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HISTOMORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE KIDNEY AND LIVER OF WISTAR RATS TREATED WITH CRUDE DECOCTION OF *NAUCLEA LATIFOLIA, ENANTIA* CHLORANTHA AND *MANGIFERA INDICA* (*AGBOIBA PONTO*)

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ABSTRACT

The histomorphological and biochemical changes in the kidney and liver of wistar rats treated with *Agboiba ponto w*as evaluated. Twenty-four male and female wistar rats weighing between 150 and 170g were divided into four groups (A-D) six rats each. Different doses of (500mg/kg, 600mg/kg, and 700mg/kg) of aqueous crude extract of *Agboiba ponto* were administered orally to the rats in groups B to D respectively, for a period of 28 days. Group A (control) received top feed and water only. Blood samples were collected from the rats for serum urea, creatinine, alanine transaminase (ALT) and aspartate transaminase (AST) estimation, while kidney and liver tissue samples were excised for histological examination. The result, showed dose dependent progressive increase in serum urea and creatinine levels of test animals compared with the control, though not statistically significant. Serum ALT and AST levels progressively decreased from groups A to C but suddenly increased in group D. The histological findings revealed similar trend, the control showed normal histo-morphological architecture while test groups had progressive morphological degeneration ranging from mild histo-architectural distortion of the cortical structures to marked tubular necrosis, moderate-severe chronic inflammatory cell infiltrates and glomerulonephritis and hepatic periportal lymphocytic infiltration. Prolonged administration of higher doses of *Agboiba ponto* may cause adverse changes in the kidney and liver of wistar rats. Therefore, caution should be observed while usage.

KEYWORDS: *Agboibaponto*, histological effects, biochemical effects, kidneys, liver, wistar rats.

INTRODUCTION

Plants have been used by man for prevention, treatment and management of diseases for a very long time without even the knowledge of the components and toxicity of these plants [1]. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicine do not differ greatly from conventional drugs in terms of mode of action. This enables herbal medicine to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.[2] Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils which possess medical values for the treatment of different disease.[1] The liver is vital organ necessary for survival of all vertebrates with currently no way to compensate for its long term absence due to dysfunction in the body. This organ plays a major role in body metabolism, such as glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, drugs metabolism and detoxification. The kidney similarly is another vital organ that is significant in filtration and excretion of toxic waste products of metabolism. The duo therefore, major role both in drug metabolism and excretion of breakdown products of drugs.

Decoction of *Nauclea latifolia*, *Enantia* chlorantha and *Mangifera indica* (*Agboiba ponto*) is a mixture of roots stems and leaves of different herbs (Table 1). These herbs were made from the plants with different abilities to synthesize a wide variety of chemical compounds that are used to perform important biological functions. *Agbo* originated from South West Nigeria, and is widely acclaimed to be efficacious and used in the treatment of malaria, typhoid fever, pain, stomach ulcers, infections, and many other related health issues in Nigeria.[3]

The decoction is hawked on the streets of Nigeria, especially at car parks, and many Nigerians consume it without recourse to dosage and effects on the body.

Individual components of *Agboiba ponto* used for typhoid fever treatment have medicinal values. The extract of mango stem bark called Vimang, isolated by a Cuban Scientist contains numerous poly-phenols with antioxidant properties in vitro [3] and on blood parameters of the elderly.[4]

The mango triterpene, lupeol, is an effective inhibitor in laboratory models of prostate and skin cancers.[5]

The individual herbs besides their medicinal implications may also have their different nephrotoxic and hepatotoxic effects. Ogenyi et al [6] studied the effect of ethanolic crude extracts of *Nauclea latifolia* smith (rubiaceae) leaves, fruits, stem and root barks on the liver of Chinchilla rabbit and reported that protracted consumption of the herbal remedy has predilection for hepatic dysfunction. This was corroborated by an earlier study by Akpanabiatu et al.[7] Similarly, a study on the acute and long-term toxicity of mango leaves extract in mice and rats, Zhang et al.[8] reported increased weight of liver and kidney of the animals after experimental procedures but with no significant morphologic effects. Phytochemical and acute toxicity of ethanol extract of *Enantia chlorantha* (oliv) stem bark in albino rats was investigated by Adebiyi and Abatan.[9] The authors reported that though significant morphological alterations were not observed in the vital organs, there was evidence of congestions in the kidney and heart and therefore, concluded that that oral administration of E. chlorantha may produce severe toxic effects at relatively high doses.

Most people who consume this mixture lack a comprehensive knowledge of its contents, efficacy, accurate prescription, dosage or illness it is intended to treat. Sequel to the wide spread consumption of this decoction, resulting from the endemic nature of malaria and typhoid fever in our locality, coupled ignorance and poverty, the need to evaluate its effect in the kidney and liver, using animal model becomes imperative. This study therefore, was aimed at determining the morphological and biochemical effects of *Agbo Iba ponto* in the liver and kidney of albino rats.

Table I: Components of *Agboibaponto*.

Local Name	Scientific Name	Family Name	English Name	Parts Used
Egboegesi or egeiba	*Nauclea latifolia*	*Rubiacea*	African peach	Stem
Awopa	*Enantia chlorantha*	*Annonnaceae*	African yellow wood	Bark
Mangoro	*Mangifera indica*	*Anacardiaceae*	Mango	Stem/bark

MATERIALS AND METHODS

Study area
This study was carried out in the College of Health Sciences, NnamdiAzikiwe University, Nnewi campus.

Experimental design and conduct
The study was an experimental design using animal model. Twenty-four (24) healthy adult albino rats comprising of both sexes were grouped into 4 (A-D) experimental groups, according to their body weights. Group (A) served as the control while groups B, C and D were the experimental groups. The experimental rats in group A (control) received feed and water only, group B were administered with 500mg/kg of the extract, group C, 600mg/kg group D 700mg/kg. The animals were administered with the extract orally for twenty eight days using orogastric tube and were fed with feed chow manufactured by vital feeds Jos, Plateau State Nigeria. All animals were allowed access to food and water *ad libitum* throughout the study period.

Collection of animal
Twenty-four (24) healthy adult (weighing between 150-180g) albino rats comprising of both sexes were procured from the animal house of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. They were housed in a well ventilated stainless steel cage and allowed 2 weeks acclimatization before experimental procedures. The animals were housed under standard animal house conditions, a controlled room with 12 hours light; 12 hours dark cycle with room temperature of 25±1^{o}C and 55±3% humidity.

Collection of plant materials
Agboiba ponto was purchased from a local market (Nkwo market) in Nnewi, Anambra State Nigeria. Hundred grams (100g) mixture of already ground plant materials was procured from a popular vendor in the market and stored in an air tight see-through glass bottle before extraction.

Extraction
The ground plant material (100g) was poured a clean and dry white plastic container with a fitting lid and mixed with 1000ml of distilled water. The mixture was kept in a cool dry environment and allowed to stand for 48 hours but was however, stirred every six hourly using a sterile glass rod. The mixture was filtered using muslin clothe and the extract measured, kept in a sterile container and refrigerated at 4+2^{0}C until when needed [10].The percentage yield was determined by evaporating 10ml of the homogenous extract to dryness, the dry weight was weighed and the ash value determined by simple proportion.

Specimen collection
The specimens were tissue and blood samples.

Blood collection
At the end of the experimental procedure, the rats were painlessly sacrificed through chloroform anaesthesia and blood collection actualized through direct cardiac punctures. The blood samples were delivered into plain test tubes, allowed to stand for 20 minutes to clot and centrifuged at 2,000rpm for 5 minutes. Sera for biochemical analysis (serum aspartate transaminase (AST) and alanine transaminase (ALT)) levels, urea and creatinine test were collected using Pasteur pipettes.

Tissue collection
Through a midline abdominal incision, the two kidneys and liver were excised and blotted with filter paper, weighed, transferred to jars of 10% formal saline and allowed sufficient time for fixation.

Tissue preparation
The fixed tissues were processed using Leica automatic tissue processor and embedded if paraffin wax using also Leica embedding centre. Tissue sections of 3µ thick were cut from the tissue blocks with the aid of a rotary microtome. Cut sections were floated out on a lukewarm Leica water bath, mounted on slides, drained, labelled and placed on Leica hot plate in order to dry and affix the tissue onto the slides.

Staining
Sections were stained by Haematoxylin and Eosin (H&E) method originally described by Drury et al [11] and stained sections were viewed under the digital optical microscope and photomicrographs were taken with the aid of an attached camera Leica ICC50.

Biochemical analysis
The renal functions of the rats were assayed using the Jaffe – Slot Alkaline Picrate method for Creatinine and Diacetyl Monoxime method for urea.[12] while serum AST and ALT levels were assed using Reitman's-Frankel method as described by Adebiyi and Abatan.[9]

Statistical analysis
Data were expressed as mean and standard error of mean (mean ± SEM). Mean values were compared using one-way analysis of variance (ANOVA) with $P<0.05$ considered significant. Data analyses were performed using statistical package for social sciences (SPSS) windows version 22 software.

RESULTS

The result of the present study showed dose dependent progressive increase in serum urea and creatinine levels of test animals compared with the control. The mean values of urea showed no statistically significant difference when group A is compared (7.05 ±0.55) with test groups, but a comparison of B and C showed decreased statistically significant difference while C and D showed increased statistically significant difference. Comparison of mean creatinine values followed a similar trend (Table I). There was a progressive decrease in the serum levels of ALT and AST amongst groups A, B and C and a sudden increase in group D. Comparison of group A and B showed no significant difference in both ALT and AST levels whereas increased statistically significant difference was observed when group A with C and D. The ALT level in Group C also showed a significant increase when compared with group D while serum AST level in group C was not significant when compared to group D (Table 2).

Histological findings
Gross
The kidney of the control and test animals showed a normal reddish brown coloration when dissected. They were smooth and are of the normal size about 2.5by 1.6cm.

Microscopy
The control sections (Group A) of the kidneys showed normal histological features. There was presence of dense-rounded renal corpuscles with the glomerulus entirely surrounded by the narrow Bowman's spaces (Figure 1). The kidney sections of the Wistar rats in group B treated with 500mg/kg of *Agboibaponto* revealed some levels of mild histo-architectural distortion of the cortical structures including hemorrhage, hypertrophy and severe chronic inflammatory infiltrate as compared to the control. The kidney sections of animals in group C treated with 600mg/kg of *Agboibaponto* crude extract revealed distortion of histo-architecture of the renal cortical structures. There were interstitial edema, mild chronic inflammatory cell infiltrate and hemorrhage while the kidney sections of Wister rats in group D treated with 700mg/kg crude extract of *Agboiba ponto* showed marked tubular necrosis, moderate-severe chronic inflammatory cell infiltrates and glomerulonephritis. The histological examination of the liver sections similarly, showed a mild periportal lymphocytic infiltration in the rats given 600 (C) and 700mg/kg (D) body weight of the extract while no morphological changes were observed in those given 500mg/kg of the extract (B) and control (A).

Table 2: Comparison of mean values of serum urea, creatinine, AST and ALT levels amongst study groups.

Dependent Variable	Study group	Mean ±Sd	p-value
Urea (Mg/dl)	**A**	7.02±0.04	0.001
	B	8.02±0.01	
	C	5.20±0.09	
	D	8.20±0.14	
Creatinine (Mg/dl)	**A**	91.30±1.69	.
	B	98.02±0.97	0.001
	C	71.36±1.85	
	D	102.00±1.41	
AST (Iu/l)	**A**	16.37±0.55	
	B	14.80±0.85	0.033
	C	19.25±1.77	
	D	23.00±2.83	
ALT (Iu/l)	**A**	13.15±0.21	
	B	11.15±0.78	0.001
	C	17.25±0.21	
	D	25.62±0.54	

Significant level: P<0.05

Table 3: Pair wise comparison of serum urea, creatinine, AST and ALT levels amongst study groups.

Parameter	Duration	Mean difference	Standard error	P-value
Urea	**A vs B**	-1.000*	0.032	0.020
	A vs C)	1.825*	0.072	0.016
	A vs D	-1.180*	0.104	0.074
	B Vs C	2.825*	0.066	0.023
	B Vs D	0-.180	0.100	0.554
	C Vs D	-3.005*	0.119	0.008
Creatinine	**A vs B**	-6.715	1.382	0.135
	A vs C)	19.945*	1.773	0.020
	A vs D	-10.700	1.562	0.055
	B Vs C	26.660*	1.474	0.021
	B Vs D	-3.985	1.212	0.216
	C Vs D	-30.645*	1.644	0.009
AST	**A vs B**	1.570	0.716	0.383
	A vs C)	-2.880	1.309	0.444
	A vs D	-6.630	2.038	0.323
	B Vs C	-4.450	1.387	0.265
	B Vs D	-8.200	2.088	0.248
	C Vs D	3.750	2.359	0.548
ALT	**A vs B**	2.000	0.570	0.285
	A vs C)	-4.100*	0.212	0.007
	A vs D	-12.470*	0.409	0.016
	B Vs C	-6.100	0.570	0.083
	B Vs D	-14.470*	0.669	0.009
	C Vs D	8.370*	0.409	0.028

*Significant level: P<0.05

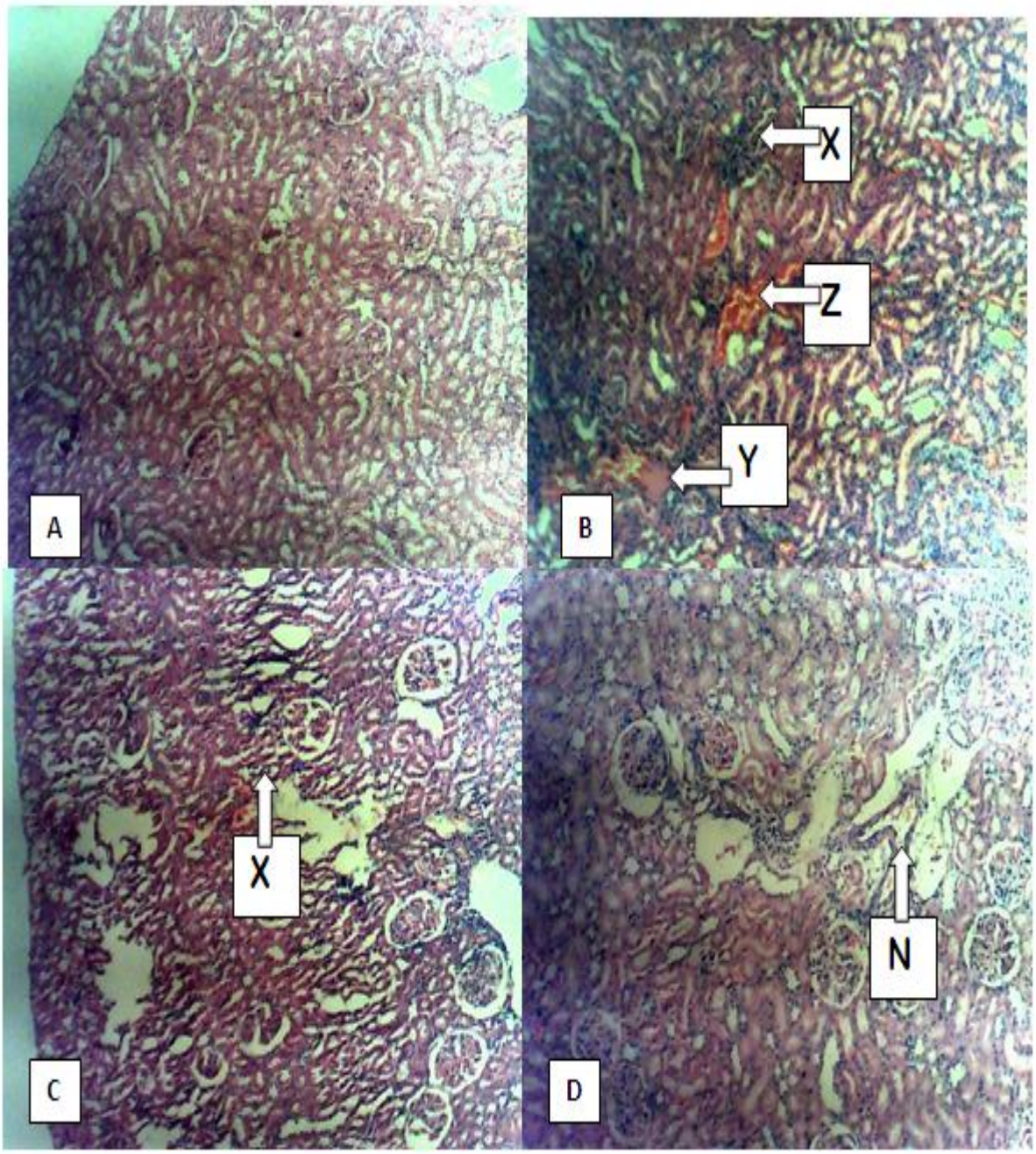

Fig. 1: Histomorphological staining features of kidney sections of albio rats treated with the decoction of *Nauclea latifolia, Enantia* chlorantha and *Mangifera indica* (*Agboiba ponto*). A: Control; B: treated with 500mg/kg body weight; C: treated with 600mg/kg body weight; D: treated with 700mg/kg body weight. A showed normal Bowman's capsules with intact glomeruli, B showed focal area of mild congestion (Z), oedema (Y) and inflammatory cellular infiltration (X) while C showed scanty inflammatory cells (X) while D showed moderate sign of glomeronephritis (N) (H&E X400).

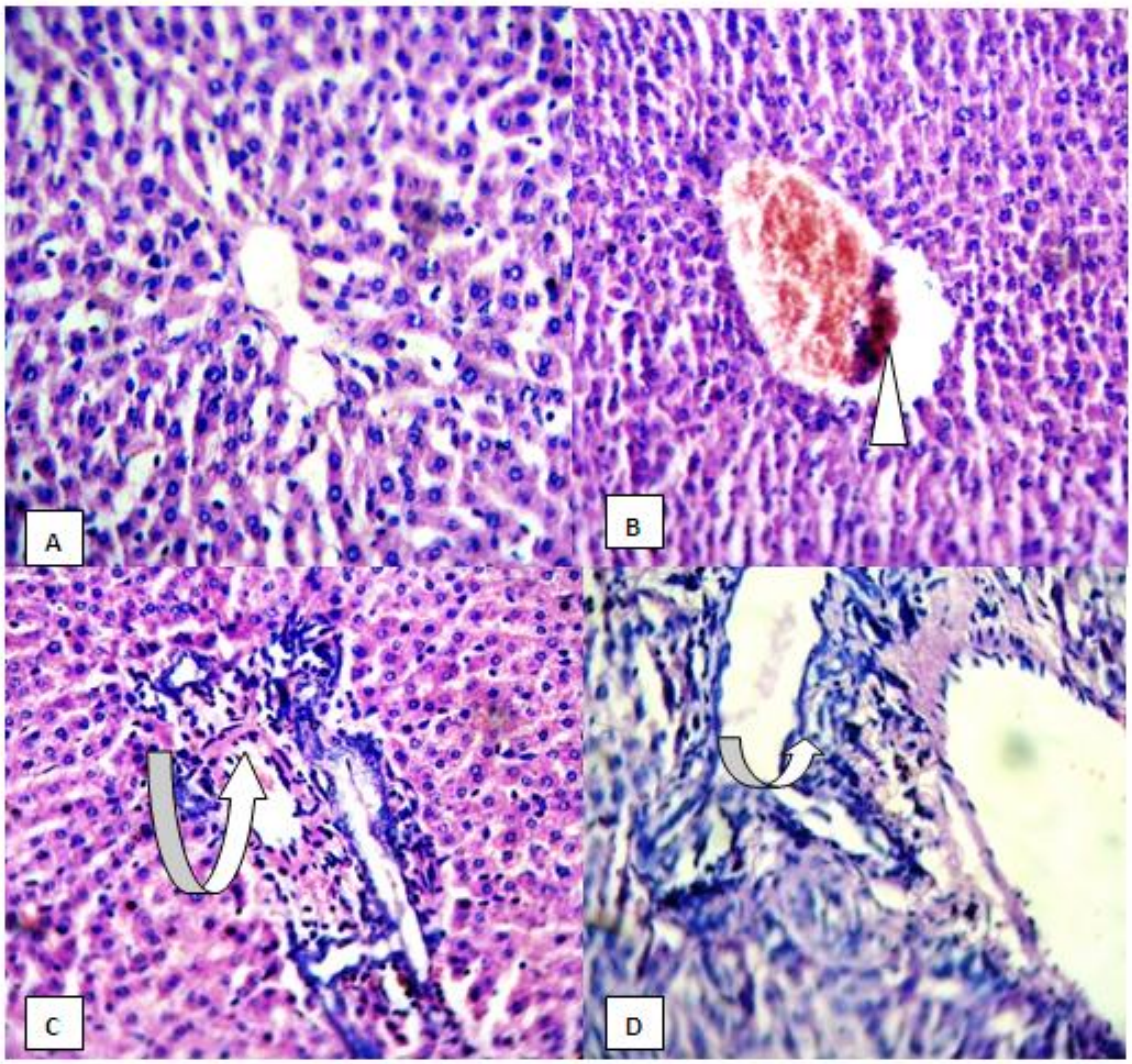

Fig. 2: Histomorphological staining features of liver sections of albino rats treated with the decoction of *Nauclea latifolia, Enantia* chlorantha and *Mangifera indica* (*Agboiba ponto*). A: Control; B: treated with 500mg/kg body weight; C: treated with 600mg/kg body weight; D: treated with 700mg/kg body weight. A showed normal hepatocytes, blood vessels and sinusoids, B showed mild congestion within the central vein (arrow head) while C and D showed moderate periportal lymphocytic infiltration (curved arrow) (H&E X400).

DISCUSSION

The result of the present study revealed that the effects of the decoction of *Nauclea latifolia* (*Agboibaponto*) on the kidney and liver to be dose-related. This could be deduced from the progressive increase in serum urea, creatinine, AST and ALT levels with increased dosage administration (group B-D). The histological findings revealed similar trend with the control showing normal histo-morphological architecture while test groups showed progressive morphological degeneration ranging from mild histo-architectural distortion of the cortical structures to marked tubular necrosis, moderate-severe chronic inflammatory cell infiltrates and glomerulonephritis and hepatic periportal lymphocytic infiltration. This finding corroborated the reports of Ogenyi et al, Akpanabiatu et al, Zhang et al, Adebiyi and Abatan and Iwuji et al.[6,7,8 9,13] A much earlier study by Akande et al.[14] and MacDonald et al [15] also reported various forms of deleterious effects in the internal milieu of some organs and cells of wistar rats administered with high dose of *Agboibaponto.* The effects included but not limited to hemolytic, membranolytic and hemorrhagic changes. It should be noted that despite the observed changes, the biochemical and morphological derangements were not severe and may not at that present stage cause any serious organ malfunction. However, the progressive changes with increase in dosage administered may result to severe organ impairment, especially with protracted usage. This may portend great danger, especially to human consumers who have no recourse to dosage and duration of intake.

It is noteworthy that *Agboigbaponto* contains high levels of alkaloids and antioxidants.[16] which expectedly effectively inhibit and ameliorate cellular components oxidation by reactive oxygen species (ROS), leading to cellular injury.[15,17] Another possible explanation of the

result is that secondary metabolites, which are largely responsible for pharmacological and therapeutic activities of medicinal plants.[18,19] may be responsible for their organ toxicity with arbitrary intake. Therefore, it could be deduced from the study that prolonged administration of higher doses of *Agboibaponto* resulted to increased hepatotoxicity and nephrotoxicity, however, the exact mechanism of action of the cellular degeneration was unknown and needs further investigation.

CONCLUSION

It could be concluded that administration of decoction of *Nauclea latifolia* (*Agboibaponto*) progressively altered the serum levels of urea, creatinine, ALT and AST which also was evident in the histo-architectural pattern of the kidney and liver of wistar rats. Though, the present study did not establish severe renal and hepatic injury, the mild changes observed especially at higher doses leaves something to worry about. This becomes more worrisome owing to the fact that the decoction is widely consumed without recourse to dosage and health status of the consumers. In some rare occasions, ethanol is used as solvent for the active ingredients. It is therefore, recommended that caution should be advocated in the intake of the decoction while further studies, involving humans should be carried out to evaluate the mechanism of action and the exact point and route of injury.

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