

Hepatic, Pancreatic, and Renal Histo-Morphologic Alterations in Administration of Aqueous and Ethanol Seed Extract of *Buchholzia Coriacea* in Alloxan-Induced Diabetic Rats

Abstract:

Physiologically, Insulin is a hormone that helps the body's cells to absorb glucose (sugar) so it can be used as a source of energy. In diabetics, however, due to the pancreatic production of insufficient amounts of insulin or failure of body's cells to respond to available insulin, blood and urine glucose levels build up to cause excessive urination, thirst, hunger, and problems with fat and protein break down. Recently, medicinal herbs have been implicated in traditional medical practice for the treatment of this ailment (Diabetes Mellitus, DM). The present study sought to investigate the effect(s) of oral administration of aqueous and ethanol extracts of *Buchholzia Coriacea* on the liver, pancreas and kidney 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 (250mg/kg) treated sub-groups respectively, Group 2 (hyperglycemic) contained the diabetic control, metformin, aqueous extract (250mg/kg), and ethanol extracted (250mg/kg) treated sub-groups dosed daily by oral gavage for 14days. At the end of treatment, rats were euthanized via cervical dislocation; with selected visceral (liver, pancreas and kidney) harvested and observed for histo-architectural changes. Blood samples were also collected and checked for sugar levels. Slight modulations were seen in the histo-morphology of the pancreas, liver and renal tissues as *Buccholzia Coriacea* apparently posed some hypoglycemic effects. Hitherto, there was an appreciable improvement and merit in the use of the extract in the management of diabetes across groups.

Keywords: *Buchholzia Coriacea*, Alloxan, histo-architecture, blood glucose

INTRODUCTION

Primarily, the effect of insulin on glucose breakdown is to aid its efficient uptake and utilization by most cells of the body, except those of the brain^{1&2}. Defects in insulin secretion or its action leads to an increase in blood glucose level with consequence in derangement of carbohydrate, protein and fat metabolism³. Diabetes is characterized by increased thirst, hunger and loss of weight^{4&5}; resulting in chronic complications like macrovascular, microvascular, and neuropathic disorders⁶. Long-term consequences of diabetes are diseases of the coronary arteries and cerebrovascular system; renal failure, neuropathy, retinopathy and premature death⁴.

There is a rapid increase in the prevalence of diabetes globally and the World Health Organization (W.H.O) has predicted that by 2030 the number of adults with diabetes would have almost doubled globally, from 177 million in 2000 to 370 million⁷. 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 people will be affected by the disease⁷. The prevalence of diabetes among adults worldwide in 2010 was 285 million (6.4%) and this value is predicted to rise to around 439 million (7.7%) by 2030⁸.

In Nigeria, according to Olatunbosun *et al.*, the prevalence of diabetes ranged from low level of 0.8% among adults in rural highland dwellers to over 7% in urban Lagos with an average of 2.2% nationally⁹. Despite the availability of several oral anti-hyperglycemic agents and insulin for the management of diabetes, there is still an increasing demand by sufferers to use herbal drugs even when their biologically active compounds are unknown due to their effectiveness, fewer side effects, and relative low cost¹⁰. This has prompted active research efforts to provide a more effective, safer and cheaper alternative agent of plant origin that possess anti-hyperglycemic properties.

Buchholzia Coriacea (Wonderful cola) is an evergreen shrub belonging to the family Capparidaceae. It is found in many tropical countries like Ghana, Gabon, Cameroon, Central African Republic, Congo, Angola, Nigeria, among others¹¹. Previous studies have shown that the ethanol extract of *Buchholzia Coriacea* elicits hypoglycemic effects that exhibits synergistic actions with the hypoglycemic agent metformin. Traditional medicine and scientific research on different parts of the plant has claimed that it is a valuable alternative therapy for treatment of Diarrhea¹², malaria¹³, worm infection^{14&15}, rheumatism^{16&17}, diabetes¹⁸, hypertension, psychiatric disorders, asthma and cough, impotence, among others.

Aim of Study

Study sought to investigate the effect of *Buchholzia Coriacea* seed extract on the histo-architecture of selected organs (liver, kidney and pancreas), in alloxan-induced diabetic wistar rats. Specifically, study:

- i. Examined the effect(s) of *B. Coriacea* on blood glucose level
- ii. Determined the effect(s) of *B. Coriacea* on body weight
- iii. Examined the effect(s) of *B. Coriacea* on liver, pancreas, and renal histo-architectures

Methodology

Study Design

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

Normoglycemic groups

Normal control: neither given Alloxan monohydrate nor administered with *Buchholzia Coriacea* extract. They received normal feed and water *ad libitum* for the duration of study.

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

Aqueous extract (Normal rats): administered 250mg/kg of aqueous *Buchholzia Coriacea* seed extract¹¹

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

Diabetic groups

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

Metformin (Diabetic rats): induced with DM from the administration of 50mg/kg Alloxan monohydrate. Treated with the standard oral hypoglycemic drug, 50mg/100g BW of metformin

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

Ethanol extract (Diabetic rats): induced with DM from the administration of 50mg/kg Alloxan monohydrate and treated with 250mg/kg of *Buchholzia Coriacea* seed ethanol extract

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 the sterilizing machine and weighed. 500g of the powder was then divided into two equal parts and macerated in 4500ml distilled water and 4500ml of ethanol respectively, following intermittent shaking for 48hours. The extract was then obtained with a rotary evaporator (an electrical evaporator extraction apparatus). The solvent was extracted at a temperature of 45°C and pressure of 60mmhg of water. The paste-like extract was obtained and oven-dried to complete solid, then, grinded to smooth powdered form and stored in a refrigerator till use.

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109 **Procedure**

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111 **Ethical Clearance**

112 Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of
113 Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta
114 State.

115 **Consent:** NA

116 **Acute Toxicity Test**

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

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122 **Sample Collection**

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

131 **Inducing Diabetes**

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

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139 **Preparation of Tissue for Microscopic Examination**

140 The process of preparation of tissue for histological examinations was done for liver, kidney
141 and pancreas; First, the tissues were processed by impregnating the specimen into embedding
142 medium to provide support and suitable consistency for microtomy sectioning using different
143 graded solutions of alcohol from 70% to 100% to dehydrate, them (tissues). Thereafter
144 tissues were processed with paraffin wax, using an automatic tissue processor. Samples were
145 then embedded in paraffin wax at 70 degrees centigrade and cut with a rotary microtome to
146 4 μ . The staining technique employed in this study was the haematoxylin and eosin staining
147 techniques.

148 **Photomicrography**

149 Stained tissue images were captured with digital microscopic eyepiece 'Scoptek' Dcm 500,
150 5.0mega pixels connected to USB 2.0 computer.

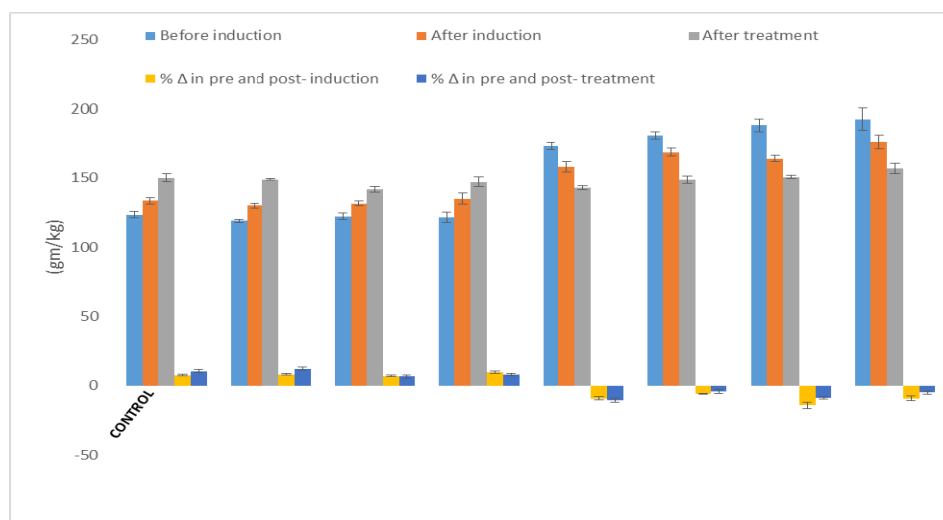
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152 **Statistical Approach**

153 Results were expressed as Mean $\pm$ SEM (standard error of the mean) and statistical
154 significance of the treatment effect was analysed with one-way analyses of variance
155 (ANOVA), followed by post Hoc LSD's test for multiple comparisons, using software social
156 science (SPSS) version 22. P-values < 0.05 were considered to be statistically non-
157 significant.

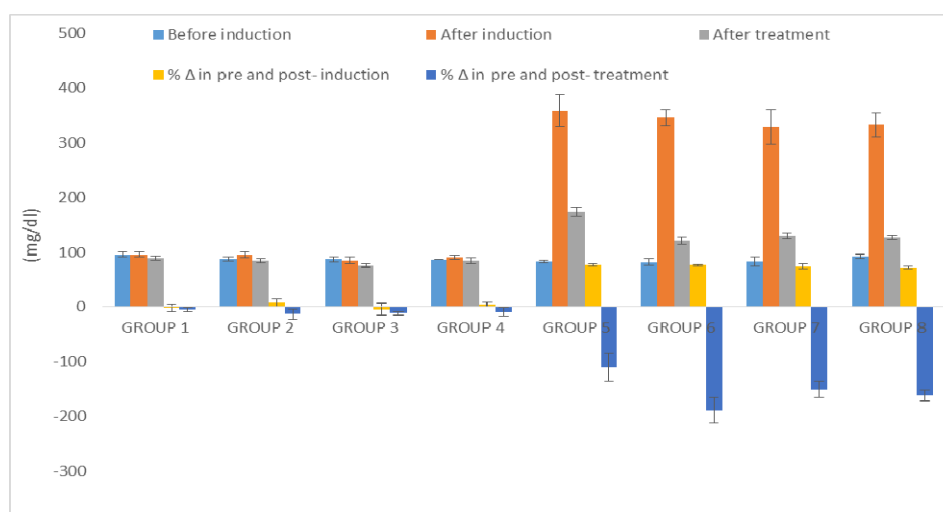
158 **Results**

159 **Figure I: Showing body weights of normal and diabetic rats before induction and after**
160 **of treatment**



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

Figure II: Changes in glucose levels of normal and diabetic rats administered with Metformin (50mg/kg) and aqueous 250 mg/kg and ethanol 250mg/kg extracts of *B. Coriacea*



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= $p<0.05$)

Histology of the Liver

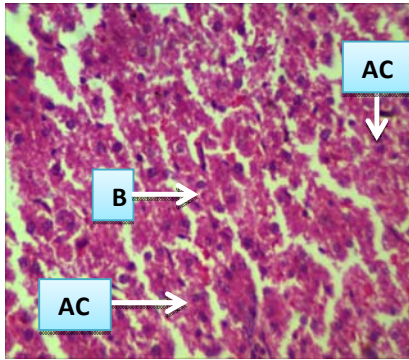


Plate 1: Coronary section of liver of normal control rats (Normal control). H&E x 400 magnification.

Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
B - Congested sinusoids.

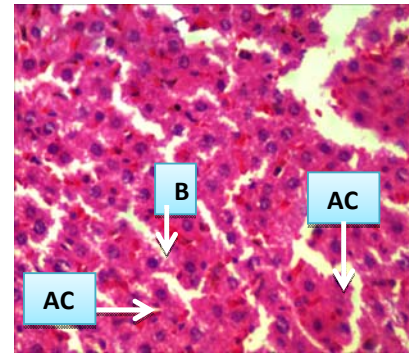


Plate 2: Coronary section of normal rats administered Metformin 50mg/kg/bw/day (Metformin). H&E x 400 magnification.

Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
B - Congested sinusoids.

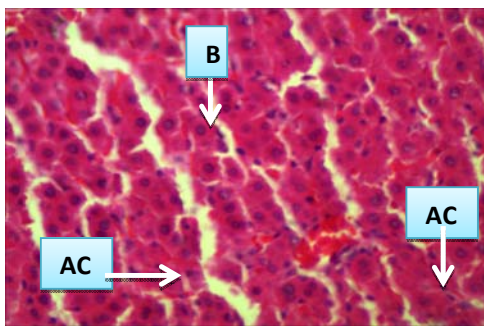


Plate 3: Coronary section of the liver of normal rats administered *Buchholzia Coriacea* seed 250mg/kg/bw/day aqueous. H&E x 400 magnification.

Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
B - Congested sinusoids.

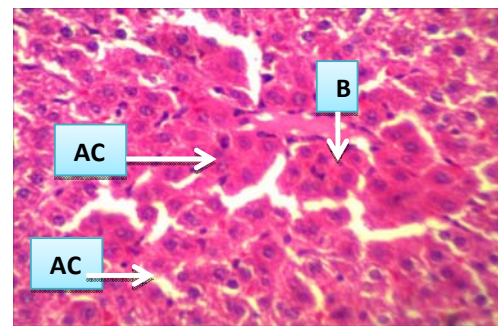


Plate 4: Coronary section of the liver of normal rats administered *Buchholzia Coriacea* seed 250mg/kg/bw/day ethanol. H&E x 400 magnification.

Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
B - Congested sinusoids.

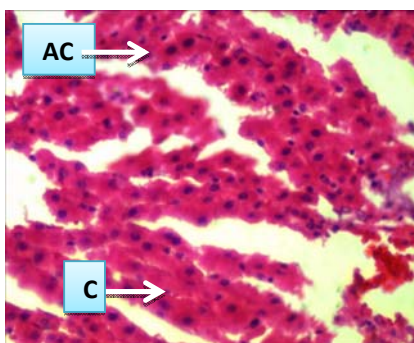


Plate 5: Coronary section of the liver of Diabetic rats Control. H&E x 400 magnification.

Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
C - Lymphatic space.

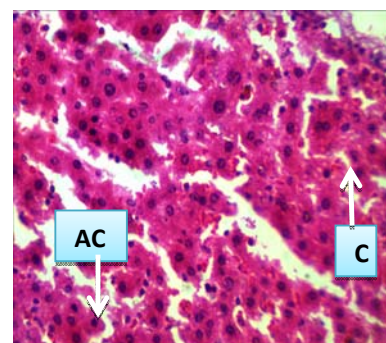


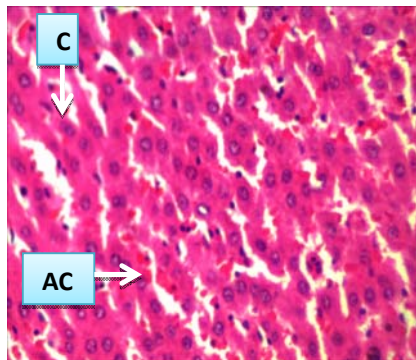
Plate 6: Coronary section of diabetic rats administered Metformin 50mg/kg/bw/day. H&E x 400 magnification.

Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
C - Lymphatic space.
B - Congested sinusoids.

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Plate 7: Coronal section of the liver of diabetic rats administered *Buchholzia Coriacea* seed 250mg/kg/bw/day aqueous. H&E x 400 magnification.

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Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
C – Lymphatic space.

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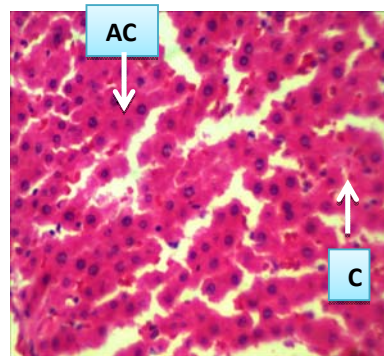
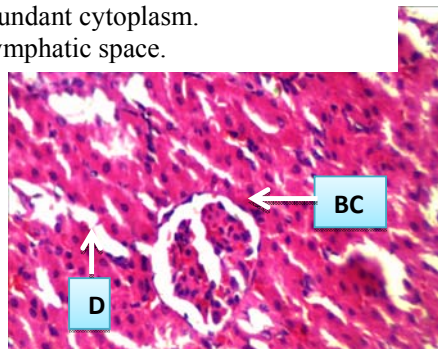


Plate 8: Coronal section of the liver of diabetic rats administered *Buchholzia Coriacea* seed 250mg/kg/bw/day ethanol. H&E x 400 magnification.

Note: AC - hepatocytes with oval nuclei and abundant cytoplasm.
C – Lymphatic space.



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Plate 9: Coronal section of the kidney of normal control rats. H&E x 400 magnification.

Note: BC - Glomerulus

D - Renal tubules lined by single layer of endothelium.

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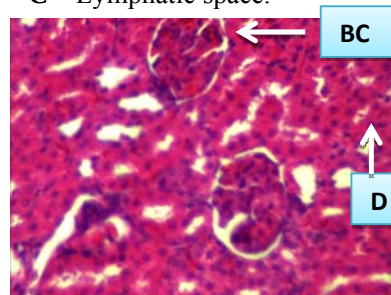


Plate 10: Coronal section of the kidney of normal rats administered Metformin 50mg/kg/bw/day. H&E x 400 magnification.

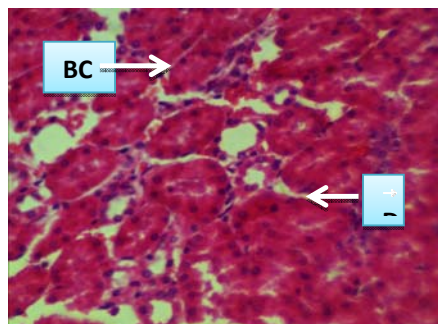
Note: BC - Glomerulus

D - Renal tubules lined by single layer of endothelium.

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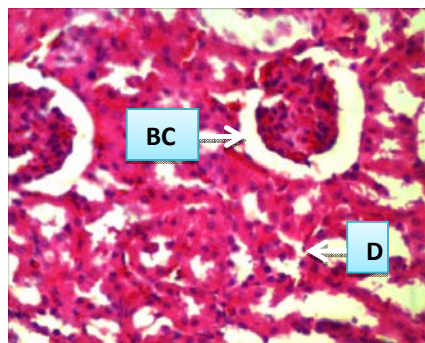


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204 **Plate 11:** Coronal section of the kidney of
normal rats administered *Buchholzia Coriacea*
205 seed 250mg/kg/bw/day Aqueous. H&E x 400
magnification.

206 **Note:** BC - Glomerulus
207 **D** - Renal tubules lined by single layer of
endothelium.

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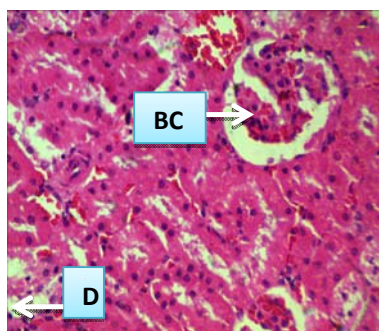


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210 **Plate 13:** Coronal section of the kidney of
diabetic control rats. H&E x 400
211 magnification.

212 **Note:** BC - Glomerulus
213 **D** - Renal tubules lined by single layer of
endothelium.

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Plate 15: Coronal section of the kidney of
diabetic rats administered *Buchholzia*
Coriacea seed 250mg/kg/bw/day Aqueous.
H&E x 400 magnification.

Note: BC - Glomerulus
D - Renal tubules lined by single layer of
endothelium.

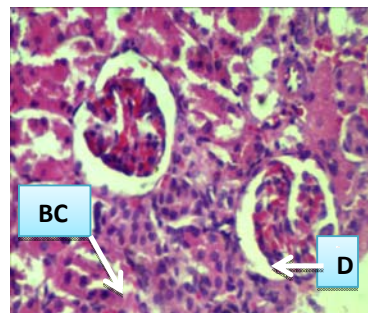


Plate 12: Coronal section of the kidney of
normal rats administered *Buchholzia Coriacea*
seed 250mg/kg/bw/day ethanol. H&E x 400
magnification.

Note: BC - Glomerulus
D - Renal tubules lined by single layer of
endothelium.

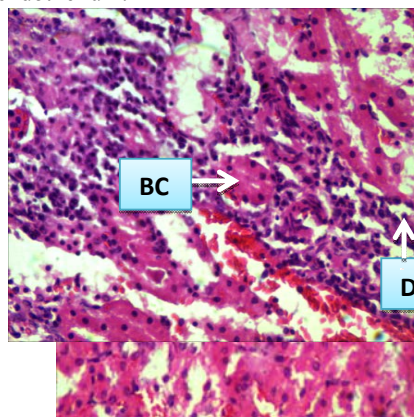


Plate 14: Coronal section of the
kidney of diabetic rats administered
Metformin 50mg/kg/bw/day. H&E x
400 magnification.

Note: BC - Glomerulus
D - Renal tubules lined by single layer
of endothelium.

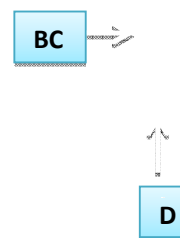


Plate 16: Coronal section of the kidney of
normal rats administered *Buchholzia Coriacea*
seed 250mg/kg/bw/day ethanol. H&E x 400
magnification.

Note: BC - Glomerulus
D - Renal tubules lined by single layer of
endothelium.

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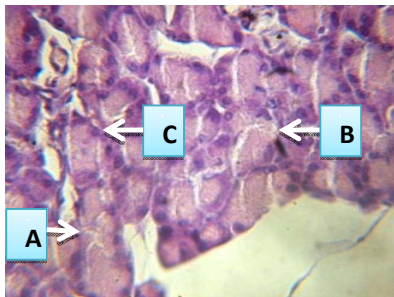
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Histology of the Pancreas



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222 **Plate 17:** Coronal section of the pancreas of normal control rats. H&E x 400 magnification.

223 A- Less karyolysis and improved islet cells with light hyaline changes in the majority of cells

224 B- Acinus

225 C- Intercalated duct

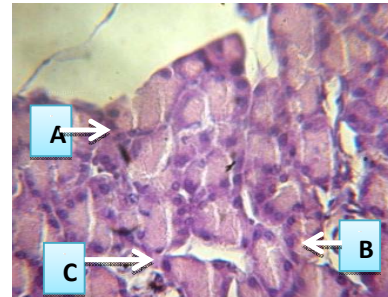
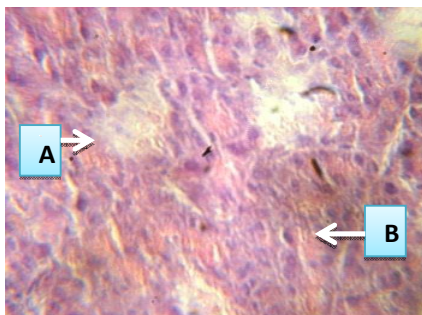


Plate 18: Coronal section of the pancreas of normal rats administered Metformin 50mg/kg/bw/day. H&E x 400 magnification.

A- Less karyolysis and improved islet cells with light hyaline changes in the majority of cells

B- Acinus

C- Intercalated duct



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227 **Plate 19:** Coronal section of the pancreas of normal rats administered *Buchholzia Coriacea* seed 250mg/kg/bw/day Aqueous. H&E x 400 magnification.

228 A- Less karyolysis and improved islet cells with light hyaline changes in the majority of cells

229 B- Acinus

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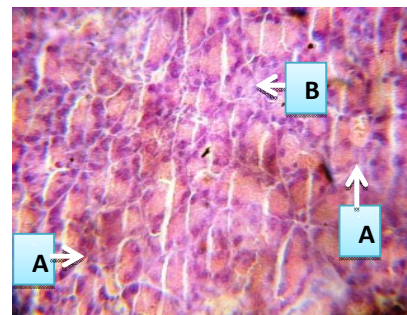


Plate 20: Coronal section of the pancreas of normal rats administered *Buchholzia Coriacea* seed 250mg/kg/bw/day Aqueous. H&E x 400 magnification.

A- Less karyolysis and improved islet cells with light hyaline changes in the majority of cells

B- Acinus

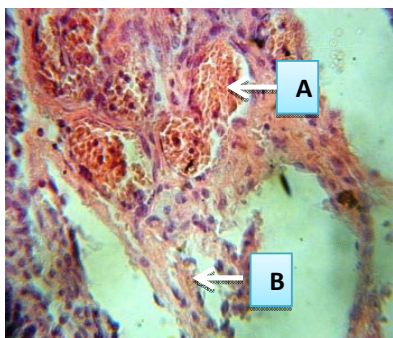


Plate 21= Diabetic control rats. Haematoxylin and eosin. x 400 magnification
A- Islets with irregular outline decrease populated cells and homogenization of the center, some islet-cells showing pyknotic nuclei, ballooning of cells and vacuolated cytoplasm
B- Pyknotic nuclei of some acinar cells with severely damaged vacuolated acini.

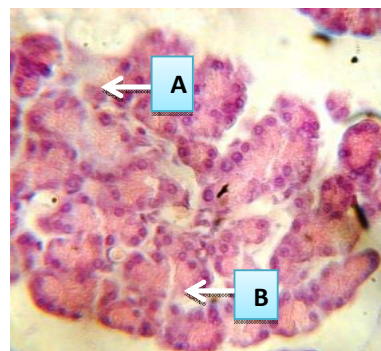


Plate 22= Diabetic rats treated with Metformin 50mg/kg. Haematoxylin and eosin. x 400 magnification
A- Less karyolysis and prominent islet cells appeared with mild enlargement in size and displayed increase in size and light hyaline changes in the majority of cells.
B- Improved pyknotic nuclei of some acinar cells with severely damaged vacuolated acini.

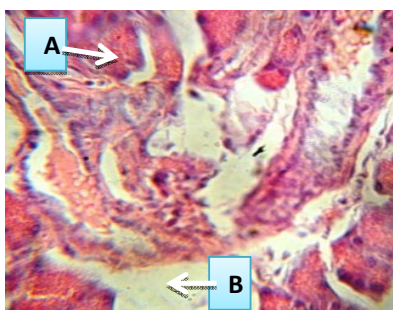


Plate 23= Diabetic rats treated with 250mg/kg of *Buchholzia Coriacea* seed aqueous Haematoxylin and eosin. x 400 magnification
A- Less karyolysis and prominent islet cells appeared with mild enlargement in size and displayed increase in size and light hyaline changes in the majority of cells.
B- Improved pyknotic nuclei of some acinar cells with severely damaged vacuolated acini.

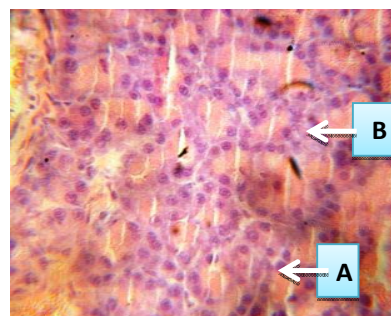


Plate 24= Diabetic rats treated with 250mg/kg of *Buchholzia Coriacea* seed ethanol. Haematoxylin and eosin. x 400 magnification
A- Less karyolysis and prominent islet cells appeared with mild enlargement in size and displayed increase in size and light hyaline changes in the majority of cells.
B- Improved pyknotic nuclei of some acinar cells with severely damaged vacuolated acini.

Discussion

Numerous advantageous effects of *Buchholzia coriacea* seed extracts have been identified in literature, earning it the name “wonderful kola”^{19&20}.

The body weight of normal and diabetic rats before induction and after of treatment is shown in figure I. There was a significant elevation in the body weight of normal control rats when compared baseline (0 days before induction) body weight to final (day 14 after treatment). The administration of Metformin (50mg/kg) and aqueous 250 mg/kg and ethanol

250mg/kg extracts also showed a moderate increase ($p>0.05$) in the body weight of normal rats as compared to day 0 and day 14 after treatment. There was a significant ($p<0.05$) reduction in the body weight of rats in the diabetic control group when compared baseline (0 days after induction) body weight to final (day 14 after treatment). The treatment with Metformin (50mg/kg) and aqueous 250 mg/kg and ethanol 250mg/kg extracts showed a significant decrease in the body weight of diabetic rats as compared baseline (0 days after induction) body weight to final (day 14 after treatment). There was no significant difference ($p>0.05$) in the body weight of normoglycemic treated groups when compared with the normoglycemic control group (normal control) before induction and after treatment. A mild decrease ($p<0.05$) in metformin diabetes treated group, aqueous extract diabetes treated group and ethanol extract diabetes treated group when compared to the diabetes control group

Figure II shows changes in glucose levels of normal and diabetic rats administered with Metformin (50mg/kg) and aqueous 250 mg/kg and ethanol 250mg/kg extracts in. Here, there was a mild change in fasting blood glucose level in normal control rats when compared with baseline (0 days before induction) body weight to final (day 14 after treatment). There was also a mild but not significant ($p > 0.05$) decrease in the metformin normoglycemic treated group, AEBC normoglycemic treated group and EEBC normoglycemic treated group when compared to the normal control group after day 14 treatment. The administration of Metformin (50mg/kg) and aqueous 250 mg/kg and ethanol 250mg/kg extracts also shows mild alteration ($p>0.05$) in the fasting blood glucose level of normal rats after day 14 treatment as compared to day 0. Findings from this study showed a significant ($p<0.05$) increase in the glucose level of diabetic rats when compared to a glucose level of the rats at the commencement (0 days before induction) fasting blood glucose level to day 4 (after induction) of the experiment ranging from 72.04 to 76.43%.

It was also observed that the values of a glucose level of the diabetic rats treated with Metformin (50mg/kg) and aqueous 250 mg/kg and ethanol 250mg/kg extracts were not up to the glucose level of diabetic control rats. Despite this, there was statistical significance ($p < .05$) in percentage changes between Metformin (50mg/kg) and aqueous ethanol (250mg/kg) extracts. The metformin treated group showed more effect ($-189.60\pm23.36^*c$), followed by ethanol extract treated group ($-161.96\pm9.81^*a$) and aqueous extract treated group ($-151.10\pm14.92^*a$) when compared to diabetic control rats (-109.78 ± 26.33).

Relevance of Study

Since diabetes became a global monster to earthlings, any study that is directed towards its amelioration is not only Jermaine, but hugely recommended. This study will provide basic information on histologic changes in pancreas, liver, and kidneys; being the major organs that directly or indirectly part-take in glucose homeostasis as a result of the graded-dose use of *Buchholzia coriacea* seed extracts. This implies that administration of this extract with dose and administration duration considered will be of being scientific merit in the folkloric use of the extract for management of this ailment.

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.

Recommendations

Being just a preliminary work, we recommend further study on this, however with more sophisticated and high-tech stains/equipment that can reveal deeper tissue structures and cellular components.

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