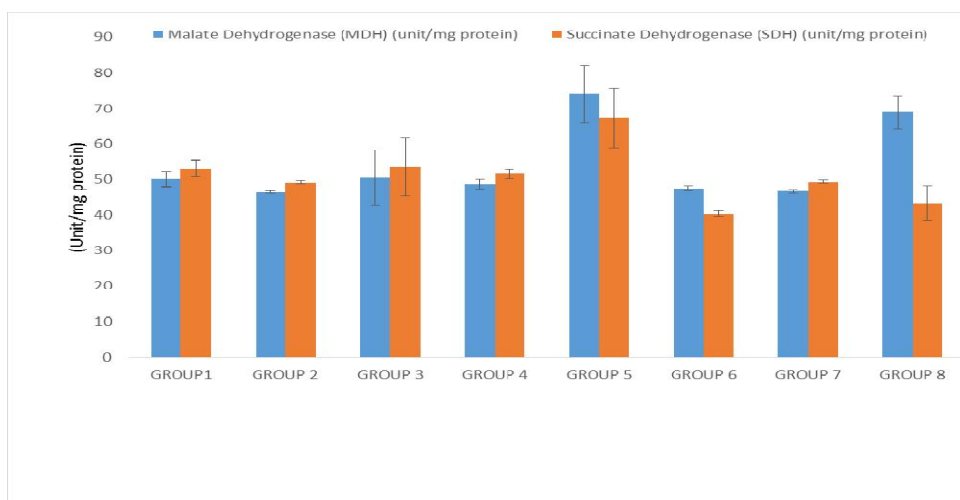


Chart 4. Showing Effect of aqueous and ethanol extract of *Buchholzia coriacea* seeds on malate dehydrogenase and succinate dehydrogenase

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$ (*= $p<0.001$; a= $p<0.005$; b= $p<0.05$)

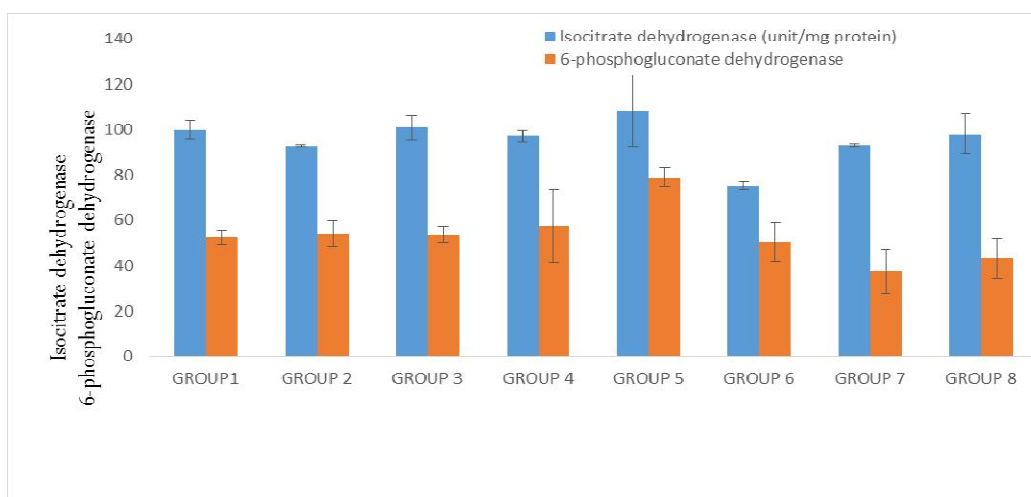


Chart 5. Showing the effect of BC extracts on lactate dehydrogenate and 6-phosphogluconate dehydrogenase levels in normoglycemic and hyperglycemic rats induced with alloxan

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$ (*= $p<0.001$; a= $p<0.005$)

Diabetes is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins [14]. As expected in diabetic control group, body weight of rats was progressively reduced; thus, alloxan caused body weight loss; which was regained to its above-initial values by *Buccholzia coriacea* seed extract treatment. This indicates the prevention of muscle tissue damage due to hyperglycemic condition reflecting an improved health of treated animals. Worth noting here is

the increase in body weight recorded in normal rats administered with the extract (aqueous and ethanol normal group). This observation gains support from the previous study of Ibrahim and Fagbohun (2012), who showed that *Buchholzia coriacea* seeds contain high percentage of carbohydrate, protein and fat and therefore the seeds could be used when considering natural food and feed additives to improve human and animal health [15].

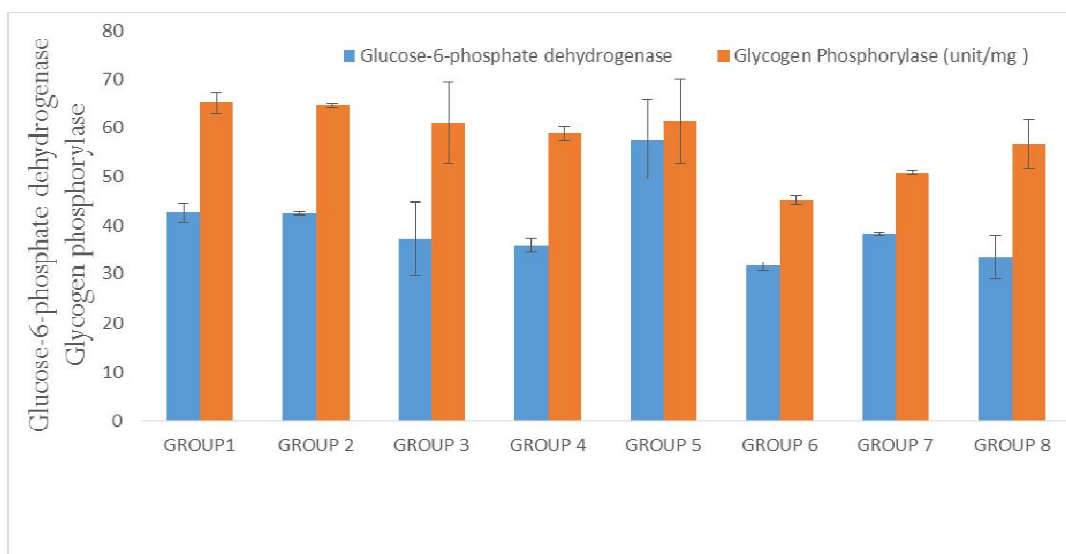


Chart 6. Showing activities of gluconeogenic and glycogen metabolic enzymes in normal and alloxan induced diabetic rat

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P < 0.05$ (*= $p < 0.001$)

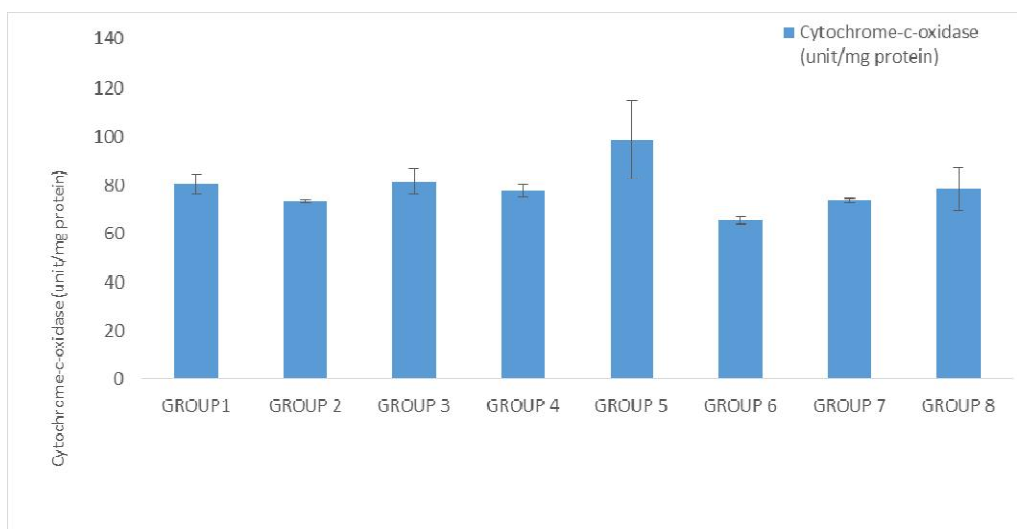


Chart 7. Showing activities of electron transport chain enzyme (cytochrome c oxidase) in normal and alloxan induced diabetic rat

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P < 0.05$ (*= $p < 0.001$)

Chart 2 shows the effect of aqueous and ethanol extract of *Buchholzia coriacea* seed on fasting blood glucose level of rats experimentally induced with diabetes mellitus and normoglycemic rats. As seen, fasting blood glucose level in animals administered alloxan monohydrate was significantly higher ($p < 0.05$) than those untreated groups. This showed that alloxan monohydrate induced hyperglycemia in

albino rats. Experimental studies conducted in alloxan-induced diabetic mice reveal that orally administered aqueous and ethanolic extracts of *Buchholzia coriacea* produced 16% antidiabetic potentials for *Buchholzia coriacea*, which is a significant fasting blood glucose lowering activity. The extracts compared favourably with the control drug, metformin. The results from this study suggest the presence of anti-diabetes

principles in *Buchholzia coriacea* aqueous and ethanolic seed extracts, which seems to confirm its traditional uses.

Chart 4 and 5 show the effect of aqueous and ethanol extract of *Buchholzia coriacea* seed on some carbohydrate metabolic enzymes of rats experimentally induced with diabetes mellitus and normoglycemic rats. As seen, levels of kidney lactic acid and liver lactic acid were insignificantly different in normoglycemic group. High level of kidney and liver lactic acids was observed in the hyperglycemic control group. This high level was reduced by the extract and metformin to values close to those of normoglycemic groups.

An increase in kidney and liver lactate dehydrogenase (LDH) was observed for hyperglycemic control group. This high level of LDH in the hyperglycemic was brought back to levels close to those of normoglycemic group as can be seen in the metformin and extract treated groups. According to the results, it can be speculated that LDH provides additional information about diabetes mellitus. To date, no study examining effect of *Buchholzia coriacea* extract on plasma levels of the enzymes have been carried out. Therefore, the results obtained could not be compared with others. Although LDH levels in diabetes have been studied, the results are mostly in conflict. Oliver et al. 1993, Tanaka et al. 1988 and Margiavichene et al. 1986 did not observe any increase in LDH between hyperglycemia and controls; also their findings are not in accordance with this finding. Melinkeri et al. Awaji et al. 1990, Jones et al. 1988 and Goldberg et al. 1977 indicated that LDH levels were higher in patients with diabetes mellitus than those of normal subjects. This report is in accordance with our findings. On the other hand, Cai, 1989 and Ryder et al. 1988 observed decreases in LDH level in diabetic subject, which contradicts our findings and those of other studies

For tri-carboxylic acid enzymes (Chart 4), there was an insignificant change in the activities of Malate Dehydrogenase (MDH), Succinate Dehydrogenase (SDH) and Isocitrate Dehydrogenase in normoglycemic treated group when compared to the normoglycemic control group after 14 days treatment duration. Significantly increased activities of Malate Dehydrogenase (MDH), Succinate Dehydrogenase (SDH), Isocitrate dehydrogenase were observed in diabetic rats. A significant

decrease in the levels of these enzymes in hyperglycemic treated groups was observed when compared to the normoglycemic treated groups. A significant reduction ($p < 0.05$) in Isocitrate Dehydrogenase level was in hyperglycemic metformin treated group when compared to the AEBC and EEBC treated groups but the values of isocitrate dehydrogenase in AEBC and EEBC hyperglycemic treated group were not significant.

4.1 Benefit of Study

Treatment of diabetes mellitus is considered a global problem, with successful treatment yet to be discovered. Therefore, any study that is directed towards amelioration of these problems is highly commendable. Data from this study will provide basic information on some of the changes in carbohydrate metabolic pathways resulting from graded-dose use of *Buchholzia coriacea* seed extracts.

5. CONCLUSION

Administration of aqueous and ethanol seed extract of *Buchholzia coriacea* for treatment of Alloxan-Induced diabetic rats modulated key carbohydrate metabolic enzymes, resulting in normal blood glucose homeostasis. This implies that administration of this extract with dose considered and the duration of administration has anti-hyperglycemic and glycolytic effects with adverse effects on liver and kidney functions. Thus, there could be scientific merit in the folkloric use of the extract in the management of diabetes.

6. RECOMMENDATIONS

Present work was a preliminary effort, therefore, further studies are needed for characterization of active compounds, and to investigate and elucidate the possible mechanism of action of the active ingredients, establish complete safety profiles, pre-formulation studies for development of a potential dosage form and evaluate the potential value of *Buchholzia coriacea* aqueous seed extract for the management of diabetes mellitus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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