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Biochemical and Histomorphometric Assessment of Aqueous Cola Nitida Seeds Extract Administration in Brain and Kidney of Wistar Rats

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Abstract
The side effects of orthodox drugs used in treatment of high blood pressure and hypertension has prompted inquiry into the use of plants and herbal remedies believed to be safe with no serious side effects as alternatives in the treatment. This study aims to evaluate the effects of 28-day *Cola nitida* seeds[CNS]aqueous extract dose[through oral gavage] on biochemical parametres, nephrotoxic potentials and tissue damage among others; in female wistar rats alongside a standard diuretic drug-Furosemide. Twenty five female wistar rats were assigned into five groups of five each: 1, 2, 3, 4and 5 which received orally 0.9% of normal saline, 40mg furosemide [standard], 400, 600 and 800 mg/kg body weight of the CNS aqueous extract respectively for 28 days. The toxicity on the kidney and serum was correlated with changes in the alanine, aspartate aminotransferases activity [ALT&AST] and in their serum and homogenized kidney samples. Biochemical indices of organ damage and toxicity were determined using standard methods. The result showed the activities of kidney and serum ALT and AST were significantly altered in rats of the 800mg/kg [P>0.05] after aqueous extract of CNS administration. Student t-test and One-way Anova was used for the statistical analysis and p-values less than 0.05 were considered statistically significant. The treatment related alterations in the present study indicates that the aqueous extract of CNS at the experimental doses caused no functional toxicity on the activities of serum ALT levels in their kidneys and serum. However, histopathological changes to the hippocampus was observed in the 400mg/kg and 800mg/kg group while interstitial nephritis was observed in the kidneys of rats from standard[reference drug] and all CNS groups.

1.0 INTRODUCTION

For several centuries, medicinal plants and herbal remedies have continued to enrich the health care needs of animals and humans. Kola nut extract consists of extract of the nuts [or seeds] from the pods of primarily two species of the Cola plant [1]. *Cola nitida* is a genus of about 125 species of trees native to the tropical rainforests of Africa, classified in the family *Malvaceae*, subfamily *Sterculioideae* [2].They are evergreen trees, growing to 20 m tall, with glossy ovoid leaves up to 30 cm long [3]. Caffeine and stimulant properties is one of its characteristic element which has prompted its frequent use by humans [4,5]. Its diuretic potentials have also been explored and reiterated in several human and animal in-vivo studies. Several literatures have asserted the diuretic potential of *Cola nitida which* could be used for treating water retention caused by diseases such as oedema or dropsy,hypertension etc [6].Flavonoids, saponins, alkaloids and organic acids constituents as been attributed as factors responsible for the diuretic activity of a plant extract [7].Also, alkaloids presence in this extract is presumed to provide an inhibitory mechanism and protection against microbial infections among others[8].Hence, the phytochemical properties of *Cola nitida* extracts indicates that the presence of these secondary metabolites might be responsible for the diuretic activity of *Cola nitida* among other pharmacokinetic potentials [9].However, medicinal plants, in spite of its their relatively safe pharmacological uses need to be authenticated by scientifically validated tests for toxicological properties and safe dose levels in order to prevent tissue damage among others. Sub-chronic studies have observed restlessness, excitement, irritability, loss of hair and appetite, and diuresis in animals receiving kola nut extract[1].Teratogenic potential of cola nitida seeds extract was also reported by [10] effects as change in locomotion behavior were observed in treated offspring and decrease in pups body weights while neuro-toxicological indices were observed in a study by[11]. Medicinal plants, in spite of being popularly claimed as naturally safe need to be authenticated by scientifically validated tests for toxicological properties before being introduced for widespread pharmacological use [12].These findings among others suggests that further research should be carried out to determine safety, dosage [to avoid the toxicity], isolation of plant constituents and determining its mode of actions as vital before adoption as standard drug in treatment of diseases.

2.0 METHODS

2.1.Experimental Design

Twenty five adult female albino rats weighing between 150 g and 180 g bred in the Animal House of Physiology Department, Afe Babalola University were used. Female rats were selected for this study as there are few reports of examination of CNS diuretic potential in female rats.They were housed under standard laboratory conditions in plastic cages with wire guaze covering under a 12-hour daylight cycle and had free access to pelletized rat chow [obtained from ABUAD Farms] and water. They were acclimatized to laboratory conditions for two weeks before the commencement of the experiments.The experimental procedures adopted in this research were in strict compliance with Experimental Animal Care and Use of Laboratory Animals in Biomedical Research Regulation of the College of Sciences, Afe Babalola University, Ado-Ekiti. They were randomly divided into five groups with each group consisting of five rats[n=5]. Group 1 [Control] received 10ml/kg of 0.9% NaCl [normal saline], Group 2[standard] received 20mg/kg of furosemide, Group 3 received 400mg/kg CNS, Group 4 received 600mg/kg CNS and Group 5 received 800mg/kg CNS.

2.2 Plant Materials

Fresh seeds of *Cola nitida* were locally sourced and authenticated in the taxonomy unit of the department of Agriculture, Afe Babalola University. Large quantities [245.00g] of the fresh seeds of *Cola nitida* were washed and cut into smaller bits and air-dried in a clean tray for three weeks, the dried specimens were pulverized using laboratory mortar and pestle. Weighed portion[232.50g] of the pulverized specimens were macerated and extracted with distilled water [1:2 wt./vol] for 48 hours at room temperature [26 – 28^0C]. The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores [0.25 mm]. The distilled water was later evaporated using rotary evaporator. Forty gram of the aqueous extract of *Cola nitida* seeds [CNS] was dissolved in 100 ml of distilled water to give a concentration of 0.4 g/ml.

2.3 Experimental controls

A loop diuretic [Mark-Furosemide, Tianjin, Xinzheng, Henan, China], was used as positive control [reference drug] and 0.9% sodium chloride [Merck, Germany] was used as control drug, respectively.

2.4 Determination of Biochemical Parameters

Activities of alanine transaminase [ALT] and aspartate transaminase [AST] were determined both in the serum and the kidney of experimental rats according to the method of [13].Tissues [kidney] were harvested from each animal and homogenized using Teflon homogenizer in ice-cold Tris-HCL buffer [pH 7.4] for AST/ALT levels determination. All the above biochemical parameters were determined using Erba Mannheim kits.

2.5 Histomorphometric Analysis

The weights of the harvested tissues:k idney and brain were documented for all groups using weighing balance. Small portions from the harvested kidneys and brain[hippocampus] were cut and fixed in 10%formo- saline in sterile plain bottles. The fixed tissues were thereafter sectioned [5-micron thickness], and embedded in paraffin. Sections were stained with Hematoxylin and Eosin [H&E] and examined under a light microscope[Olympus BX-51, Japan] by a pathologist. Tissues of 1 mm in thickness were used for ultra-structural investigations. Light microscopic examination of multiple tissue sections from each organ in all groups were carried out as described by [14].

2.6 Statistical Analysis

The mean and standard error of mean [S.E.M.] were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance [ANOVA] with Duncan's Multiple Range Test[15]. Differences were considered statistically significant at $p<0.05$ performed in all groups and images representative of the typical histological profile were examined.

3.0 RESULTS

3.1. Serum AST/ALT levels

Table 1 below shows the result of AST and ALT levels assayed in the serum of experimental rats used in this study after 28 days aqueous CNS oral dose.

Table 1: **Effect of 28days treatment with varying doses of Cola nitida seeds on rats' serum**

GROUPS	AST	ALT
1-Control	2.16±0.59	3.02±0.86
2-Furosemide	3.03±0.71	3.89±0.73
3-400mg/kg	2.81±1.00	4.75±1.43
4-600mg/kg	3.07±0.61	3.24±0.90
5-800mg/kg	3.10±0.90	3.02±0.99

3.2. Tissue [kidney]AST/ALT levels

Table 2 below shows the result of AST and ALT levels assayed in the homogenized kidney of experimental rats used in this study after 28 days aqueous CNS oral dose.

Table 2: **Effect of 28days treatment with varying doses of Cola nitida seeds on rats' kidney**

GROUPS	AST	ALT
1-Control	5.18±1.30	3.60±1.58
2-Furosemide	2.91±0.96*	2.30±0.65
3-400mg/kg	3.37±1.64*	3.45±0.93
4-600mg/kg	4.22±1.60*	3.50±1.25
5-800mg/kg	5.84±2.32*	1.83±0.40

n=5, p>0.05 significance level

3.3 Histomorphometric Analysis of rats brain [hippocampus] using H&E technique

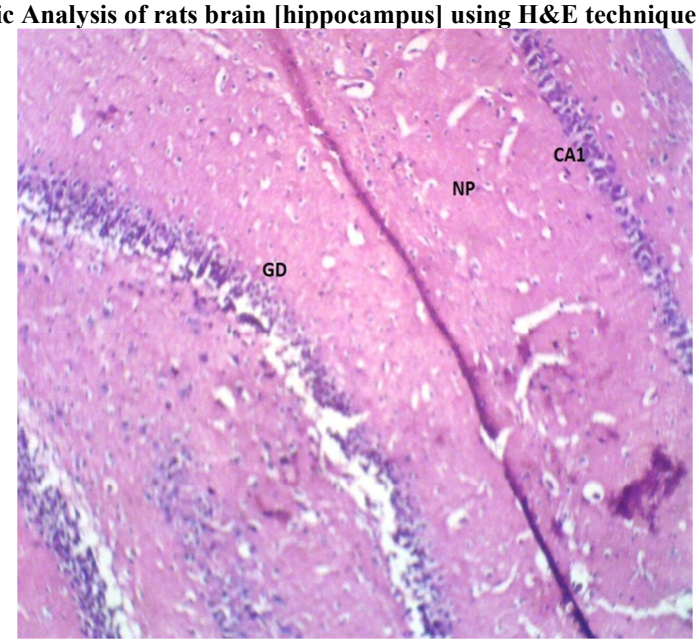

Fig 1: CONTROL [Group 1]

Photomicrograph shows section of the hippocampus[**X400**] showing distinct region of the hippocampus [CA1-CA4 and dentate gyrus GD] and the neural parenchyma [NP]. The cytoarchitecture of the neurons, glia cells [granular and pyramidal cells] and capillaries appear essentially normal and unremarkable. No inflammatory response observed.

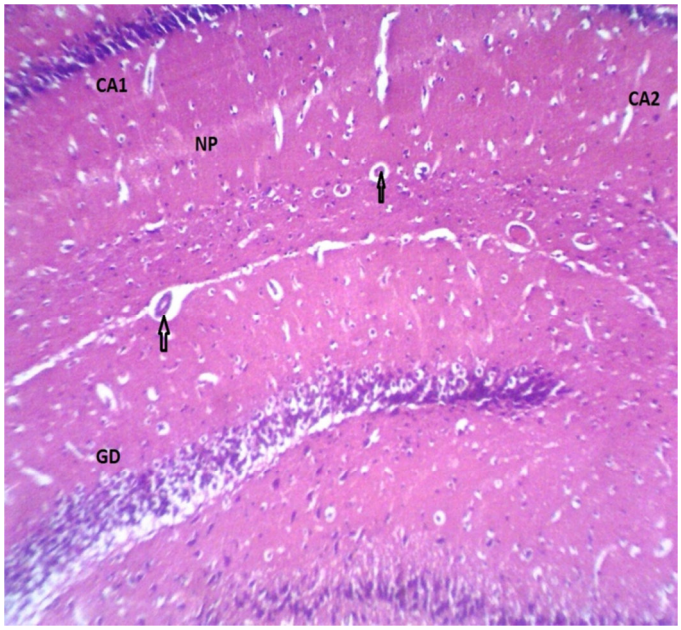

Fig 2:Furosemide [Group 2]

Photomicrograph shows section of the hippocampus[X400] showing distinct region of the hippocampus [CA1-CA4 and dentate gyrus GD] and the neural parenchyma [NP]. The cytoarchitecture of the neurons, glia cells [granular and pyramidal cells] and capillaries appear essentially normal and unremarkable. No inflammatory response observed.

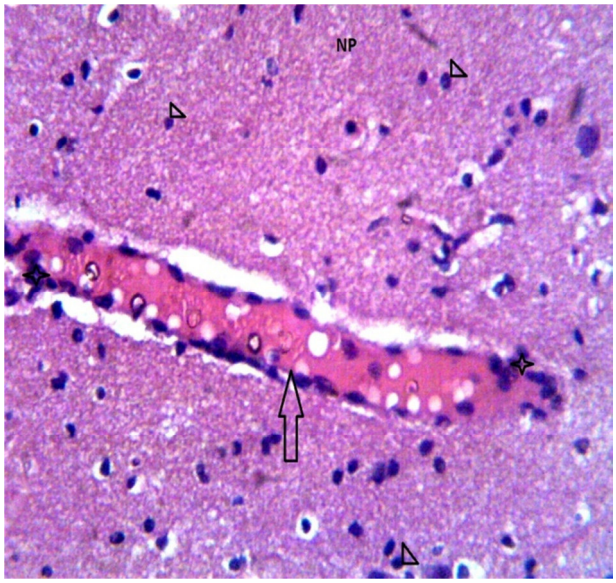

Fig 3:400mg/kg Aqueous extract CNS[Group 3]

Section[X400] shows capillary congestion [arrow] and mild peri capillary leukocytosis [Star].the neuroparenchymal [NP] and glia cell [Arrow Head] appear unremarkable.

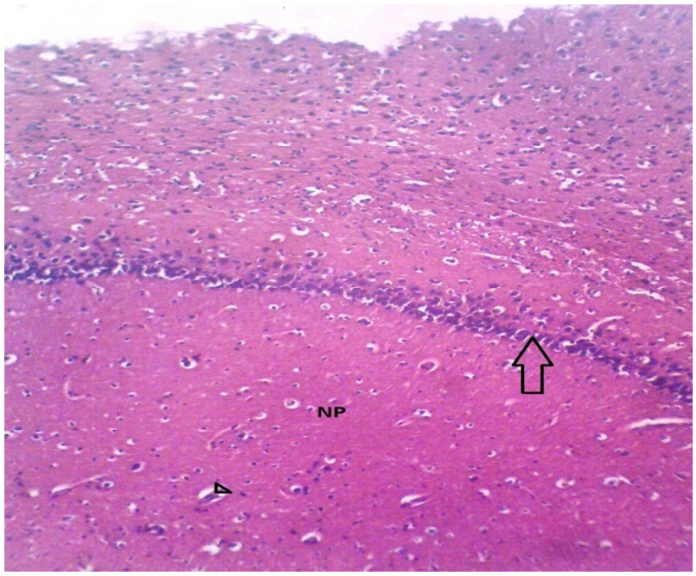

Fig 4:600mg/kg Aqueous extract CNS[Group 4]
Section [x400]appear as in control, normal and unremarkable

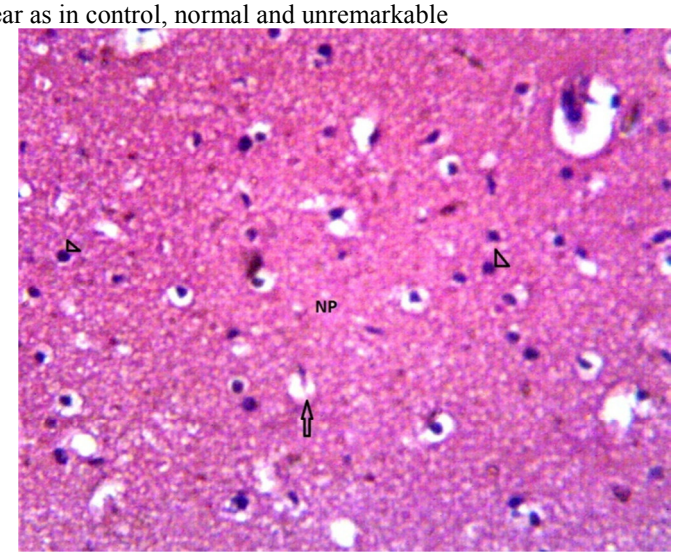

Fig 5: 800mg/kg CNS
Section[X400] appear normal and essentially remarkable parenchyma [NP] and glia cells [arrow]. Arrow showed perinuclear vacoulation [arrow].

3.4 Histomorphometric Analysis of rats kidney using H&E technique.

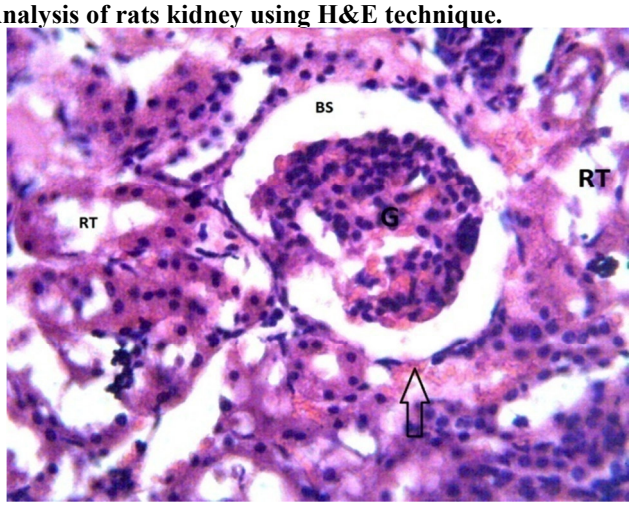

Fig 6:CONTROL [Group 1]

Photomicrograph[x400] shows the renal corpuscle [arrow] composed of the glomerulus [G], well defined bowman's space. The renal tubules are lined by regular epithelium; the interstitium is free from collection and inflammatory cells**.**

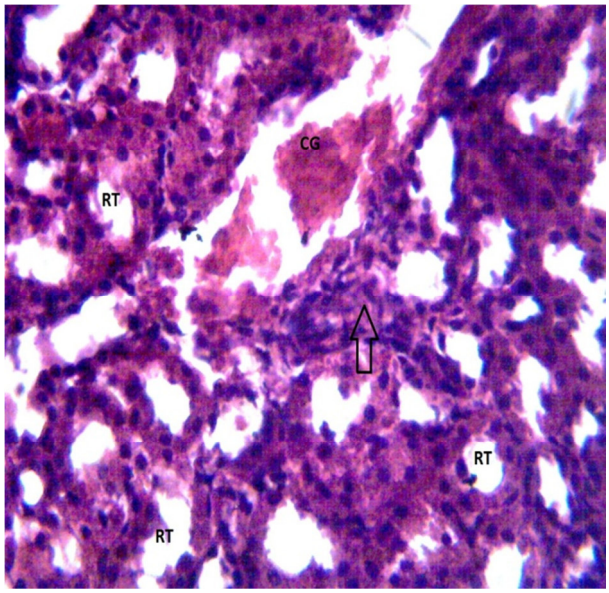

Fig 7: Furosemide [Group 2]

Section[x400] shows interstitial congestion and mild leucocytes infiltrates. The renal tubules appear unremarkable.

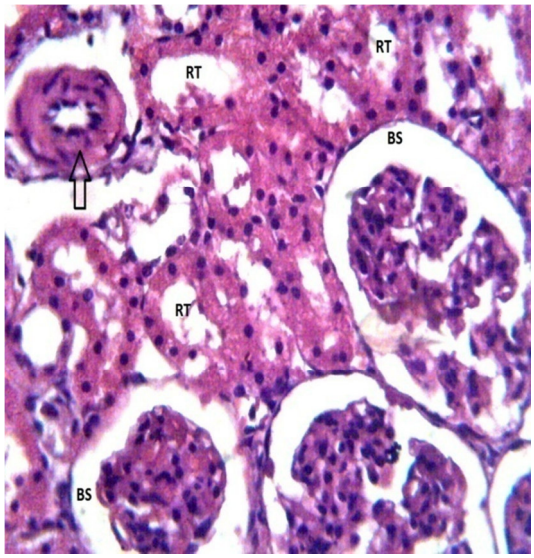

Fig 8: 400mg/kg CNS[Group 3]

Section[x400] appears as in control. Vessel shows mild medial hypertrophy. Renal tubules appear normal.

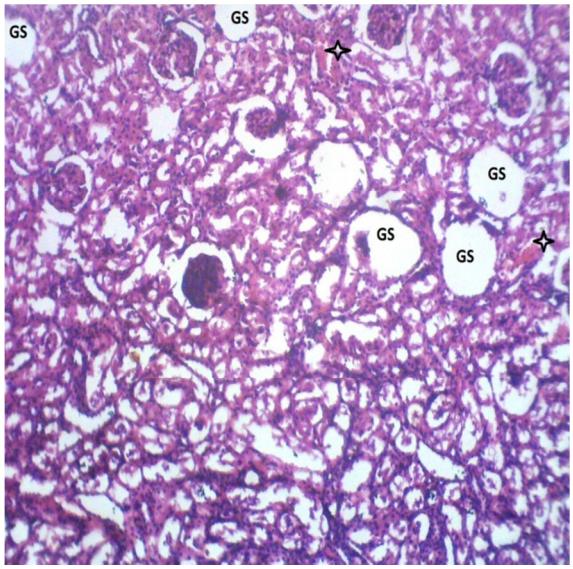

Fig 9:600mg/kg [Group 4]
Section[x400] shows Glomeruloscleriosis [GS] and interstitial congestion [Star].

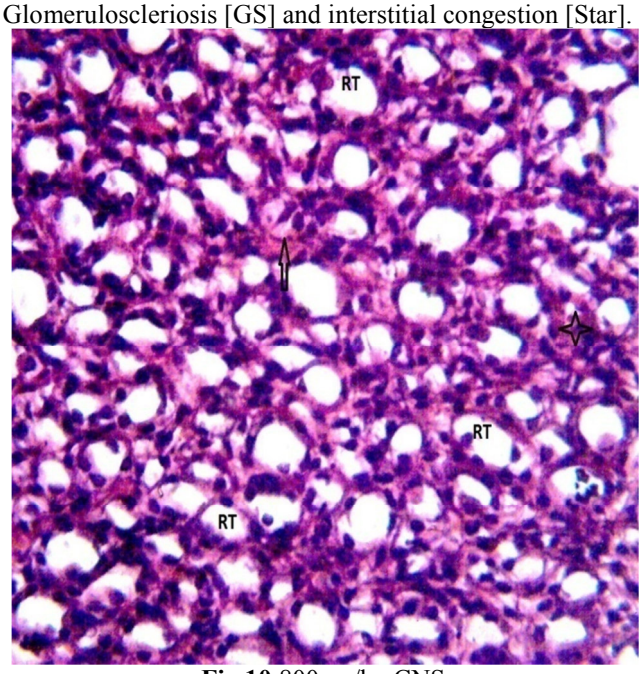

Fig 10:800mg/kg CNS
Section[x400]shows interstitial congestion**.**

4.0 DISCUSSION

AST levels in the kidneys of rats after twenty eight days CNS administration was significantly lower in the furosemide group[reference drug group] when compared to the CNS groups at high doses: 400mg/kg,600mg/kg and 800mg/kg and normal saline[negative control].However, there was no significant difference in the AST levels assayed in the serum of rats from furosemide,600mg/kg and 800mg/kg aqueous extract CNS when compared to normal saline and 400mg/kg AECON group which were significantly lower. Hence, treatment of rats with 600mg/kg and 800mg/kg caused significant [$p>0.05$] changes similar to reference drug [furosemide] in the activities in the serum AST levels of rats. However, the 800mg/kg CNS group showed a significantly higher AST levels in their kidneys but lowest ALT levels in serum. This finding conforms to the results of [16] who found an increase in the activity of alanine aminotransferase [AST] and a decrease in the activity of aspartate amino transferase[ALT] with administration of various doses *cola nitida* in the kidney. Serum ALT is known to increase in serum when there is a tissue/cellular damage-usually the liver and also when functions of the nephrons have been compromised[17] .AST levels in the kidney was significantly lower when compared to the control rats in the Furosemide,400mg/kg and 600mg/kg groups. However, AST levels in the 800mg/kg[higher than the aforementioned groups]was not significant when compared to the control groups. Hence, the results of

this study are in consonance with the report of [18] with no significant change in the activities of ALT and AST in the serum during administration of *Cola nitida* at doses .

Histopathological changes to the hippocampus was observed in the 400mg/kg[capillary congestion and peri-capillary leukocytosis –Fig 3] while perinuclear vacoulation was observed in the hippocampus of brains harvested from rats in 800mg/kg group[Fig 5] which indicates blood–brain barrier disruption, neuro-degeneration and neuro-inflammatory response as described by [19]. The nephrons of the kidneys in animals from standard [reference drug-Fig 2] and CNS dosed groups had histological alterations as well.

Leukocyte interstitial infiltration in kidneys is linked to coordinated action of both kidney chemokine expression and leukocyte chemotaxis to kidney-expressed chemokines. Interstitial leukocyte infiltration depicts deterioration of renal function as described by [20].

Swelling of kidney tubules is observed [acute interstitial nephritis] in groups 2,4 and 5 and most reported cases of acute or chronic interstitial nephritis has been linked to drug adverse reactions in patients. Also ,a link has been found between infiltration of renal interstitium in kidneys and uveitis syndrome[an autoimmune disorder] which causes immunologic dysregulation with serious health consequences and subsequently degenerates into renal disorder[21].

In histological studies, end-stage renal disease manifests itself as glomerulosclerosis-hardening of the blood vessels in the kidneys [seen in 600 and 800mg/kg groups] has also been reported in adverse drug reactions[22] which correlates with the observations seen in animals in this study.Hence, cola nitida seeds consumption for therapeutic purposes at high doses can result in serious health consequences .

5.0 CONCLUSION

This study examined the impact of treatment of rats with varying doses of *Cola nitida* on serum and kidney levels of electrolytes [sodium, potassium, chloride], AST, ALT activities, in an attempt to evaluate the nephrotoxic potential of this plant extract. The results obtained revealed animals treated with high doses of the aqueous extract of *Cola nitida* seeds compared with a standard reference drug with respect to the toxicological biochemical indices and histomorphometric changes revealed slight biochemical indices modulations . However, acute to chronic adverse drug reactions were observed in histomorphometric analyses of harvested brain [hippocampus] and kidneys[drug-induced interstitial nephritis] in standard drug[furosemide] group and experimental rats dosed with aqueous extract of CNS at three different dose levels.

Conflicts of Interest

The authors hereby declare no conflict of interest for this research work.

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