**ABSTRACT**

Diabetes a chronic condition that is characterized by elevated blood glucose level and various heart related diseases. The *Buchholzia coriacea* (Wonderful Kola) seed extract effect on cardiac tissue, pancreas and lipid-profile parameters in alloxan induced Wistar rats was evaluated. Mature fruits of Wonderful Kola were gathered and authenticated. The seeds were processed, dried, and extracted into a methanolic extract. Twenty-five albino rats were obtained, acclimatized, and randomly divided into five groups; The normal control group was fed with water and feed only, positive control group was induced diabetes with alloxan and was not treated throughout the study, the known standard drug group was induced and treated with metformin daily, while the test group was divided into low dose test group and high dose test group. The low dose test group was administered with 250mg/kg of *Buchholzia coriacea* (Wonderful Kola) and the high dose test group was treated with a high dose of 1000mg/kg of the *Buchholzia coriacea* (Wonderful Kola) plant extract for 14 days. After two weeks, animals were euthanized, and blood samples, pancreas and heart tissues were collected. The lipid profile examination revealed notable reductions in HDL cholesterol, along with elevated LDL cholesterol and Triglycerides in the alloxan-induced group, indicating toxicity (p < 0.05). Metformin partially restored these parameters, while both low
and high doses of Wonderful Kola extract exhibited beneficial effects, suggesting potential protection against alloxan (p < 0.05) revealing reduction in the elevated levels of LDL cholesterol and Triglycerides and increase in the HDL cholesterol. Differential counts and indices also showed positive responses to Wonderful Kola extract. Histological analysis revealed severe inflammation in the alloxan induced group, partial mitigation with metformin, and potential protective effects with Wonderful Kola, particularly at higher doses. Wonderful Kola extract demonstrated protective impacts on lipid profile parameters and cardiac tissue histology in alloxan induced group, indicating its potential as a therapeutic remedy. Further investigation is necessary to elucidate the underlying mechanisms and optimize dosage.

**KEYWORDS:** Alloxan, Cardioprotective, Pancreatic, Lipid and *Buchholzia Coriacea*.

**INTRODUCTION**

Diabetes has been recognized as the major risk factor for cardiovascular diseases (CVD) such as Atherosclerosis, Heart failure, Stroke and other related heart diseases. Epidemiologic and clinical data from the last 2 decades have shown that the prevalence of heart failure in diabetes is very high (WHO, 2021). Data from Triposkiadis, F et al., (2021) suggest that more than one-third of patients who are hospitalized for heart failure without a diagnosis of diabetes exhibit impaired fasting glucose or impaired glucose tolerance. Diabetes Mellitus is a major risk factor of the onset of Heart failure and structural heart disease through cellular, myocardial, and systemic processes. High blood sugar level from diabetes can lead to arterial hypertension, heart attack, and peripheral neuropathy and other heart diseases may result from this damage over time Triposkiadis, F et al., (2021). The aim of this study is to investigate the *Buchholzia coriacea* (wonderful kola) seeds extracts effects on some cardioprotective, pancreatic and lipid profile values in alloxan induced wistar rats. Triposkiadis, F et al., (2021) and Edo refer to it as "Owi". It has been discovered to have medicinal benefits for many years as herbal remedy to treat various ailments or disorders in the body all over the world. *Buchholzia coriacea* (wonderful kola) have numerous medicinal benefits, because of this significance, the seeds earned its popular name “Wonderful kola”. The seeds can be eaten raw or cooked. Studies have shown that the extract of *Buchholzia coriacea* has strong analgesic effects, when rubbed on the forehead, thereby curing migraines. Epa, C., (2019). Hyperglycemia increases the risk of diabetes and associated complications including heart
attack, stroke, kidney disease, limb amputations, poor vision, and nerve damage M. Piero et al., (2015).

**MATERIALS AND METHODS**

**Sample Collection**

The *Buchholzia coriacea* (wonderful kola) were purchased from the street of Eketa Community in Ahoada East Local Government area of Rivers State in the month of October and were authenticated at the herbarium of Plant Science Biotechnology department of the University of Port-Harcourt, Nigeria, where it had a specimen voucher number UPH/P/409 assigned to it. The pulp of *Buchholzia coriacea* (wonderful kola) were removed and its seeds were air dried for one week in order to remove moisture. Then, the seeds were sliced in to small bits, shade dried, grinded and stored in an air-tight container ready for extraction. The fine powder was immediately taken to the University of Port-Harcourt Pharmaceutical Laboratory for extraction into a methanolic extract. The extraction used in this process was cold maceration, which involved macerating 1392g of the powdered plant material in 3.5 liters of methanol, soaking it for 48 hours. It was filtered using Whatman No 1 filter paper. The resulting filtrate was concentrated to dryness using a rotary evaporator, under reduced pressure at a temperature of 60 degrees Celsius and then dried using a water bath at 50 degrees Celsius. The crude extract obtained, *Buchholzia coriacea* (wonderful kola) seed extract, was stored in airtight container in a refrigerator for screening. The weight of the obtained methanolic extract was determined, and the percent yield was calculated. The extract was highly soluble in water then was preserved in a refrigerator until use. A number of twenty-five (25) adult female albino rats were obtained from the Animal House of the College of Health Sciences, University of Port-Harcourt. All experimental animals were handled and housed in accordance with the guidelines of both the University’s ethical committee and the International Guidelines for Handling of Laboratory Animals. These twenty-five (25) adult male wistar rats (130–200 g) between the ages of five to eight weeks were housed in well-ventilated and disinfected cage with a perforated floor which contained saw dust as bedding in a controlled environment with 12 hours’ light and 12 hours’ dark cycle and a room temperature of 28 degrees in 60% humidity. The animals were acclimatized for two weeks (14 days) prior to commencement of the experiment. The animals were allowed to acclimate for seven days. Alloxan monohydrate was obtained from Sigma Aldrich Chemical Company, St. Louis, U.S.A. All other chemicals and reagents used were of analytical grade and were obtained from reputable scientific and chemical companies.
Metformin, each tablet of metformin was obtained from a pharmaceutical store in the University of Port Harcourt Teaching Hospital, Port Harcourt Nigeria. A digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) was used for the determination of the blood glucose levels of the animals.

**Animal Grouping and Treatment**

After acclimatization the animals were further grouped into five (5) groups

The twenty-five albino wistar rats of both sex were divided into five groups of five rats per groups and two was kept as backup for any unaccepted mortality. Animals were grouped and labelled a follows:

**Group 1:** The Non-diabetic control group, in this group the experimental animals were nondiabetic rats that received a normal feed and water.

**Group 2:** Diabetic control; the rats were induced with 150mg/kg of alloxan via Intraperitoneal injection. The experimental animals developed diabetes after alloxan injection as evidenced hence sustaining hyperglycemia and glycosuria 7 days after the induction.

**Group 3:** Non-diabetic treated received *Buchholzia coriacea* (wonderful kola) extract low dose of 250 mg/gram (not induced with alloxan but treated with *Buchholzia coriacea* (wonderful kola) 1ml).

**Group 4:** The experimental animals received 150mg/kg of alloxan via Intraperitoneal injection and treated with 1000mg/gram of *Buchholzia coriacea* (wonderful kola) extract.

**Group 5:** The experimental animals received 150mg/kg of Alloxan via Intraperitoneal injection and 100ml/gram Metformin as the standard drug. All the animals in the group received food and grounded pellets ad. Libitum.

**Induction of Alloxan on Experimental Animals**

The powdered form of Alloxan was dissolved in sterile normal saline to a concentration of 150mg to create an injectable form. The animal conducted an overnight fasting for sixteen (16) hours to eighteen (18) hours. The dissolved alloxan monohydrate was induced by a single intraperitoneal injection at a dose of 150mg/kg.

**Monitoring after Induction**

After twenty-four (24) hours administration, the induced rats were monitored for the development of diabetes. The blood glucose levels were checked by collecting blood
samples from the tail vein while using the glucometer to determine blood glucose level at intervals.

**Determination**

After monitoring at intervals, the induced-rats having fasting blood glucose level greater than 9mmol/L were considered to be diabetic.

**Phytochemical Screening**

The Phytochemical screening analysis was carried out to detect the phyto-constituents such as alkaloids, tannins, flavonoids, steroids, saponins, terpenoids, glycosides, etc from the sample.

**Test for Alkaloids:** About 0.3 gram of the dried powdered sample was warmed with 3 ml of 10% aqueous sulphuric acid and filtered. The filtrates were divided into three different test tubes.

- **Dragendroffs test:** Two drops of dragendroffs reagent were added into the first test tube containing a portion of the filtrate. A brick red precipitate coloration indicates the presence of alkaloid.
- **Meyer’s test:** Two drops Meyer’s reagent was added to the second portion of filtrate. A reddish brown precipitate coloration assures the presence of alkaloid.
- **Hager test:** To the third portion of filtrate, two drops of Hager’s reagent was added to it. The presence of reddish-brown precipitate coloration indicates the presence of alkaloid.

**Test for Anthraquinone**

**Test for free anthraquinone derivatives:** Procedure: 0.2 gram of the powdered crude material was put in a 100 ml conical flask. A 10 ml of chloroform was added and it was warmed gently on water bath (<40˚c) for 5 minutes with intermittent shaking at intervals. The mixture was filtered into a clean test tube after allowed to cool. To 2ml filtrate, 1ml of 10 per cent ammonia solution was added and shaken. A bright pink coloration in the upper aqueous ammonical layer indicates the presence of the free anthraquinones (Harborne, 1998).

**Test for the combined anthraquinone derivatives:** 0.2 gram of the crude material was transferred into 100 ml conical flask. A 10 ml of aliquot 10% sulphuric acid and 10 ml aqueous ferric chloride were added to it and boiled for 5 minutes with intermittent shaking at intervals. The filtrate was partitioned with equal volume of chloroform. The chloroform layer was collected into a test tube and 10% ammonia solution equivalent to half the volume of the
filtrate was added and was shake. A bright pink color in the upper aqueous ammonical layer confirms the presence of combined anthraquinones (Harborne, 1998).

**Test for Carbohydrate**

**Molisch Test:** 0.2 gram of the powdered sample was transferred into a test tube. A 5ml portion of distilled water was added to it and warmed on a hot water bath at 100˚c for 5 minutes with intermittent shaking. The mixture was allowed to cool, filtered into a clean test tube and 1ml of αnaphthol solution was added to it. In a slanting manner, 1ml of conc. H$_2$SO$_4$ was added down the test tube. A purple ring at the interface of the liquids indicates the presence of carbohydrates. **Fehling test for reducing sugar:** 0.2 g of the powdered material was put into a test tube and allowed to boil at 100˚c for 5 minutes on water bath and shake at interval. The mixture was filtered into a clean test tube. 0.1 ml of Fehling’s solution was added to the filtrate. A brick redcolored precipitate at the bottom of the test tube indicates the presence of free reducing sugar.

**Test for cardiac glycosides:** A 200 milligram of the powdered material was boiled in 95% alcohol for 2minutes and filtered after cooling. The filtrate was partitioned with 5ml of chloroform in a separating funnel. The lower chloroform layer was divided into small evaporating dishes and allow to dry.

**Keller-killiani test for De-oxo sugar:** One of the chloroform residues above was transferred into a test tube and was dissolved in 1ml of glacial acetic acid containing a trace of ferric chloride solution. A 0.4ml of conc. Sulphuric acid was carefully poured at angle 45˚c of the test tube. A reddish-brown color at the interface of the liquids indicates the presence of De-oxo sugar.

**Kedde test for De-oxo sugar:** A portion of methanol filtrate was mixed with Kedde’s reagent A, and then an equal volume of Kedde’s reagent B was added. A non-observance color which changes from violetto purplish-blue confirm the absence of a cardenolide aglycone.

**Test for triperpenoids:** 500 milligrams of the powdered *Buchholzia coriacea* sample was macerated with 10ml of anhydrous chloroform and filtered. The filtrate was divided into two equal portions.
Salkowski test: The first portion of the filtrate was mixed with 2ml of concentrated sulphuric acid carefully so that the sulphuric acid formed a lower layer. A reddish-brown coloration at the interface indicates the presence of a steroidal ring.

Liebermann-Burchard test: The portion of chloroform filtrate above was mixed with 1ml of acetic anhydride, followed by the addition of 1ml of concentrated Sulphuric acid to form a layer underneath. The formation of a reddish violet colouration at the interface of the two liquid and a green or violet coloration in the chloroform layer indicates the presence of triterpenoids.

Test for phenolic compounds
Ferric chloride test: 200 mg of the powdered sample was boiled in 50 ml of distilled water for 3 minutes on a hot plate, and filtered after cooling. A few drops of 10% ferric chloride solution were added to the filtrate. A blue or green color indicates the presence of phenolic compounds.

Test for Phlobatannins or condensed tannins
Hydrochloric acid test: 1 ml of the water extract of powdered seeds was boiled with an equal volume of 1% aqueous hydrochloric acid was added to it. A deposit of red precipitate indicates the presence of phlobatannins.
Gelatin test for tannins: To the water extract of powdered seeds a few drop 10% gelatin solution was added. A deposit white precipitate shows the presence of tannins.
Flavonoids: 500 milligramme of the powdered seeds material was extracted with distilled water and warm on a water bath and was filtered. The filtrate was divided into two portions.
Sodium hydroxide test: A 2 ml aliquot of 10% NaOH was added to an equal volume of the first portion. An intense yellow solution which disappear on addition of dilute hydrochloric acid confirms the presence of flavonoids.
Shinoda Test: A 200 mg of the grinded sample was extracted in ethanol by boiling in a water bath for 5 minutes, and filtered after allowing to cool. Four pieces of magnesium fillings was added, followed by 2 to 3 drops of concentrated hydrochloric acid. An orange to red-crimson color indicates the presence of flavonoids.

Test for Saponins
Forthing test: A 0.2 g of the grinded sample was extracted with 10 ml of distilled water and filtered in a clean test tube. The filtrate was shaken vigorously for 30 seconds. It was allowed
to stand for over half an hour after shaking. A persistent honey-comb froth indicates the presence of saponins.

**Emulsion Test:** A 0.2 g of the powdered seeds material was transferred into a 100 ml conical flask. A 10ml aliquot of normal saline was added and heated in a boiling water bath for 5 minutes and was shaken at intervals. After 5 minutes it was filtered into two clean test tube: R1 and R2. To R1, 2 ml of the filtrate solution was transferred into it while to R2 (negative control), 2 ml of normal saline was also transferred. 2 ml of the olive oil was added to R1 and R2 and they were mixed well. There is a presence of saponins when there is formation of emulsion in test tube R1.

**Cyanogenic glycosides:** A 0.2 g quantity of dried powdered of *Buchholzia coriacea* (wonderful kola) in an Erlenmeyer flask was properly moistened with water. A strip of sodium picric paper was placed inside without touching the moistened plant sample and corked. The flask was heated. A change in color of the sodium picrate paper inserted between a split on the cork stopper of flask from yellow to various shades of red shows the presence of Cyanogenic glycoside.

**Biochemical and Histological Examination**

Lipoprotein profile test involves the measurement of the levels of various types of cholesterol including LDL (low-density lipoprotein) cholesterol, HDL (high-density lipoprotein) cholesterol and the triglycerides. This test assesses the risk of heart diseases and it recommended as part of the cardiovascular risk assessment.

The pancreas and heart tissues collected were histologically examined following the standard procedure by Baker (1985), the Pancreas and cardiac of the rats were excised and fixed in 10% formal saline for 24 h. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome, and stained with hematoxylin and eosin (H and E). Microscopic examination was performed to evaluate cellular morphology, tissue architecture, and any pathological changes. Photomicrographs of lesions were taken with an Olympus photo microscope (Olympus Scientific Equipment, Ashburn, VA) for observations of the histopathological lesions.
RESULTS AND DISCUSSION

Table 1: Result of phytochemical screening on Buchholzia coriacea (wonderful kola).

<table>
<thead>
<tr>
<th>Screened phytochemical test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td></td>
</tr>
<tr>
<td>Dragendorff test</td>
<td>+</td>
</tr>
<tr>
<td>Mayer’s test</td>
<td>+</td>
</tr>
<tr>
<td>Hager’s test</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td></td>
</tr>
<tr>
<td>Fehling</td>
<td>+</td>
</tr>
<tr>
<td>Mollisch</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
</tr>
<tr>
<td>Shinoda test</td>
<td>-</td>
</tr>
<tr>
<td>AlCl₃ test</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td></td>
</tr>
<tr>
<td>Free anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Combined anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
</tr>
<tr>
<td>Frothing test</td>
<td>+</td>
</tr>
<tr>
<td>Emulsion test</td>
<td>+</td>
</tr>
<tr>
<td>Test for Cyanogenic glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Test for Phlobatanin</td>
<td>-</td>
</tr>
<tr>
<td>Test for Fixed Oil</td>
<td>-</td>
</tr>
<tr>
<td>Test for Steroid Salkowski</td>
<td>+</td>
</tr>
<tr>
<td>Test for cardiac glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Keller killer test</td>
<td>-</td>
</tr>
</tbody>
</table>

Kedde’s test -

Phenolic Constituent

Tannins: FeCl₃ test +

Test for triterpenoids

Lieberman test -

Key: + means positive
- means negative

Table 2: Effect of Buchholzia coriacea (wonderful kola) on lipid profile markers in alloxaninduced diabetes in Wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>T. CHOL (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>VLDL (mmol/L)</th>
<th>TG (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>2.40±0.30#</td>
<td>5.10±0.11#</td>
<td>1.18±0.08#</td>
<td>1.68±0.27#</td>
<td>4.60±0.10#</td>
</tr>
<tr>
<td>Negative control</td>
<td>6.01±0.44*</td>
<td>1.37±0.06*</td>
<td>3.45±0.07*</td>
<td>5.90±0.19*</td>
<td>9.20±0.92*</td>
</tr>
<tr>
<td>Metformin</td>
<td>3.40±0.24#</td>
<td>3.37±0.05#*</td>
<td>1.77±0.05#*</td>
<td>3.02±0.17#*</td>
<td>5.60±0.31#</td>
</tr>
<tr>
<td>Low dose WKE</td>
<td>3.27±0.18#</td>
<td>3.19±0.30#*</td>
<td>1.25±0.04#*</td>
<td>2.56±0.15#*</td>
<td>6.10±0.38#</td>
</tr>
<tr>
<td>High dose WKE</td>
<td>3.17±0.41#</td>
<td>3.32±0.20#*</td>
<td>1.33±0.14#*</td>
<td>2.38±0.35#*</td>
<td>5.78±0.45#</td>
</tr>
</tbody>
</table>
DISCUSSION

Alloxan is a chemical compound used in medical research, it is known to have cytotoxic effect on the β-cells of the pancreatic islets of Langerhans, which lowers the body's production of endogenous insulin and decreases the body's ability to use glucose. It functions by causing necrosis of the pancreatic beta cells via the generation of free radicals. Alloxan-induced diabetes model seems to be the best known drug induced diabetes as it appears to be the easiest, most reliable and most practicable method of inducing diabetes, although chemical induction of diabetes with streptozotocin is most widely used. The phytochemical screening of the aqueous seed extract of *Buchholzia coriacea* revealed the presence of Alkaloids, Tannins, Saponins, Terpenes, Steroids, cardiac Glycosides, Carbohydrate, Phenols and Resins (Table 1). There have been investigations on the hypoglycemic effects of flavonoids and alkaloids. Among these phyto-constituents are as well responsible for the glucose lowering ability of the aqueous seed extract of *Buchholzia coriacea* (wonderful kola) seen in this study.

Diabetes can significantly impact the lipid profile, individuals with diabetes often have abnormalities in their lipid profile characterized by elevated levels of triglycerides, low density lipid cholesterol and decreased level of high lipid cholesterol. This dyslipidemia contributes to the increased risk of cardiovascular diseases seen in people with diabetes. Result from the lipid profile analysis revealed significant increase in triglyceride and total cholesterol and a decrease in HDL levels in the diabetic control group compared to the normal control group. The low and high dose of wonderful kola extract administration resulted in lowering of these values significantly as seen in the diabetic treated group to near normal levels. High density lipid level decreased significantly in the negative control group compared to normal, extract administration.

Diabetes have significant impacts on the cardiac tissues and myocytes, overtime it can lead to a changes in the structure of the heart muscles and this can result to decreased and impaired heart function. The histological results revealed significant changes and differences from the various treatment given. In the normal control group, it showed a well-organized myocardial structure- this served as a baseline illustration. In contrast, the negative control group induced
with alloxan displayed hemorrhagic deposit and inflammatory cells infiltration within the myocardium tissue. The metformin treated group displayed a reduced inflammatory activities and vacoulation within the interstitium. This indicated a limited degree of protection effect of metformin on the diabetic cardiac muscles. The 250mg/kg Wonderful Kola treated group showed a moderate inflammation of the cardiac tissue indicating a possible protective effect. The 1000mg/kg *Buchholzia coriacea* (wonderful kola) treated group demonstrated an incredible protective effect showing a moderately reduced mononuclear activities and a near-normal appearance of cardiac tissue, demonstrating the possible effectiveness of a larger Wonderful Kola dose in reducing the pathogenic changes induced on by alloxan.

The Pancreatic histological examination of various treatments revealed several characteristic changes; the normal control group served as a baseline example, it demonstrated a well-organized myocardial structure showing arranged glandular acini, Centro acinar cells, interlobular ducts with a high number of beta cells. The negative control group showed a result in contrast to the normal, it displayed hypo cellularity of beta cells and a reduced size of the islet of Langerhans, indicating a degeneration of islet of Langerhans and beta cells. The metformin treated group demonstrated a regeneration of beta cells within the islets proving to be a potential effect to a degenerated beta cells. The 250mg/kg *Buchholzia coriacea* (wonderful kola) extract treated group showed a hyper cellularity of beta cells and increased size of the islet of Langerhans suggesting a potential use. Incredibly the 1000mg/kg *Buchholzia coriacea* (wonderful kola) extract treated group showed a near normal cellularity of the beta cells with the Langerhans proving a positive effectiveness in diminishing the pathogenic abnormalities brought about by alloxan.

**CONCLUSION**

The result above demonstrated that treatment with higher doses of *Buchholzia coriacea* (wonderful kola) can serve as better protective effects against the adverse effect of all oxan. The lipid profile examination demonstrated its treatment as better alternative in reducing high level of Low density lipid cholesterol and triglycerides and increasing High density lipid cholesterol. The histological examination also showed that *Buchholzia coriacea* can as well regenerate beta cells in the islet of Langerhans and reduce inflammation within the cardiac tissues there by preventing heart attack and stroke.
REFERENCES