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Cardioprotective and Hematological Effects of Wonderful Kola (*Buchholzia coriacea*) in Wistar Rats Exposed to Aluminum Chloride-Induced Toxicity

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ABSTRACT: Aluminium pervades ecosystems, posing a nuanced risk of intoxication for both animal and human cohorts. In this paper, cardioprotective and haematological effects of wonderful kola (Buchholzia coriacea) in Wistar rats exposed to aluminum chloride-induced toxicity was investigated by different methods including haematological analysis revealed significant decreases in PCV, HB, RBC, and PLT, with increased WBC in the aluminum chloride-induced group, indicating hematological toxicity ($p < 0.05$). Vitamin C partially restored parameters, while both low and high doses of Wonderful Kola extract showed positive effects, suggesting potential protection against aluminum chloride-induced toxicity ($p < 0.05$). Histological assessment demonstrated severe inflammation in the aluminum chloride-induced group, partial protection with vitamin C, and potential protective effects with Wonderful Kola, especially at higher doses. Wonderful Kola extract exhibited protective effects on cardiac tissue histology in aluminum chloride-induced toxicity, highlighting its potential as a therapeutic agent. These results contribute to the understanding of the potential therapeutic benefits of natural products in mitigating cardiovascular diseases. Further research on natural remedies for cardiovascular diseases is warranted, and caution should be exercised when considering treatments that may have detrimental effects on cardiac structure.

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Cardiovascular disease (CVD) stands as a formidable global health challenge, encompassing a range of conditions that affect the heart and blood vessels. According to the World Health Organization (WHO), CVD remains the leading cause of death worldwide, responsible for an estimated 17.9 million fatalities in 2019 alone (WHO, 2021). The spectrum of CVD includes coronary artery disease, heart failure, stroke, and peripheral vascular diseases, all of which contribute significantly to the global burden of disease (Roth *et al*., 2020). In this context, the exploration of alternative therapeutic approaches becomes paramount. Herbal remedies, derived from plants with purported medicinal properties, have gained attention for their potential in preventing and ameliorating cardiovascular and hematological diseases. One such plant of interest is "Wonderful Kola" (*Buchholzia coriacea*), a tropical rainforest tree that is native to West Africa. Wonderful Kola is renowned for its rich content of bioactive compounds with reputed medicinal properties (Adepoju *et al.*, 2021). The aim of this research study is to investigate the cardioprotective and hematological effects of Wonderful Kola (*Buchholzia coriacea*) on Wistar rats exposed to aluminum chloride-induced toxicity. Wonderful Kola which belonging to the

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Capparidaceae family is an evergreen shrub found in some African countries including Cameroon, Central African Republic, Gabon, Congo, Angola, Nigeria, Ghana, Liberia, etc. (Ibrahim and Fagbohun, 2014; Ijarotimi 2015; Umeokoli *et al.,* 2016). The medicinal efficacy of the seed earned the plant its common name "wonderful kola" (Ibrahim and Fagbohun, 2014; Nwachukwu *et al*., 2014; Ijarotimi *et al*., 2015). Wonderful kola bears edible fruit which taste peperish (Nwaichi and Olua, 2015) The fruit is usually about 5 inches in length and 2-3 inches in width which contain 1-inch seed. The seed is consumed by humans, and studies have demonstrated that it medicinal properties to several disease conditions (Nwachukwu *et al*., 2014; Eze *et al*., 2015; Umeokoli *et al*., 2016). Bioactive constituents have been reported in the seed of Wonderful Kola. Authors have reported the presence of alkaloids, saponins, tannins, flavonoids, oxalates, phytates cardiac glycosides, steroids, resins, carbohydrates, anthraquinone, glycosides in the seed of wonderful kola (Ibrahim and Fagbohun, 2012; Nwachkwu *et al*., 2014; Obiudu *et al*., 2015; Okere and Ladeji, 2016; Umeokoli *et al.,* 2016). Authors have variously reported that different extracts of wonderful kola have different effects depending on the specific bioactive ingredients. For instance, Umeokoli *et al.* (2016) reported seed extracts of wonderful kola contain weak alkaloids using methanol and strong alkaloids using n-hexane, chloroform and aqueous extract. The authors have also reported that chloroform extract does not contain steroids. In a quantitative determination of dried seed of wonderful kola, Ibrahim and Fagbohun (2012) reported that the methanol extract of wonderful kola has a superior effect compared to ethanol extract with regard to alkaloids, glycosides, saponins, steroids, tannins, flavonoids and sugar content while ethanol extract is superior with regard to phenols and terpenes. Nwaichi *et al.,* (2017) described hyperlipidemia as an anomalistic increase of lipids in the blood, abundance of triglycerides and cholesterol. This is due to uneven rise in lipoproteins that play essential role in the transportation of lipids in the blood, branded as hyperlipoproteinemia (Nwaichi *et al.,* 2017). Nwaichi *et al.,* (2017) reported that hypercholesterolemia is the most prevalent variety of dyslipidemia that could predispose an individual to cardiovascular diseases such as atherosclerosis, and pancreatitis. Botanicals such as wonderful kola have demonstrated clinic efficacy for the management of hyperlipidemia using male wistar rats as test organisms (Nwaichi *et al*., 2017). Therefore, the focus of this paper was based on the cardioprotective and haematological effects of wonderful kola (*Buchholzia coriacea)* in Wistar rats exposed to aluminum chloride-induced toxicity.

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.

Sample Preparation and Extraction: 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 1392g of the powdered plant material in 3.5 liter 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 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.

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.

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 in Table 1.

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	$AlCl_3$ + 250mg/kg of *Buchholzia Coriecea* for 14 days
Group 5	High Dose	$AlCl_3$ + 1000mg/kg of *Buchholzia Coriecea* 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.

Phytochemical screening: Phytochemical screening was carried out on the dried powdered sample extract obtained. The 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 indicate 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 assure 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 5minutes 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 indicates the presence of the free anthraquinone.

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.

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 α-naphthol solution was added to it. In a slanting manner, 1ml of conc. H_2SO_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 thepresence 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-oxy 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-oxy sugar.

Kedde test for De-oxy 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 addedto 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. Thefiltrate 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: R1and 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 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.

Haematological Analysis: Full blood count (FBC) includes hemoglobin content, red blood cells (RBC), white blood cells (WBC), was done by using Automated Hematology Analyzer, ready–made kits and platelets (PLT) counts.

Determination of packed cell volume (PCV): The blood in the EDTA bottle was used for the PVC. The blood was collected into a capillary tube containinganticoagulant. Plug one end of the tube with soft wax to a depth of about 2mm by heating it carefully over aflame. Place the capillary tube in the numbered slots in heaematocrit centrifuge. After centrifuge at high speed (13000 rpm) for 5 minutes. The percentage of PVC is determined using haematocrits was calculated based on the following formula

$$H_t(\%) = \frac{L_1}{L_2} * 100$$

Where; L1 = is the height of RBC column; L2 = is the total length of the column (RBC + Plasma + buffy coat) in millimeter and expressed in percent

Determination of total white blood cell counts: The counting of total white blood cells was done by using a diluting fluid (Turk's fluid) in a ratio of 1:20 whichhaemolyses the RBCs leaving the WBCs to be counted. The leukocytes are the counted in a counting chamberunder the microscope, and the number of cells in a litre of blood is calculated.

Determination of heamoglobin (Hb): Sahli's haemoglobinometer was employed for estimation of haemoglobin (Hb) content of the blood. Shahi'spipette was filled with mice blood exactly up to 20 mm3 mark. The excess of blood was removed by blotting thetip with soft absorbent tissue. The blood was expelled into a calibrated (transmission) test tube containing 1mlof 0.1 N HCl acid solutions and the pipette was rinsed several times in the acid solution. The sample wasallowed to stand for 3 minutes. This method involves conversion of hemoglobin to acid haematin. The amountof haemoglobin in the blood sample was directly read in gram percent from the graduated haemoglohinometertube.

Other blood indices: Haematological indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular HaemoglobinConcentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated from the values of Hhcontent (%) and Ht (%) using the following formula

- MCV (fL) = PCV (%) x 10

RBC count

- MCH (pg) = Hb (g/dl) x 10

RBC count

- MCHC (g/dl) = Hb (g/dl) x 100

Differential blood counts (DC): The differential counting was done as described in clinical haematology. The blood smears were made, airdried,fixed in 100% methanol and stained with May and Grunwald stain and counted under oil immersionobjective. Smears were examined for macrophages and abnormal RBC morphology (size, shape, colour,maturity, inclusions) and to determine the differential count of white blood cells (WBC). Total of 1000 bloodcells of all types was counted from each smear and then percentage of each cell type was calculated.

- Since the May-Grünwald staining solution is made up in MeOH prior fixation is not necessary.
- Place slide on a flat surface and pipet 500 µl May-Grünwald Stain on the slide, leave for 3 min.
- Dilute Stain by adding 500 µl 10mM NaPi 7.0, leave for 7 min.
- Lift slide to drain the staining solution and place in a tray with H2O for 1 min.
- Dry slide vertically for 5 min.
- Mount coverslips using an aqueous-based mounting medium.

Histological Assessment: The heart tissues collected were histologically examined including fixation, dehydration, embedding, sectioning, and staining for H & E staining method. Microscopic examination was performed to evaluate cellular morphology, tissue architecture, and any pathological changes.

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.

RESULTS AND DISCUSSION

The data obtained for the Phytochemical Screening on *Buchholzia coriacea* is presented in Table 1. Alkaloid as tested using Dragendorff test, Mayer's test and Hager's test respectively.

Each of the three (3) test used for the alkaloid screening gave a positive, confirming that alkaloid is present in the *Buchholzia coriacea*. The data obtained for the effect of Wonderful Kola extract on Haematological parameters in aluminum chloride toxicity is presented in Table 2.

Packed Cell volume, Haemoglobin, Red blood cell Counts, White Blood Cell Counts and Platelet count results showed significant values when compared Normal Control with test groups and with Aluminum Chloride Group. The data obtained for the effect of Wonderful Kola extract on Haematological Differential Counts/Indices in aluminum chloride-induced toxicity in Wistar rats is presented in Table 3.

MCHC, MCH, MCV, M, L and E results showed significant values when compared Normal Control with test groups and with negative control.

Table 1: Phytochemical Screening on *Buchholzia coriacea*

Screened phytochemical test	Results
Alkaloid	
Dragendorff test	+
Mayer's test	+
Hager's test	+
Carbohydrate	
Fehling	+
Mollisch	+
Flavonoids	
Shinoda test	-
$AlCl_3$ test	-
Anthraquinone	
Free anthraquinones	-
Combined anthraquinones	-
Saponins	
Frothing test	+
Emulsion test	+
Test for Cyanogenic glycosides	+
Test for Phlobatanin	-
Test for Fixed Oil	-
Test for Steroid Salkwoski	+
Test for cardiac glycosides	-
Keller killer test	-
Kedde's test	-
Phenolic Constituent	
Tannins: $FeCl_3$ test	+
Test for triterpenoids	
Lieberman test	-

Key: *+ means positive; - means negative*

Table 2: Effect of Wonderful Kola extract on Hematological Parameters in Aluminum Chloride-Induced toxicity in Wistar Rats

Group	PCV (%)	HB (g/dL)	RBC (million/µL)	WBC (thousand/µL)	PLT (thousand/µL)
Control	41.00±1.00#	13.65±0.35#	4.65±0.35#	5.90±0.20#	473.50±37.50#
$AlCl_3$ induced	28.33±1.45*	8.80±0.49*	2.40±0.21*	16.80±1.53*	818.67±75.04*
Vitamin C	36.67±1.76*#	12.90±0.59#	6.00±0.37*#	8.10±0.38#	703.67±48.75*#
Low Dose (250mg/kg)	38.67±2.40#	12.20±0.59#	6.93±0.52*#	6.60±1.37#	699.33±70.66*#
High dose (1000mg/kg)	38.72±1.76#	13.20±0.96#	7.43±0.48*#	5.53±0.64#	641.67±55.13*#

* = value is significant when compared to Normal control at p<0.05; # = value is significant when compared to negative control (Aluminum chloride group) at p<0.05

Table 3: Effect of Wonderful Kola extract on Hematological Differential Counts/Indices in Aluminum Chloride-Induced toxicity in Wistar Rats

Group	MCHC (g/dL)	MCH (pg/cell)	MCV (fL/cell)	M (%)	L (%)	E (%)
Control	30.60±0.30#	17.15±1.35#	53.95±7.25#	3.50±0.50#	35.50±7.00#	1.50±0.05#
$AlCl_3$ induced	24.17±0.63*	25.63±0.71*	64.37±8.53*	6.67±1.20*	76.33±4.18*	3.33±0.67*
Vitamin C	30.23±0.83#	19.46±0.50#	53.73±7.62#	4.10±0.05	38.33±3.08#	2.67±0.33*
Low Dose (250mg/kg)	30.43±0.15#	18.50±0.85#	55.03±5.76#	3.00±0.21#	44.00±4.41*#	2.65±0.67*
High dose (1000mg/kg)	33.30±1.12#	17.03±1.09#	52.80±6.74#	3.52±0.06#	36.33±5.61#	2.33±0.88*

* = value is significant when compared to Normal control at p<0.05: # = value is significant when compared to negative control (Aluminum chloride group) at p<0.05

Effect of Wonderful Kola extract on the Histology of the heart of Aluminum Chloride-Induced toxicity in Wistar Rats: Aluminum Chloride has been reported to have prooxidant activity, it is considered to be a non-redox active metal that promotes biological oxidation. In biological systems, redox-active metals often play a role in electron transfer reactions. While aluminum can undergo redox reactions, it is not a biologically essential metal like iron or copper. Aluminum's involvement in biological oxidation is more associated with potential toxic effects rather than promoting essential biological processes. It's important to note that the biological interactions of metals can vary based on specific conditions and contexts. Hematological parameter namely PCV, HB, RBC, WBC, platelets and differentials were monitored during the chronic toxicity study in rats because of their role in providing reliable information concerning hematological changes toxicants could cause. Our study results indicated that $AlCl_3$ administration showed significant decreases in the concentration of PCV, HB, RBC, and PLT, alongside a significant increase in WBC. It is suggestive that altered peripheral blood composition is a reflection of disrupted hematopoietic process and interfering with different stages of red cell synthesis and mature red blood cells. Packed Cell Volume (PCV) is used to measure the blood carrying capacity and it is directly associated with the HB and RBC ability to conduct tissue oxygenation activity. The changes in the blood PCV value have often been shown to be a good indicator of aluminum chloride toxicity. Treatment with 250mg/kg and 1000mg/kg of *Buchholzia coriacea* resulted in increase in PCV, HB, RBC, and PLT, alongside a significant decrease in WBC. Also, there was an increase in MCHC and decreases in MCH, MCV, monocytes, lymphocytes and eosinophils.

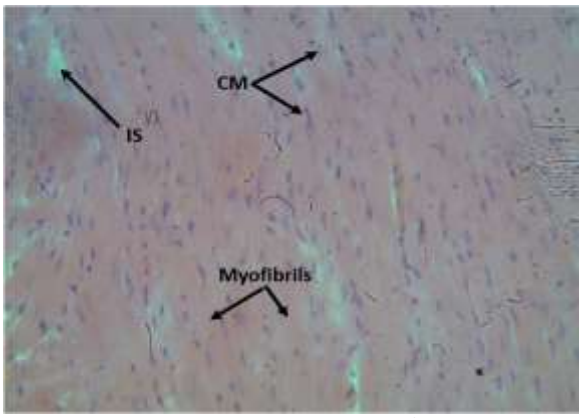

Plate 1. Photomicrograph (H and E × 400) of the myocardium architecture of the Control group showing the normal layers of straited cardiac myocytes (CM) arranged in a spiral fashion interspersed with interstitium (IS) and myofibrils. [Diagnosis: Normal myocardial tissue].

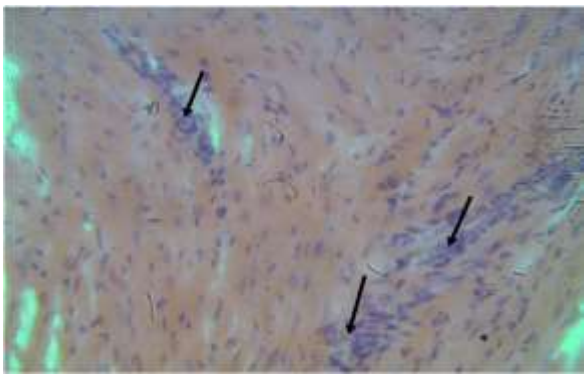

Plate 2. Photomicrograph (H and E × 400) of the cardiac tissue of positive Control group (aluminum chloride-induced) showing multifocal inflammatory cell infiltration within the interstitium of the myocardium tissue (arrows) [Diagnosis: Severe Inflammation of the cardiac tissue].

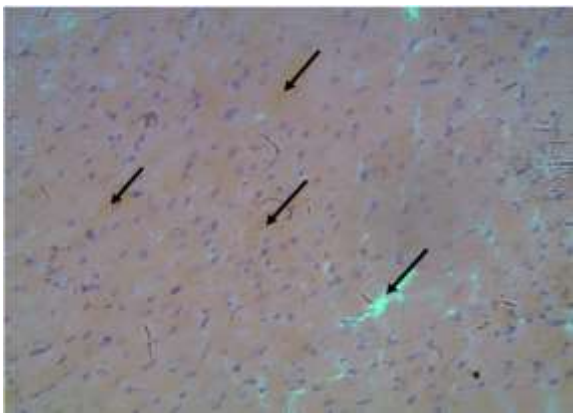

Plate 3. Photomicrograph (H and E × 400) of the myocardium of vitamin C treated group showing reduced mononuclear activities with mild interstitial distortion (arrows) [Diagnosis: Minimal distortion with normal appeareance of the cardiac tissue].

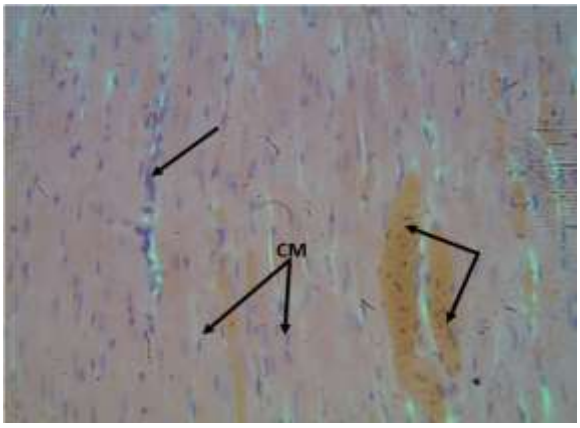

Plate 4. Photomicrograph (H and E × 400) of cardiuc of the 250mg/kg Wonderful kola treated group showing mild hemorrhagic deposit within the interstitiumwith reduced inflammatory cells activity (arrows). [Diagnosis: Mild hemorrhage with reduced inflammation of the cardiac tissue].

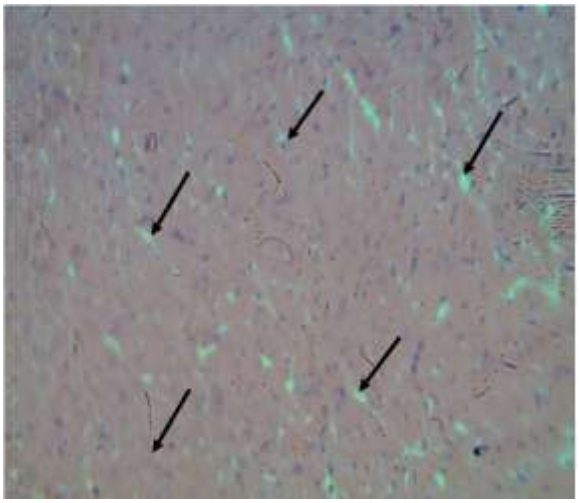

Plate 5. Photomicrograph (H and E × 400) of the myocardium 1000mg/kg Wonderful kola treated group showing myocytes (CM), myofibrils; minimal interstitial vacoulation with no mononuclear activities (arrows): cardiac tissues appear normal. [Diagnosis: Normal myocardial tissue].

Lymphocytes count in the blood following increase in white blood cells count plays an important role in stimulating the immune system of animals. The histological analysis revealed distinct changes in cardiac tissue induced by various treatments. In the control group, a typical and well-organized myocardial structure was observed, serving as a baseline representation. Conversely, the positive control group, induced with aluminum chloride, exhibited severe inflammation, characterized by multifocal inflammatory cell infiltration within the myocardial interstitium. The vitamin C treated group showed a partial protective effect against inflammation, indicated by reduced mononuclear activities with mild interstitial distortion.

The 250mg/kg Wonderful Kola treated group displayed mild hemorrhagic deposits and reduced inflammatory cell activity, suggesting a potential protective effect. Remarkably, the 1000mg/kg Wonderful Kola treated group demonstrated pronounced protection, with decreased mononuclear activities, minimal interstitial vacuolation, and a near-normal appearance of cardiac tissue, highlighting the potential efficacy of a higher Wonderful Kola dose in mitigating pathological changes induced by aluminum chloride. The findings of the study are consistent with existing literature. Aluminum chloride induced severe inflammation in the cardiac tissue, as supported by a study which showed marked morphological changes in the heart tissue such as necrosis and disarrangement of the muscle cell nucleus (Komal, Rapheal & Ova, 2022).

On the other hand, the protective effect of vitamin C against inflammation is in line with research that demonstrated the cardio protective effect of vitamin C by reducing oxidative/nitrosative stress, inflammation, and apoptosis (Akolkar *et al.,* 2017). Additionally, the potential protective effect of Wonderful Kola is supported by the traditional medicinal uses of the plant, which include treating various health *issues and its widely documented beneficial effects (Ore et al.,* 2021).

Conclusion: In conclusion, the study on Wonderful Kola highlights the potential protective effect of natural products on cardiovascular diseases. Aluminum chloride-induced toxicity adversely affected hematological parameters and cardiac tissue, leading to significant alterations. Vitamin C and Wonderful Kola extract, especially at higher doses, demonstrated potential protective effects against these induced changes. These results contribute to the understanding of the potential therapeutic benefits of natural products in mitigating cardiovascular diseases.

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