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Histopathological effect of *Buccholzia coricea* on some liver and kidneys in alloxan-induced diabetic Wistar rats

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Abstract

Liver and kidneys are vital organs that play several important roles such as detoxification, storage of blood, blood filtration and purification, urine excretion and secretion of hormones among others. This study was conducted to determine the histopathological effects of *Buccholzia coricea* on the liver and kidneys of female wistar rats, Twenty-five female wistar rats were divided into five groups of five rats per group. The five rats in group one served as the normal control group and were fed with food and water without inducement and treatment. Test group two, the positive control were induced with alloxan (150 mg/kg) without any treatment throughout the study. Test group three, the known drug group were induced with diabetes (150 mg/kg) and administered metformin daily. Test group four were induced with diabetes and were treated with low dose of 250 mg/kg of *Buccholzia coriacea* seed extract daily throughout the study. Test group five were induced with diabetes and were treated with high dose of 1000 mg/kg of *Buccholzia coriacea seed extract* daily throughout the study for 14 days and the animal models were checked daily with glucometer to ascertain their blood sugar level before the animals were sacrificed and organs harvested and preserved for histological analysis. At the end of the 14 days, hematoxylin and eosin staining methods of histological methods were used. The results of the histology of the five groups showed that group one had normal liver and kidney tissues. Group two showed cytoplasmic vacoulation and cellular infiltration of liver but an inflammation of the kidney tissue. Group three had mild sinusoidal dilation in the liver and distention of the renal tubules with reduced inflammation of the kidney tissue. Group four had cytoplasmic vacoulation of liver and reduced inflammatory activities of the kidney tissue. Group five had mild cellular degeneration of liver, hemorrhage and inflammation of the kidney tissue. Thus, *Buccholzia coriacea* can improve the functioning of the liver and kidneys of a diabetic rats when it is consumed below 1000mg and its equivalent daily by mitigation of the histopathological alterations by alloxan-induced diabetes. *Buccholzia coriacea* is a natural panacea in the management of diabetes-related organ damage.

Keywords: Histopathological; Diabetic; Kidneys; Lipid and *Buchholzia Coriacea*

1. Introduction

Diabetes Mellitus (DM) has risen to become one of the most common disorder in developed and developing countries (Ukpabi, Amanoh, Esihe, *et al.*, 2019). Same authors opined that diabetes mellitus is characterised by hyperglycaemia [high blood sugar level] over a long period of time, due to the inability of pancreas to produce enough insulin or cells of the body not responding properly to the insulin produced. According to Gbagbeke *et al.* (2018), the primary 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, which when not attained results in an increase in blood glucose level with consequent derangement of carbohydrate, protein and fat metabolism and in long term cases, coronary arteries and cerebrovascular system diseases; renal failure, neuropathy, retinopathy and premature death ensues. Ifeanyi (2018) noted that despite the

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existing pharmacotherapy, it has been difficult to attain adequate glycaemic control amongst many diabetics due to the progressive decline in beta-cell function of the pancreas. Hence, the need to discover and develop more effective hypoglycaemic agents with minimum side effects on the heart, liver and kidney has become apparent. On the other hand, humans use roots, stem, leaves, flowers, and seeds as primary source of medicine, fibres, food, shelter, et cetera on daily basis Gbagbeke *et al.* (2018). Ifeanyi (2018) noted that the uses of plants in traditional medical practice have a long drawn history and is the mainstay of primary health care in most of the third world, with several plants being studied and found to possess anti-diabetic and anti-oxidant properties. According to Gbagbeke *et al.* (2018), the use of plants in the management of diseases and their complications is currently on the increase and notable among these plant seeds are the seeds of *Buccholziacoriacea*popularly known as "wonderful kola". As Gbagbeke *et al.* (2018) puts it, the seeds of wonderful kola are used traditionally for treating diabetes, hypertension, rheumatism, cold, cough and catarrh as well as in the prevention of premature aging and migraine headache. Equivocally, same author noted that the stem and barks of the tree exhibit high concentration dependent antibacterial and antifungal activity when subject to ethanol extract. Thus, Ifeanyi (2018) asserted that it is referred to as "Wonder plant", because almost every part of it has been found to be of medicinal importance, possessing antioxidant, antibacterial, antiviral, antifungal and anti-inflammatory properties. The aim of this study is to identify the histopathological effect of *Buccholzia coriacea* (wonderful kola) on liver and kidneyin alloxan-induced diabetes wistar rats. *Buccholzia coriacea* (wonderful kola) is well known for its medicinal benefits and it has been used to treat countless problems across Africa in countries and beyond (James, 2022). According to Izah, *et al.* (2018), the seed of the *Buccholzia coriacea* (wonderful kola) contain phytochemicals, bioactive, compounds, while the essential oil contains several chemical compounds that depict the medicinal properties of the plant. As a matter of fact, Lazarus (2019) asserted that it has been utilized to treat smallpox and cure skin itches and as well to alleviate sinusitis, nasal congestion and headache. Equivocally, Izah, *et al.* (2018) also noted that the wonderful plant have analgesic, anti-depressant, anti-malaria, anti-anxiety, anti-diabetes, anti-microbial, anti-oxidants, anti-helminthes, anti-ulcer, anti-inflammatory, anti-oxidants, hyperlipidemia, anti-hypercholesterolemic, anti-atherogenic, anti-trypanosomal, anti-modulatory, anti-spasmodic, anti-diarrhoea and anti-fertility activities. Same authors also claim that the plant is effective for the treatment of fever, cough, hypertension, headache, sinusitis, catarrh, small pox, scabies, chest pains, boils, syphilis, earache, headache, gonorrhoea, rheumatism, cold and catarrh.

2. Materials and Methods

2.1. Sample Collection

The *Buchholzia coriacea* (wonderful kola) were purchased from the street of Ula-Ikata 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 storedin 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 1392 g of the powdered plant material in 3.5 liter of methanol, soaking it l for 48 hours. It was filtered using Walt man 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 acclimatize 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.

Table 1 A table showing the groups, concentration of alloxan-inducement and treatment

Groups	Class	No. of rats	Alloxan inducement	Treatment (mg/kg)
1	Control	5	NIL	NIL
2	Positive control	5	150	NIL
3	Known Drug control	5	150	150 mg/kg of metformin
4	Low dose	5	150	250 mg/kg of seed extract
5	High dose	5	150	1,000 mg/kg of seed extract

2.2. Hematoxylin and Eosin Staining Methodology

The female wistar rats were anaesthetized under the influence of chloroform vapour and dissected. After dissection, liver and kidney tissues were removed and immediately fixed. The tissues were trimmed down to a size of 3mm x 3mm thick. For every study of sections under microscope, the tissues were passed through several processes which included:

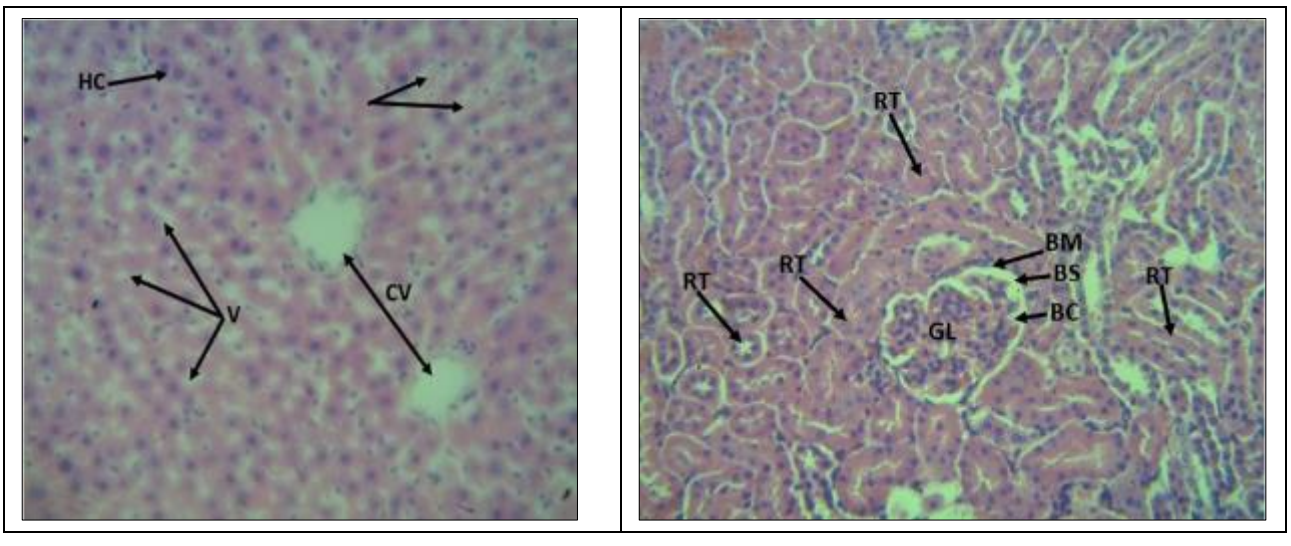
- **Fixation**: Fixation was carried out using formal saline fluid for four hours. After fixation, the tissues were washed over night under a stream tap water.
- **Dehydration**: Dehydration of the fixed tissue was done to remove water from the tissue using 50%, 70%, 90%, 95% and absolute alcohol.
- **Clearing**: After dehydration, tissues were cleared in xylene for two hours. This was aimed at removing the alcohol from the tissues.
- **Impregnation**: This removed trace of the clearing agent in the tissues. The tissues transferred from xylene to solution of molten paraffin was at a temperature of 60^{o}C for two hours each in two changes.
- **Embedding**: Tissues are immersed in molten paraffin wax at a temperature of 60^{o}C and allowed to solidify, Metallic embedding molds were used for this process. After embedding, the tissue blocks obtained were cast into wooden works. This supported the tissues and made them easier for cutting.
- **Sectioning**: The cast tissue blocks were taken to a microtome for sectioning. Sectioning ribbons ranging from 2 microns to 5 microns were floated in warm water bath of about 37°c and the best ribbons were picked with forceps and placed on slides. These slides were labelled using diamond pencil and transferred to slide racks. These were put in an embedding oven for one hour (1 hour), this was done to make the wax on the slides to melt and also to keep the sections on the slides to melt and also to keep the sections on the slides warm.
- **Staining**: Haematoxylene and Eosin (H & E) was used to stain the tissues and the slides was sent to histopathology laboratory for and evaluation of histological changes and the procedure are as follows: Xylene was added for 5 to 10 minutes. The slides were transferred to absolute alcohol, then to 95% alcohol and finally to 70% alcohols in seconds before it was rinsed in water. Then it was stained with Haematoxylene for 15 to 20 minutes and rinse in water to remove excess stain before it was differentiated in 1% acid alcohol for 5 seconds so that excess stain was removed and enhanced the nucleus to absorb the stain. It was then rinsed in two changes of water for 3 to 5 seconds by a process called bluing and it gave the stained tissue its characteristic background. The slides were stained with eosin for 5 to 10 minutes and rinsed in water to remove excess stain before it was dehydrated in absolute alcohol. After that, the slides were mounted with DPX and cover slip and views under the microscope.

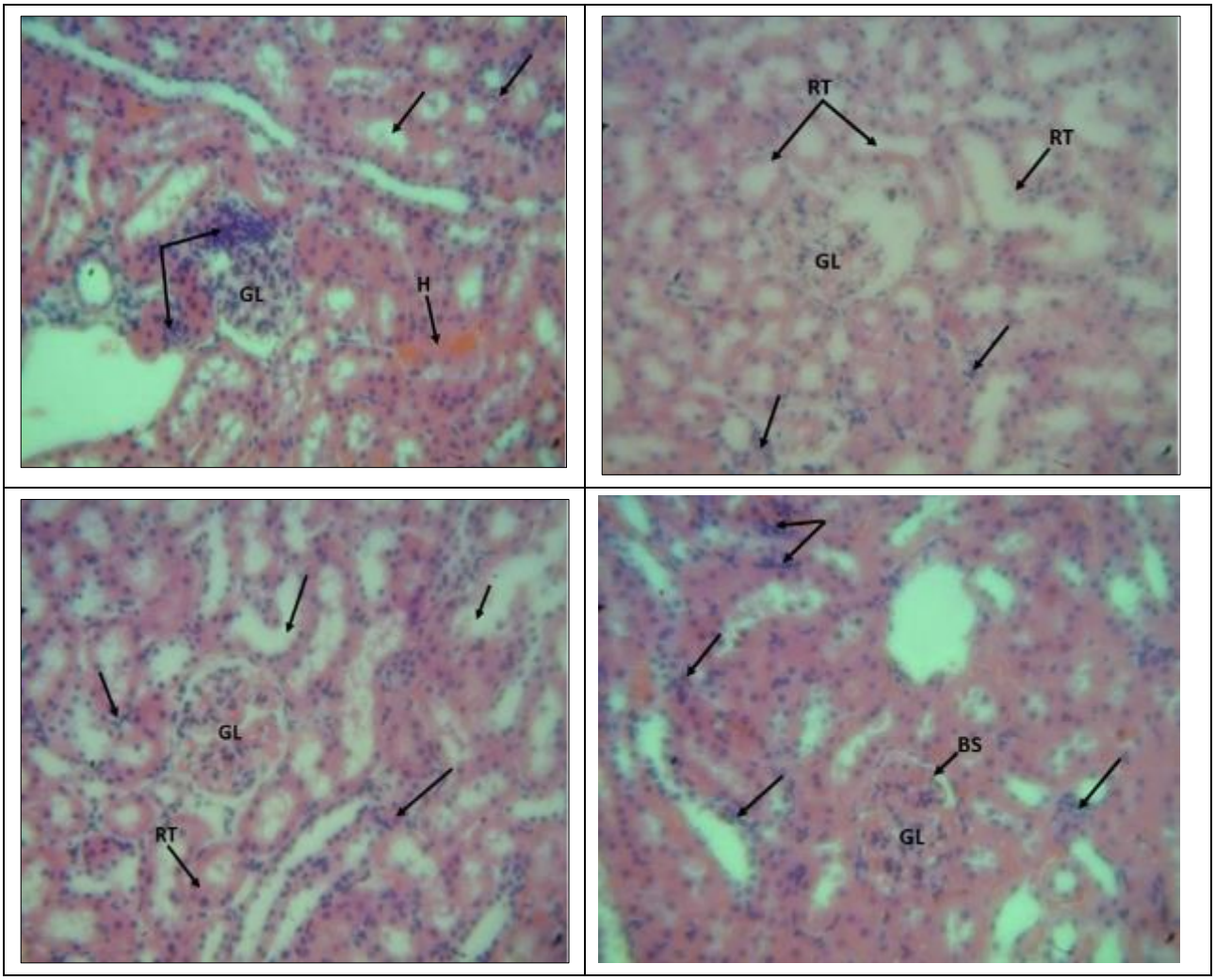
3. Results

Table 2. The result on the phytochemical screening of wonderful kola (*Buccholziacoriacea*)

S.N	Chemical constituents	Results
1	Alkaloid	
	Dragger droff	Positive
	Hager	Positive
	Meyer	Positive
2	Carbohydrates	

	Fehling	Positive
	Molisch	Positive
3	Sapolin	
	Frothing	Positive
	Emulsion	Positive
4	Phenolic constituent	
	$FeCl_3$	Negative
5	Flavonoids	
	Slinode	Negative
	$AlCl_3$	Negative
6	Cynogenicglucosides	Positive
	Phlelobatanin	Negative
	Fixed oil	Negative
7	Arthroquinone	
	Free	Negative
	Combined	Negative
8	Triterpenoid	
	Lieberman	Negative
9	Steroids	
	Salwoski	Positive
10	Cardiac glucoside	
	Kedde	Negative
	Killer-killani	Negative





Figures 1 Results on the Histopathological Examination of the Kidneys of wistar rats

4. Discussion

After the histopathological examination of both the liver and kidneys of the wistar rats from the five groups, the photomicrograph of the liver of wistar rats samples in the normal control group showed regressive changes of the hepatocytes with mild sinusoidal dilation which was an appearance and indication of a normal liver tissue and this result is in line with the findings Lenka *et al.* (2016) and Adisa *et* al. (2011). The reason was because the rats of the normal control group were not induced with diabetes, a disease insufficient insulin secretion due to liver dysfunctionality. However, the liver photomicrograph from the sampled wistar rats belonging to the positive control showed diffused cytoplasmic vacoulation within the liver parenchymal with visible central vein (CV): hepatocytes (HC), and sinusoids (SS) and mild cellular infiltration (ICI) and cytoplasmic vacoulation within the liver parenchymal. This opined that there were cytoplasmic vacoulation and cellular infiltration.of the liver tissue. This reason could be the inducement of diabetes without treatment.

The liver of the metformin control group (figure 1a and 1b) showed regressive changes of the hepatocytes with mild sinusoidal dilation (SS) and portal triad; tissue appears normal with evenly diffused hepatocytes in the hepatic lobules (HC), with mild sinusoidal dilation (SS). The mild sinusoidal dilation is an indication of a normal liver tissue. This was as a result of the 250mg of metformin administered to treat the rats after the inducement of diabetes because metformin has a high rate of 40.7% of blood glucose reduction (Adeniji et al. 2016) and it is a drug of choice for type 2 diabetes (Ismail *et al.,* 2015). The result of this study is similar to the research of Almuttairi, S.R. (2023) that revealed that metformin have significant blood glucose lowering properties and the capacity to protect several organs from the negative consequences of diabetes through the inhibition of hepatic gluconeogenesis, anti-glucagon activity, and insulin-sensitizing effect, hence, an effective hypoglycemic drugs. The result of the low dose group fed with 250mg of the extract of the plant extract after diabetes induction showed that there was diffused cytoplasmic vacoulation surrounding the

hepatocytes with mild haemorhagic deposit in the liver tissue purporting cytoplasmic vacuolation while a few showed regressive changes of the hepatocytes with mild cytoplasmic vacoulation and sinusoidal dilation (SS) which is the appearance of a normal liver tissue. *Buchholzia coriacae* contains flavonoid and alkaloid which has been discovered to have hypoglycemic effects (Adisa *et al,* 2011). A study carried out by Lapshak *et al* (2016) on alloxan induced diabetic rats showed that Buchholzia coriacae is anti-hyperglycemic. In the study of Adisa *et al.* (2011) *Buchholzia coriacae* showed a significant anti-hyperglycemic activity but glucose levels remained within the normal range for normal rats treated with the *Buchholzia coriacae*. Thus, indicating that the aqueous extract did not cause any alteration in glucose levels of normal rats which are in line with the observation from this current study. Also, Okoye *et* al. (2012) affirmed that *Buchholzia coriacae* anti-hyperglycemic activity. Differentially, the high dose group fed with 1000mg of the extract of the plant extract after diabetes induction showed regressive change of the hepatic lobules: mild hepatocyte drainage via the sinusoid into the bile duct; diffused cytoplasmic vacoulation, thus, implying mild vacuolation where as some other of same group showed that the centrilobar area of the central vein (CV): mild hepatocytes (HC) degeneration and sinusoidal dilation which indicate mild cellular degeneration and this findings are consistent with the work of Lenka*et al.* (2016). The reason for cellular degeneration of some hepatocytes was because *Buccholzia coriacea* potentiates insulin from beta cells, increase peripheral glucose uptake, decrease the absorption of sugar from the intestinal gut or by decrease the release of glucose from the liver, thus, a consistent high dose of *Buccholzia coriacea* can lead to cellular degeneracy. From this study, the phytochemical contents from the phytochemical screening of wonderful kola *Buccholzia coriacea* are Alkaloids, Carbohydrates, Saponins and Cardiac glycosides which is in line with the study of Lenka *et al*. (2016) that revealed Alkaloids, Flavonoids, Tannins, Saponins, Terpenes, Steroids, cardiac Glycosides, Carbohydrate, Phenols and Resins as phytochemical contents of *Buccholzia coriacea* and it is also consistent with the research by Ibrahim and Fagbohun (2012) where they showed that *Buchholzia coriacea* seeds contain high percentage of carbohydrate, protein and fat. The hypoglycaemic effects of alkaloids and flavonoids have been reported Lenka *et al.* (2016) that opined that some of these phyto constituents among others are presumably responsible for the glucose lowering ability of the aqueous seed extract of *Buchholzia coriacea* seen in this study.

5. Conclusion

In conclusion, wonderful kola (*Buccholzia coricea*) from studies shows it improves the integrity of the organs. It boosts the organized architecture of hepatocytes of the liver and alleviate cellular damages, hepatic steatosis and inflammation through its hepatoprotective effects. This medicinal plants improves renal function by reducing glomerular hypertrophy, tubular necrosis, interstitial inflammation and fibrosis. The therapeutic relevance of wonderful kola in cardiac histopathology is significant in decreasing myocardial fibrosis and hypertrophy. Thus, *Buccholzia coriacea* can improve the functioning of the liver and kidneys of a diabetic when it is consumed below 1000mg or its equivalent daily. *Buccholzia coriacea* mitigates the histopathological alterations induced by alloxan-induced diabetes. Therefore, wonderful kola is a natural panacea in the management of diabetes-related organ damage. Alkaloids, Carbohydrates, Saponins and Cardiac glycosides are the phytochemical contents of *Buccholzia coriacea*.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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