



(RESEARCH ARTICLE)



Effects of *Buchholzia coriacea* (wonderful kola) on hepatoprotective, nephroprotective and oxidative stress of aluminium chloride induced wistar rats

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Abstract

Buchholzia coriacea seeds has been reported to have various medicinal properties. This study evaluates the effect of the aqueous extract of *Buchholzia coriacea* seed (wonderful kola), on Aluminium chloride induced liver toxicity in adult male Wistar rats. 24 rats weighing between 150 g - 210 g were divided into five groups. Group A- (the control group) received water and animal chow. Group B received 200mg/kg of AlCl₃ only, Group C received aluminum chloride and vitamin C for 14 days. Group D received 250 mg/kg body weight (Low Dose) of aqueous extract *Buchholzia coriacea* and (200 mg/kg) AlCl₃. Group E received 1000mg/kg body weight (High Dose) of aqueous extract of *Buchholziacoriacea* and (250mg/kg) AlCl₃. The animals were sacrificed, the liver and kidney was excised and processed for routine paraffin sections at 5micromes thick. Sections were stained with Haematoxylin and Eosin to demonstrate histoarchitecture. Biochemical analysis was also carried out to determine the effects on liver and kidney antioxidant enzymes. Histological assessment showed narrowed central vein, degeneration of hepatocytes and distortion of sinusoids in the AlCl₃ alone treated groups. The histoarchitecture of the liver in the other groups showed improvement especially in Group E (High Dose of *B. coriacea*) and Group C (vitamin C and aluminum chloride only). The biochemical analysis on the other hand showed significantly reduced activities in GSH, SOD, CAT and significantly increased level of MDA in the AlCl₃ only group. In the other groups, MDA levels reduced, GSH, SOD and CAT also increased enzymatic activities following the administration of *B. coriacea*. The study concludes that *Buchholziacoriacea* seed extract plays a role in protecting liver and kidney from AlCl₃ toxicity.

Keywords: *Buchholzia Coriacea*; Hepatoprotective; Aluminum Chloride Nephroprotective; Oxidative Stress

1. Introduction

The patronize of traditional medicine attendants is associated to low income size, unavailability of medical facilities to the inhabitants of rural dwellers in many developing countries. Furthermore, (Kalunta, 2017) also attributed the use of herbal remedies to inaccessibility of modern drugs probably due to economic factor. Medicinal plants have been widely been use as stimulants, analgesic, anti-pyretic, anti-inflammatory, anti-convulsant, anti-microbial, anti-malaria, anti-leukemia, anti-hypertensive, anti-platelet, anti-oxidant, anti-tumor, anti-asthmatics, anti-inflammatory, anti-diarrhoea, anti-spasmodic, anti-depressants, anti-rheumatism, immunomodulatory, anti-epilepsy, anti-convulsant, anti-thyroids, hepato-protective, anti-apoptotic, anti-metastatic, anti-mutagenic/anti-tumor, anti spermatogenic, anti-colon toxin, pesticidal efficacy against insect, treatment of skin diseases, catarrh, headache, ear ache, memory booster, cough and eye effects. Medicinal plants have also been applied in the management of diabetes (Kalunta, 2017; Kigigha and Kalunta, 2017). The efficacy of some medicinal plants has been confirmed against specific ailment. While traditional medicine

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practitioners also claim that some of the plants have specific activities which have not been scientifically proven. Some of the medicinal plants can be used to treat more than one disease condition. In addition, some are also major food resources to humans. Some medicinal plants that are taken have some traditional backing. For instance, several species of Kola nut is used to receive visitors in several part of Nigeria. Furthermore, some Kola used in Nigeria has been validated to have medicinal plants against some ailment. Some of this Kola nut include *Garcinia kola* (bitter kola) (Ezeigbo et al., 2016), *Buchholzia coriacea* (Wonderful Kola) (Nwachukwu et al., 2014; Eze et al., 2015; Umeokoli et al., 2016), *Cola nitida* and *C. acuminata*. For instance, Buba et al. (2016), Faphonda et al. (2017) have reported that *Garcinia kola* have anti-oxidant, anti-microbial, anti-inflammatory, anti-hypercholesterolemic, anti-viral, anti-diarrhoea, anti-proliferative, anti-androgenic and anti-coronary activities. Like, nutmeg, clove, cinnamon, lime, garlic, moringa and pepper fruit, wonderful kola have been used as alternative medications to promote good health in Nigeria through treatment of different ailments. This practice is still on till date (Ejikeugwu et al., 2014). The aim of this study is to assess the hepatoprotective, nephroprotective and antioxidant effects of wonderful kola in aluminium chloride-induced toxicity in wistar rats, and to explore the potential mechanism of action of wonderful kola in relation to its protective effects. Several decades ago psychiatric conditions are managed with herbs, and it was discovered that some patients showed significant relief from severe psychotic depression. Approximately 70% of the depressed patients respond to treatment using available therapies but with some level of disappointmen. The pathophysiology of depression is associated with the deficiency of one or more monoamines among psychiatric patients (Onasanwo et al., 2016). Diarrhoea is one of the major disease conditions that cause morbidity and mortality (Kalunta, 2017). Diarrhoea is caused by diverse group of organisms including viruses, fungi, parasites and bacteria (Nguyen et al., 2006). Some of the bacteria associated diarrhoea includes *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia enterocolitica*, *Vibrio cholera*, diarrheagenic *E. coli* pathotypes (Varela et al., 2015). Diarrhoea is transmitted from person-to-person and consumption of foods and water contaminated with human fecal materials with a pathogenic strain (Ali et al., 2014; Gautam et al., 2015).

2. Materials and Methods

2.1. 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 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.

2.2. Administration of Aluminum Chloride

At the end of the acclimatization, the animals will be randomly selected into 5 groups (Group A-E)(n-5) 10 % of Aluminum chloride solution will be made (Ig Aluminum chloride 100ml of distilled water), 2mls of the drug(Aluminum chloride solution) would be administered to the experimental group C-E for a week and group B for 21 days to enablethe

determination of Toxicity and oxidative effects on experimental animals. After these processes, the rats will be observed for one week, at the end of which the proper administration of plant extract commences.

2.3. Administration of Extract

A total of Twenty-five (25) Albino Wistar rats consisting of five (5) groups with five (5) animals in each group were used for the study. Administration of extract and aluminum chloride commenced after one week of acclimatization. The experimental process lasted for 3 weeks (21days). The administration process of extract and aluminum chloride are as shown below;

Group	Identity	Treatment
Group 1	Normal Control	Nil
Group 2	Positive Control	Nil
Group 3	Vitamin C	AlCl ₃ + Vitamin C for 14 days
Group 4	Low Dose	AlCl ₃ + 250 mg/kg of <i>Buchholzia Coriacea</i> for 14 days
Group 5	High Dose	AlCl ₃ + 1000 mg/kg of <i>Buchholzia Coriacea</i> for 14 days

The rats were sacrificed on the last day of the third week of the experiment with the help of anesthesia by an incision made on the midline of the ventral surface of the rats with the heart exercised and blood samples collected from the jugular vein.

2.4. Histological Analysis and Photomicrography

Pieces of the liver, kidney were fixed and embed in 10% formalin, Sections of 5 µm thickness were obtained using a rotary microtome before staining using haematoxylin and eosin. Histological slides were viewed using a digital microscope with objective lens x 400. Slides micrograph were viewed and photomicrographically taken at objective lens x 40.

2.4.1. Biochemical analysis

Tissue samples from liver, kidney were taken for determination of values of liver antioxidant; malondialdehyde (MDA), reduced glutathione (GSH) levels, and the activities of Superoxide dismutase (SOD) and catalase (CAT).

2.4.2. Estimation of MDA

This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm, an MDA standard was used to construct a standard curve against which readings of the samples were plotted.

2.4.3. Estimation of GSH

The method is based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit.

2.4.4. Determination of tissues CAT

It is assayed by the method of Sinha which based on formation of chromic acetate from dichromate and glacial acetic acid in presence of hydrogen peroxide, chromic acetate that was produced and measures colorimetrically at 570 nm, one enzyme unit was defined as the amount of enzyme which catalyzed the oxidation of 1 µmole H₂O₂ per minute under assay conditions.

3. Results

Table 1 Effect of methanol extract of *Buccholzia Coriacea* on kidney function of albino rats

Parameters	Normal Control	Positive Control	Vitamin C (Known Drug)	Methanol Extract
Urea (mmol/L)	4.57±0.77	3.530 ± 1.40	3.13 ± 1.50	4.58 ± 1.02
p-value	-	0.4448	0.4148	0.9939
Creatinine (Umol/)	81.51 ± 5.34	89.33 ± 27.43	131.91 ± 62.57	177.9±52.85
p-value	-	0.7867	0.3572	0.0543

Values are measured in mean±SEM = p< 0.05*

Table 2 Effect of methanol extract of *Buccholzia Coriacea* on Liver Function test of albino rats.

Parameters	Normal Control	Positive Control	Vitamin C	Methanol Extract
AST	59.86±10.79	89±0	89±0	89±0
ALT	67.57±8.89	52.83±14.8	41.6±21.4	49.6±15.14
p-value	-	0.03	0.03	0.03
p-value	-	0.3958	0.2378	0.3006
T.protein	19.63±2.91	22.84±1.52	24.09±1.34	23.47±1.26
ALK.Phos(u/L)	49.25±0.21	33.78±8.79	45.68±2.87	37.56±8.12
p-value	-	0.3732	0.2515	0.3168
Albumin (g/dL)	5.53±0.32	5.49±0.31	5.24±0.41	5.06±0.46
p-value	-	0.911	0.7132	0.5114
T. Bilirubin (Umol/L)	1.06±0.15	0.50±0.08	0.50±0.24	0.23±0.06
p-value	-	0.110	0.1212	0.3141
Conj. Bilirubin	0.42±0.14	0.39±0.1	0.29±0.15	0.34±0.07
p-value	-	0.1211	0.4211	0.5660

Values are measured in mean± SEM = P < 0.05 *

4. Discussion

The seed extract of *B. coriacea* is reported to be hepatoprotective and to also possess antioxidant properties (IbiamUdu, Ugwuja Emmanuel et al.; 2022). Studies have also shown that the hepatotoxic effect of aluminium chloride is mediated by the generation of free radicals via oxidative stress, (Abdel et al.; 2014). Hence, the present study investigated the protective effects of *Buccholzia coriacea* against Aluminium Chloride induced toxicity in the liver of adult male wistar rats via biochemical analysis of antioxidant enzymes and histological analysis of the liver. Oxidative stress is the disturbance that exists between the balance in free radical generation and antioxidative enzymes and molecules, in favor of radical production (Abdel et al.; 2014). Lipid peroxidation is a known hallmark of oxidative stress. The free radical mediates a chain reaction which results in oxidative deterioration of polyunsaturated lipids with malondialdehyde (MDA) as one its major toxic by-products. Accordingly, malondialdehyde is used as a biomarker of oxidative stress induced lipid peroxidation. The level of lipid peroxidation increased by the aluminum chloride administration clearly shows an imbalance between pro-oxidant and antioxidant system, which automatically induces oxidative stress. The increase in MDA in the liver, as recorded in the present study, could be due to the resultant increase in the production of free radicals like hydrogen peroxide and hydroxyl radicals in the liver of aluminum chloride treated rats. The value for MDA in the AlCl₃ group is higher and statistically significant than the other groups. The increased lipid peroxidation as a result of AlCl₃ administration is in line with the observation of other authors.(IbiamUdu and Ugwuja Emmanuel et al; 2022)(Abdul wahab et al; 2014).The aqueous seed extract of *Buccholzia coriacea* may have

attenuated the AlCl₃ induced increase in liver homogenate concentrations of MDA due to its antioxidant potential (Egba et al.; 2022)(Okolie et al.; 2019). In this present study elevated levels of malondialdehyde (MDA) but reduced levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in the liver samples of aluminum chloride (AlCl₃) - treated rats was recorded. The significant decrease recorded in the activities of liver antioxidant protein and enzymes (GSH, SOD and CAT) reflect the failure of antioxidant defense system to overcome the influx of ROS precipitated by AlCl₃ administration. These observations are in accordance with (Kumar, et al.,2009) who observed significant drop-in activities of SOD, GSH and CAT after AlCl₃ treatment. The glutathione peroxidase system comprises of several components, one of which is GSH. GSH, is a very essential component of the oxidative system. It serves as a cofactor for glutathione transferase, which is known to help in the removal of certain drugs, chemicals and other reactive molecules, from cells by Ebrahim and Kambiz. Moreso, GSH can interact directly with certain ROS, i.e., hydroxyl radicals to detoxify them, as well as performing other critical activities in the cell. Hence, GSH may probably be the most important antioxidant molecule present in cells. In our study GSH was lowest in the AlCl₃ treated group, but it increased significantly in the groups involving *Bucchozia coriacea* seeds (wonderful kola) administration. Aluminum might also affect GSH synthesis by decreasing glutathione-synthase activity, which would result in reduced GSH levels (Kumar et al.; 2009). A study carried out by (Ore et al.; 2019) showed significant amelioration of liver function markers and levels of pro-inflammatory proteins by Hydroethanolic extract of defatted Buchholziacoriacea seeds (HEBCS). Histopathological studies showed a reduction in inflammatory cells and improvement in liver structure in animals treated with HEBCS. Hydroethanolic extract of defatted *Bucchozia coriacea* seeds (HEBCS) also protected against High Fat Diet-induced inflammation, oxidative stress and hepatocellular damage (Ore et al.;2022). The enzymatic antioxidant defense system, which includes SODs and CATs, can decompose superoxide and hydrogen peroxide in the cells and are the main defense against oxidative injuries. SOD catalyzes the rapid removal of superoxide radicals, generating H₂O₂. Therefore, SOD works in collaboration with H₂O₂ removing enzymes (Orihuela et al.; 2005). The level of Superoxide dismutase (SOD) also decreased significantly in the AlCl₃ only treated groups, and like GSH the administration of *Bucchozia coriacea* increased the levels of SOD. CAT is present in the peroxisomes of nearly all aerobic cells and functions to protect the cell from the toxic effects of hydrogen peroxide through catalyzing its decomposition into molecular oxygen and water without the production of free radicals Al-Hashem.⁴ In this study the values for catalase (CAT) in all the groups are not statistically significant except for the Wonderful Kola and Aluminium Chloride, only groups. Also, treatment with the extract was able to ameliorate altered liver function.

5. Conclusion

In conclusion, the present study has provided supportive evidence that the oral administration of Aluminium chloride in male rats at a dose of 250 mg/kg body weight daily for a period of 30 days induces hepatic, nephrotic dysfunction as evidenced in significant alterations in some biochemical and histological parameters. The use of aqueous extract of *Bucchozia coriacea* seeds in combination with Aluminium chloride was observed to attenuate some of the harmful effects of this element. Therefore, supplementation with *Bucchozia coriacea* seeds may prove useful as protective therapy against the hepatotoxic effects of Aluminium chloride. The *Bucchozia Coriacea* (wonderful kola) has been scientifically found to have analgesic, anti-depressant, anti-plasmodial, anti-anxiety, anti-diabetes, anti-inflammatory, anti-oxidants, anti-hypercholesterolemic, anti-atherogenic, anti-trypanosomal, anti-modulation, anti-spasmodic, anti-diarrhoea, anti-ulcer, anti-helmintics, anti-fertility activities. The scientific studies validates the claim of traditional medicine practitioners on the potency of wonderful kola. Therefore, further studies need to be carried out to elucidate on the mode/mechanisms of action, the dose that is required, purification of the chemical constituents, most suitable solvent to be used for extraction, age of the plants for the leave extract, and environmental conditions suitable for cultivation of wonderful kola with regard to its nutritional values. Also, studies need to be focused on effect on stimulant, tonic, aphrodisiac, fever, cough, hypertension headache, sinusitis, and catarrh, small pox, scabies, chest pains, boils, syphilis, earache, headache, gonorrhoea, rheumatism, cold and catarrh potentials of the wonderful plants so as to validate its efficacies as widely claimed by traditional medicine practitioners.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

- [1] Abdel Wahab WM. Aluminium chloride-induced toxicity and oxidative stress in liver of male rats: Protection by melatonin. *Life Science Journal*. 2012;9(4):1173–1181.

- [2] Ali, M. M. M., Ahmed, S. F., Klena, J. D., Mohamed, Z. K., & Moussa, T. A. A. (2014). Ghenghesh KS, Enteroaggregative *Escherichia coli* in diarrheic children in Egypt: molecular characterization and antimicrobial susceptibility. *J Infect Dev Ctries*, 8(5), 589-596. <https://doi.org/10.3855/jidc.4077>
- [3] Buba, C. I., Okhale, S. E., & Muazzam, I. (2016). *Garcinia kola*: the phytochemistry, pharmacology, and therapeutic applications. *International Journal of Pharmacognosy*, 3(2), 67-81.
- [4] Egba SI, Famurewa AC, Omoruyi LE. *Buchholziacoriacea* seed extract attenuates mercury-induced cerebral and cerebellar oxidative neurotoxicity via NO signaling and suppression of oxidative stress, adenosine deaminase and acetylcholinesterase activities in rats. *Avicenna J Phytomed*. 2022;12(1):42–53
- [5] Ejikeugwu, C., Umeokoli, B., Iroha, I., Ugwu, M., & Esimone, C. (2014). Phytochemical and antibacterial screening of crude extracts from leaves and wonderful kola. *American Journal of Life Sciences*, 2(6), 9-12.
- [6] Eze, J. I., Ekelozie, C. F., & Nweze, N. E. (2015). Immunomodulatory Activity of the Methanolic Extract of *Buchholzia coriacea* Seeds on *Trypanosoma brucei* Brucei Infected Mice. *Global Veterinaria*, 14(4), 582-588.
- [7] Ezeigbo, O. R., Ejike, E. N., Nwachukwu, I., & Ejike, B. U. (2016). Comparative antibacterial activity of methanolic, ethanolic, and aqueous extract of *Garcinia kola* (bitter kola) and *Cola nitida* (Kola nut). *International Journal of Plant Research*, 6(3), 53-56.
- [8] Fapohunda S., Awoyinka A., Oluchi A., Banji A., David J., Aderiike A., Abimbola A., and Adebayo O. (2017). Evaluation of the nutraceutical potentials of *Garcinia kola* seed. *J. Pharm. Phytochem*, 6(5), 1894-1901.
- [9] Gautam K., Pokhrel B.M., Shrestha C.D., and Bhatta D.R. (2015). Prevalence of Diarrheagenic *E.coli* (DEC) Determined by Polymerase Chain Reaction (PCR) in Different Tertiary Care Hospitals in Nepal. *JSM Microbiology*, 3(2), 1021.
- [10] IbiamUdu, Ugwuja Emmanuel. Antioxidant effect of *Buchholziacoriacea* ethanol leaf extract and fractions on freund's adjuvant-induced rats. 2022;59(1):31–45.
- [11] Kalunta C.G. (2017). Antimicrobial effect of different seed extracts of *Piper nigrum* against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. *Biotechnol Res*, 3(3), 71-76.
- [12] Kigigha L.T. and Kalunta C.G. (2017). Antimicrobial efficacy of leaf extracts of *Piper nigrum* against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. *Journal of Basic Pharmacology and Toxicology*, 1(2), 32-36.
- [13] Kumar A, Dogra S, Prakash A. Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: Possible behavioral and biochemical alterations in rats. *Behav. Brain Research*. 2009;205(2):384–90.
- [14] Nwachukwu M.I., Duru M.K.C., Amadi B.A., and Nwachukwu I.O. (2014). Comparative evaluation of phytoconstituents, antibacterial activities and proximate contents of fresh, oven dried uncooked and cooked samples of *Buchholzia coriacea* seed and their effects on hepatocellular integrity. *International Journal of Pharmaceutical Invention*, 3(6), 41-49.
- [15] Onasanwo S.A., Faborode S.O., and Ilenre K.O. (2016). Antidepressant-like Potentials of *Buchholzia coriacea* Seed Extract: Involvement of Monoaminergic and Cholinergic Systems, and Neuronal Density in the Hippocampus of Adult Mice. *Niger. J. Physiol. Sci.*, 31, 093-099.
- [16] Ore A, Akinloye OA, Adeogun AI, et al. *Buchholziacoriacea* seed (wonderful kolanut) alleviates insulin resistance, steatosis, inflammation and oxidative stress in high fat diet model of fatty liver disease. *J Food Biochem*. 2022;46(3):e13836.
- [17] Ore A, Ugbaja RN, Adeogun AI, et al. Hydroethanolic extract of *Buchholziacoriacea* seeds alleviates LPS induced liver injury in rat via antioxidant and anti-inflammatory actions. *Journal of Complementary and Alternative Medical Research*. 2019;8(2):1–15.
- [18] Umeokoli, B. O., Abba, C., Ezeh, P., & Ajaghaku, D. (2016). Antimicrobial, anti-inflammatory, and chemical evaluation of *Buchholzia coriacea* seed (Wonderful kola). *American Journal of Life Sciences*, 4(5), 106-112.
- [19] Varela, G., Batthyány, L., Bianco, M. N., Pérez, W., Pardo, L., Algorta, G., Robino, L., Suárez, R., Navarro, A., Pérez, M. C., & Schelotto, F. (2015). Enteropathogens Associated with Acute Diarrhea in Children from Households with High Socioeconomic Level in Uruguay. *International Journal of Microbiology*.