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EVALUATION OF THE CHEMICAL CONSTITUENTS AND TOXICOLOGICAL POTENTIALS OF *ASYSTASIA VOGELIANA* BENTH. LEAF EXTRACT

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ABSTRACT

Majority of the tradomedicinal uses of Asystasia vogeliana lack scientific backing. Therefore, this study sought to investigate the phytochemical constituents and the toxicological potentials of methanolic leaf extract of Asystasia vogeliana in Swiss albino mice. Air-dried pulverized leaves (1248 g) of Asystasia vogeliana were extracted with methanol for 48 h. The extract was concentrated using rotary evaporator to obtain crude methanol extract (99.8 g). The extract was screened for phytochemicalconstituents and partitioned with n-hexane and dichloromethane (DCM). The two fractions were subjected to GC-MS analysis. Acute toxicity study was conducted in Swiss albino mice based on standard procedures. Biochemical parameters were determined in the liver, kidney and blood of the animals. Flavonoids, steroids, saponins and triterpenes were detected in the extract. GC-MS analysis of the n-hexane fraction showed presence of n-hexadecanioc acid (24.01%) and 6-octadecenioc acid (36.68%) as major constituents while oxypurinol (15.68%), and 2,2-dimethylcyclohexan-1,3-dione dioxime (11.21%) were the main constituents in dichloromethane fraction. No mortality was recorded in the acute toxicity study. Liver and blood serum aspartate transaminase (AST) and alanine transaminase (ALT) activities increased across the group. The creatinine and urea content in the kidney and blood serum increased. The insignificant increase in creatinine and urea levels in the kidney and blood showed mild toxicity. Also, increase in AST and ALT level was noticed in the blood which showed liver injury. Therefore, the study concluded that the extract at the tested concentration is mildly nephrotoxic and hepatotoxic.

Keywords: *Asystasia vogeliana,* extract, leaf, methanolic, oxypurinol, toxicological.

INTRODUCTION

Globally, there is increase in the use of natural remedies in the treatment of life-threatening diseases like cardiovascular, neurodegenerative and other associated diseases. Plants and marine organisms are the main sources of these natural healing products.

In-time memorial, decoctions of plant materials were being used without adequate measurement or prescribed dosage (Abubakar and Hague, 2020). The biosafety and toxicity of these extracts were not a major concern because their side effects are minimal and the cumulative effects of long-term use of the medicinal plant extracts are also being neglected (Xulu et al., 2022). With increased interest in herbal therapies, scientific safety evaluation of the medicinal plants should be a major concern (Sofowora, 2008). The evaluation of the toxic action of plants extracts is indispensable in order to consider it safe for treatment of any ailments (Vaghasiya *et al.,*2011). Also, toxicity assessment needs to be carried out for the plant extracts use in food, pharmaceutical and cosmetics production. Also, no drug must be used clinically without its toxicity studies and clinical trials (Anisuzzaman *et al.,*2001).

Asystasia vogeliana is widely distributed in West and Central Africa and listed as one of the multipurpose medicinal plants (Uno *et al.,* 2018).It is also used in traditional medicine to treat various chronic ailments like diabetes, epilepsy, cancer, hypertension, gastric disorder, and gonorrhea (Popoola *et al.,*2017). There are reports of toxicity effects of the leaf extract of *A. vogeliana* and other ichthyotoxic plants on African Catfish (*Clarias gariepinus*) and Zebrafish (*Danio rerio*) embryo (Ekanem *et al.,*2003; Uno *et al.,*2018). In spite of its toxic effect in fish model, the leaf decoction is still consumed extensively in various folklore medicines for the affirmed health benefits. Apart from these, there is shortage of scientific information on biological activities especially the toxicity effects of *A. vogeliana* and its chemical constituent. There is need to evaluate the toxicological potentials of *A. vogeliana* on murine model and examine chemical constituents that are inherent in the plant that may be responsible for the toxicity.

MATERIALS AND METHODS

Plant Collection

Fresh leaves of *A. vogeliana* Benth were collected from the neighborhood of Aserifa, old Ibadan-Road, Ile-Ife Osun State, Nigeria. The plant was identified, authenticated and deposited with a voucher number (18310) at Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of Plant Extract

The leaves of *A. vogeliana* Benth were harvested, air-dried and pulverized. The powdered leaf sample (1248 g) was soaked in 6 l of methanol for 48 h and filtered. This process was repeated trice to effect maximum extraction. The filtrates were pooled and concentrated *in-vacuo* using rotary evaporator at 40°C to give the crude methanol extract.

Chemical Studies

Phytochemical Screening of the Crude Methanol Extract

The crude extract was examined for the presence of phytochemicals like cardiac glycosides, tannins, saponins, alkaloids, flavonoids, triterpenes, and steroids using the procedures described by Trease and Evans (2002) and Sofowora (2008).

Partitioning of Crude Methanol Extract

The crude methanol extract (34.8g) was partitioned with n-hexane and DCM using Soxhlet extractor to afford the respective fractions, which were subjected to GC-MS analysis for chemical constituent determination. The extracts were also subjected to Infrared spectroscopy analyses.

Biological Studies

Experimental Animals

Swiss albino mice (25) of both sexes (20-25g) were purchased from Animal Breeding

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Laboratory, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State. The animals were allowed to acclimatize for two weeks at room temperature with 12:12 h light-dark cycle; with free access to commercial food pellets and water *ad libitum*.

Toxicological Evaluation of the Methanolic Crude Extract

Twenty- five male Swiss albino mice (20-25 g) used for this study were obtained from Animal Research Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The mice were housed in a plastic cage in a well-ventilated environment maintained at 25 ± 2ºC at 12/12 h day/light cycles. The animals were acclimatized for one week period and were fasted for 12 h before commencement of the treatment.

Twenty- five Swiss albino mice were randomly distributed into 5 groups of 5 mice per group

Group I served as control and received 0.2 ml distilled water

Group II received 0.2 ml 200 mg/kg body weight of extract

Group III received 0.2 ml 400 mg/kg body weight of extract

Group IV received 0.2 ml 600 mg/kg body weight of extract

Group V received 0.2 ml 800 mg/kg body weight extract

The mice were treated orally with extract every day for 14 consecutive days. On the 14th day of treatment, mice were fasted overnight and then sacrificed the next day. Blood samples were collected by cardiac puncturewith a syringe and needle for estimation of biochemical parameters. Liver and kidney were also excised, rinsed with normal saline, weighed and stored for preparation of homogenates for further biochemical analysis.

Preparation of Blood Serum

The whole blood samples collected were allowed to clot and then centrifuged at 3000 revolution per minute (rpm)for 10 min.The supernatant containing the serum was collected and kept frozen for further biochemical analyses.

Preparation of Liver and Kidney Homogenate

Frozen tissue (1 g each) was weighed and homogenized in 10 ml of freshly prepared 100 mM Phosphate Buffer (pH 7.4) to obtain a 10% homogenate of the tissue sample. The homogenate was then centrifuged at 3000rpm for 10 min, the supernatants were collected into clean bottles and kept frozen for biochemical analyses.

Biochemical Assays

Alanine Aminotransferase (ALT) Activity

The activity of alanine aminotransferase was assayed based on colorimetric method of Reitman and Frankel (1957) using commercially available Randox^{TM}kit. To 250 µl buffered substrate R1 (consisting of 100 mM phosphate buffer, pH 7.4, 200 mM L-alanine, 2 mM α-ketoglutarate), 50µl serum (or tissue homogenate) was added and replicated in triplicate. The reaction mixture was thoroughly mixed and incubated at 37ºC for 30 min. Then, 250 µl reagent R2 (2 mM 2, 4-dinitrophenylhydrazine) was added. The mixture was shaken properly and allowed to stand at room temperature for 20 min after which 2500 µl 0.4 M NaOH solution was added to terminate the reaction. Absorbance of the mixture was read at 546nm against the reagent blank and activity of the enzyme was extrapolated from calibration curve obtained from absorbance-enzyme activity table of values provided by the manufacturer of the kit. Enzyme activity was expressed in U/l.

Aspartate Aminotransferase (AST) Activity

The activity of aspartate aminotransferase was assayed based on the colorimetric method of Reitman and Frankel (1957) using Randox^{TM} kit. To 250 µl buffered substrate R1 (consisting of 100 mM phosphate buffer, pH 7.4, 200 mM L-aspartate, 2 mM α-ketoglutarate), 50µl serum (or tissue homogenate) was added and replicated in triplicate. The reaction mixture was thoroughly mixed and incubated at 37ºC for 30 min. Then, 250 µl reagent R2 (2 mM 2, 4 – dinitrophenylhydrazine) was added. The mixture was stirred properly and allowed to stand at room temperature for 20 min after which 2500 µl of 0.4 M NaOH solution was added to terminate the reaction. The absorbance was read at 546nm against blank. Activity of the enzyme was extrapolated from the calibration curve (7.0 – 89.0 U/l) obtained from absorbance-enzyme activity table of values provided by the manufacturer of the kit. Enzyme activity was expressed in U/l.

Determination of Creatinine Concentration

The creatinine concentration was determined using Randox^{TM} kitbased on the method described by Bartels and Bohmer (1972). Creatinine in alkaline solution reacts with picric acid to form a red coloured complex. The amount of the complex formed is proportional to the creatinine concentration. Creatinine standard solution (0.1 ml) and 0.1 ml of the serum was dispensed into separate cuvettes labeled standard and sample respectively. This was followed by addition of 1.0 ml of the working reagent (picric acid and NaOH). The absorbances of the mixtures were then taken after 30 s (A1) and at exactly 2 min later (A2) using a colorimeter at 520 nm.

Serum creatinine concentration was calculated using the formula:

$$Creatinine\ conc\ (mmol/l) = \frac{\Delta Absorbance\ of\ Sample}{\Delta Absorbance\ of\ Standard} \times Conc. of\ Standard$$

The ΔA of the sample or standard was calculated by subtracting A2 from A1 i.e. A2 – A1 = ΔA of sample or standard.

Determination of Urea Concentration

The urea concentration was determined using Randox^{TM} kitbased on the method of Berthelot's reaction (Fawcett and Scout 1960). Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia formed is then measured spectrophotometrically. Reagent 1 (100 µl) containing 6 mmol/L of sodium nitroprusside and 1g/l of urease was dispensed into three separately labeled test tubes: reagent blank, standard and sample containing 10 µl of distilled water, 10 µl urea standard and 10 µl test serum respectively. The mixture was then incubated at 37°C for 10 min in a water bath. After the period of incubation, 2.5 ml of reagent 2 (120 mmol/l of phenol) was added to the contents of each test tube followed by 2.5 ml of reagent 3 (27 mmol/l of sodium hypochlorite). The mixture was incubated for 15 min at 37°C in a water bath. The absorbance of the sample and that of the standard was read against the reagent blank using a colorimeter at 546 nm.

Serum urea concentration was calculated using the formula:

$$Urea\ concentration\ (mmol/l) = \frac{\Delta Absorbance\ of\ Sample}{\Delta Absorbance\ of\ Standard} \times Conc. of\ Standard$$

Statistical Analysis

All the results of biochemical assays were expressed as Mean ± SD, n = 3. The differences between groups were determined by One-way analysis of variance (ANOVA). The analyses were carried out using Graph Pad Prism 8and mean values were considered to be significant ($p<0.05$).

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RESULTS

Percentage Yield of the Methanolic Leaf Extract of *A. vogeliana*

The methanolic extract obtained from the leaf of *A. vogeliana* was 99.8g which represented 7.99% of the starting materials.

Phytochemical Constituents of *A. vogeliana* Leaf Extract

The screening revealed the presence of alkaloids, flavonoids, saponins, triterpenes and steroids while Tannins were not detected (Table 1)

Infra-Red(IR) Spectra of n-Hexane and DCM Fractions

The IR absorption spectra (Fig. 1) showed the presence ofstretching absorption bands at 3600 – 3300 cm^{-1} (broad); 2975 – 2840 cm^{-1} and 1000 – 1200 cm^{-1} representing hydrogen bonded O–H of carboxylic acid; C–H sp^3 of saturated group and C–O of ester respectively.These absorption peaks are indicative of the presence of fatty acids and their esters in both fractions but more in n-hexane fraction (Fig. 1A) because of the relatively higher intensities of the absorption peaks. This implies that n-hexane fraction contains more of fatty acids and fatty acid esters. However, DCM fraction (Fig. 1B) contains more of aromatics as indicated by the more intense absorption peaks in the finger-print region 600–900 cm^{-1} describing aromatic substitution pattern.

GC-MS Analyses

The results of GC-MS analyses of the two fractions showing the chemical profiles, retention time (RT) and percentage composition of each constituent are as shown in Tables 2 and 3.

Fifteen compounds were identified in the n-hexane fraction, by matching the sample chromatogram with National Institute of Standard and Technology (NIST 11) library of compounds. The main constituents are n-hexadecanioc acid and 6-octadecenioc acid, with retention time (RT in min) 37.295 and 38.497, peak area (a function of concentration) 24.01 and 36.68% respectively (Table 2).

A total of forty compounds were identified in the GCMS of the DCM fraction out of which two are the major constituents (oxypurinol and 2,2-dimethylcyclohexan-1,3- dioxime), with RT (min) of 13.717 and 20.001, peak area 15.68 and 11.21 % respectively (Table 3). They constitute 26.89% of the Fraction.

Effect of *A. vogeliana* Leaf Extract on Creatinine Concentration

The effect of *A. vogeliana* leaf extract on the liver and kidney creatinine level is shown in Fig.2.. There was a non-significant increase in the liver creatinine level for groups treated with 200 mg/kg and 400 mg/kg body weight of extract buta significant increase in group treated with 600 mg/kg body weight of extract(63.24 mmol/L) was observed. Also, there was a non-significant decrease in the kidney creatinine level across all the treated groups as compared with the control (50.45 mmol/L). The effect of the extract on the blood creatinine level is shown in Fig. 3. The level of creatinine steadily increases as the dose increases but with no significant different except at the final dose of 800 mg/kg bwt.

Effect of *A. vogeliana* Leaf Extract on Urea Concentration

The effect of *A. vogeliana* leaf extract on the liver and kidney urea level is as shown in Fig. 4. There was an increase in the level of urea in the liver across the treated groupsbut was lower than the control value (1.84 mmol/L). Also, a dose-dependent and non-significant steady increase in the kidney urea level was observed when compared with the control (1.44 mmol/L). Fig. 5 shows the effect of the extract on the blood urea nitrogen concentration. There is a significant increase

from across the groups when compare with the control.

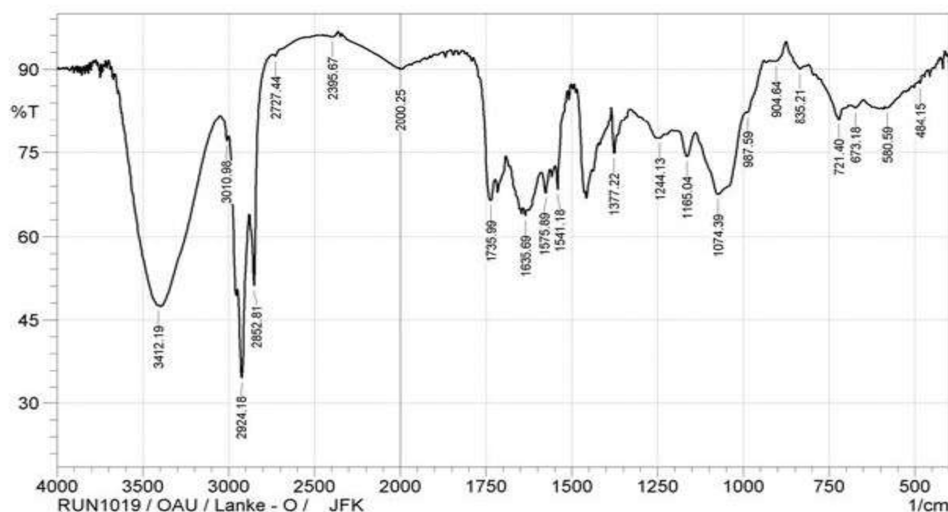
Effect of *A. vogeliana* Leaf Extract on the Activity of AST

The effect of *A. vogeliana* leaf extract on the liver and kidney AST level is as shown in Fig. 6. There was also a significant dose-dependent increase in the liver AST level as against the control (46.14 U/L). The kidney also had a significant dose-dependent increase in AST activity when compared with its control (42.21 U/L). There was a common trend in liver and kidney AST activity; the group 4 that received the highest concentration of the extract (800 mg/kg bwt) exhibited 2-3 fold increase in AST activity. AST activity in the blood serum showed no significant different in all the dose concentrations tested. At 400 and 600 mg/kg bwt, the activity of the AST was lower than that of control while at the 800 mg/kg bwt a non-significant increase was noticed. (Fig. 7)

Effect of *A. vogeliana* Leaf Extract on the Activity of ALT

The effect of *A. vogeliana* leaf extract on the liver and kidney ALT level is as shown in Fig. 8. There was a significant dose-dependent increase in the liver and kidney ALT activity level as against their control. The same effect was consistent in the activity of ALT in both liver and kidney. In the blood serum, the same trend of AST activity was observed for ALT. Highest dose concentration (800 mg/kg bwt) produced highest ALT activity which was greater than the control but the ALT activity obtained from the groups treated with 400 mg/kg bwt and 600 mg/kg bwt were lower than that of control (Fig. 9).

A



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B

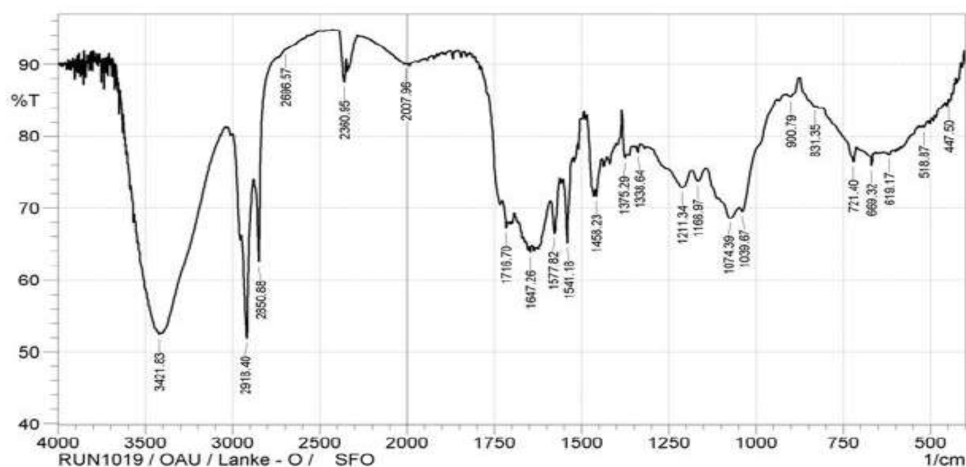

Figure 1. Infra Red (IR) Spectra of (A) n-hexane and (B) Dichloromethane (DCM) Fractions

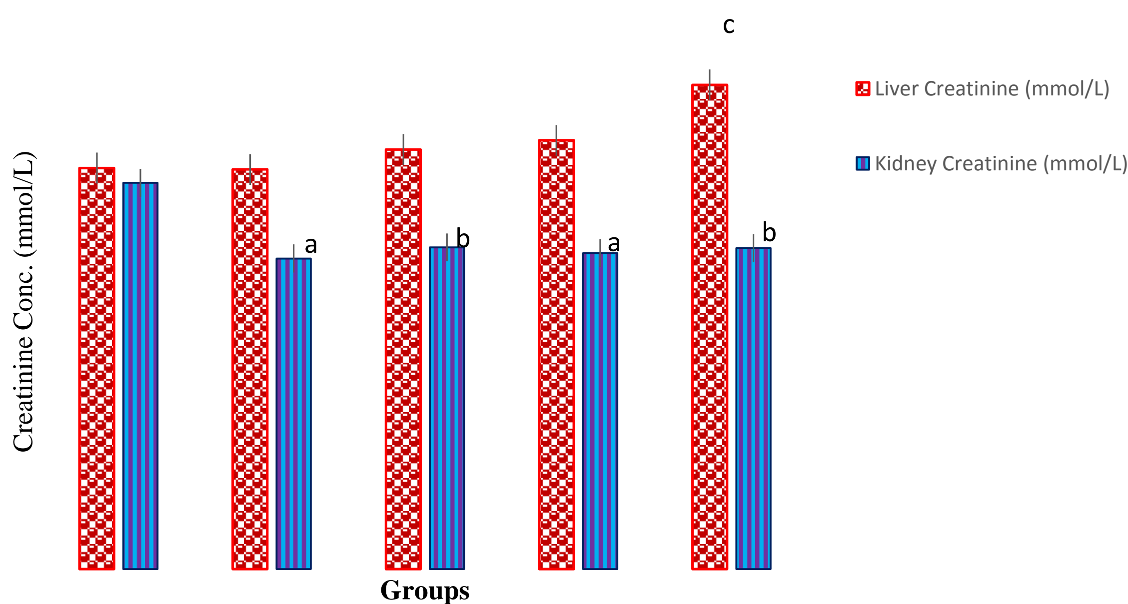

Figure 2: **Effect of *A. vogeliana* leaf extract on creatinine level in liver and kidney.**

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control ($p<0.05$)

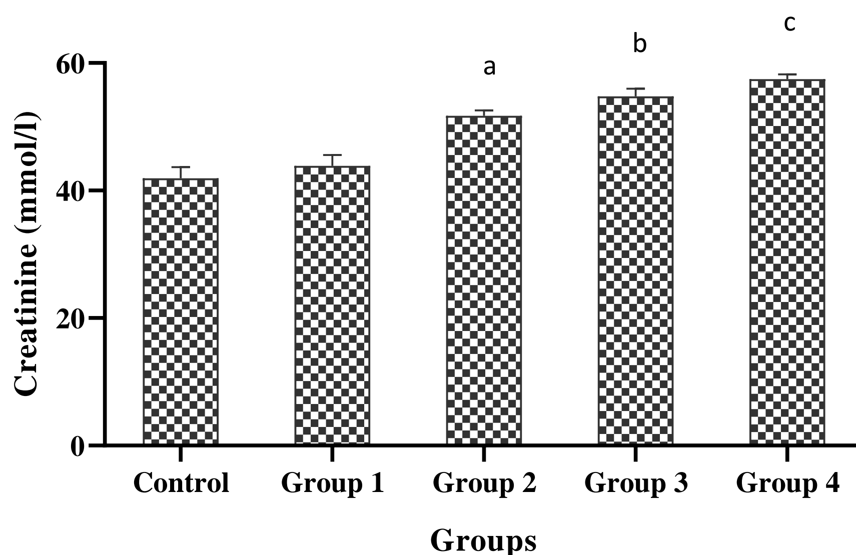

Figure 3:

Effect of *A. vogeliana* leaf extract on the blood creatinine level

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control (p<0.05)

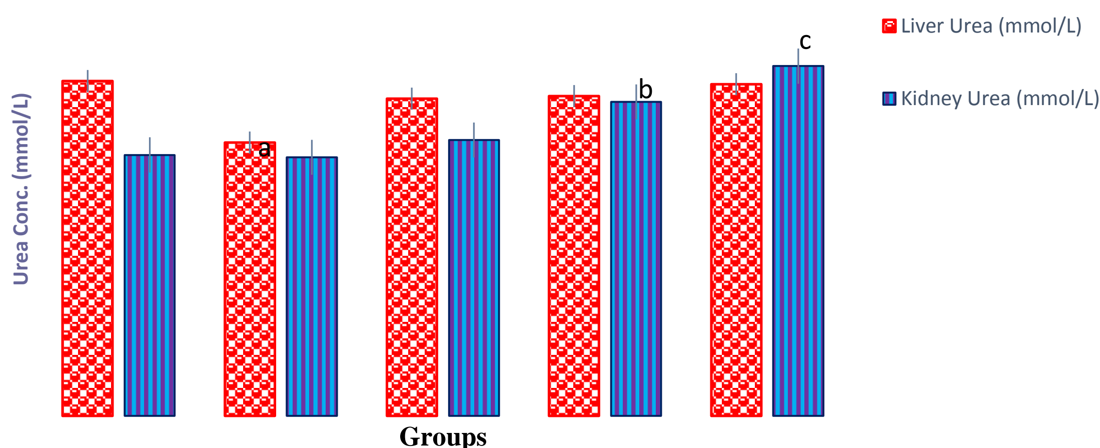

Figure 4: **Effect of *A. vogeliana* leaf extract on urea level in liver and kidney.**

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control (p<0.05)

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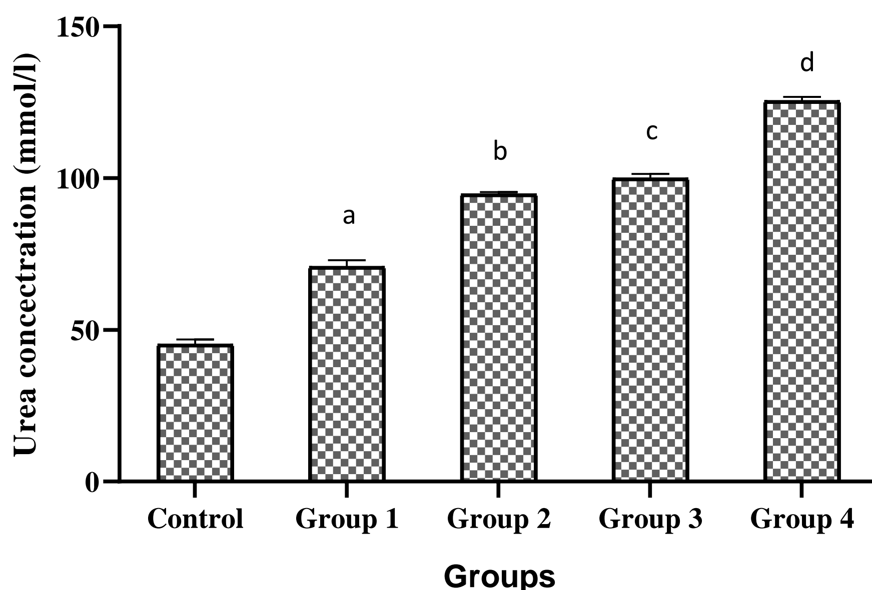

Figure 5: **Effect of *A. vogeliana* leaf extract on the blood Urea level**

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control ($p<0.05$)

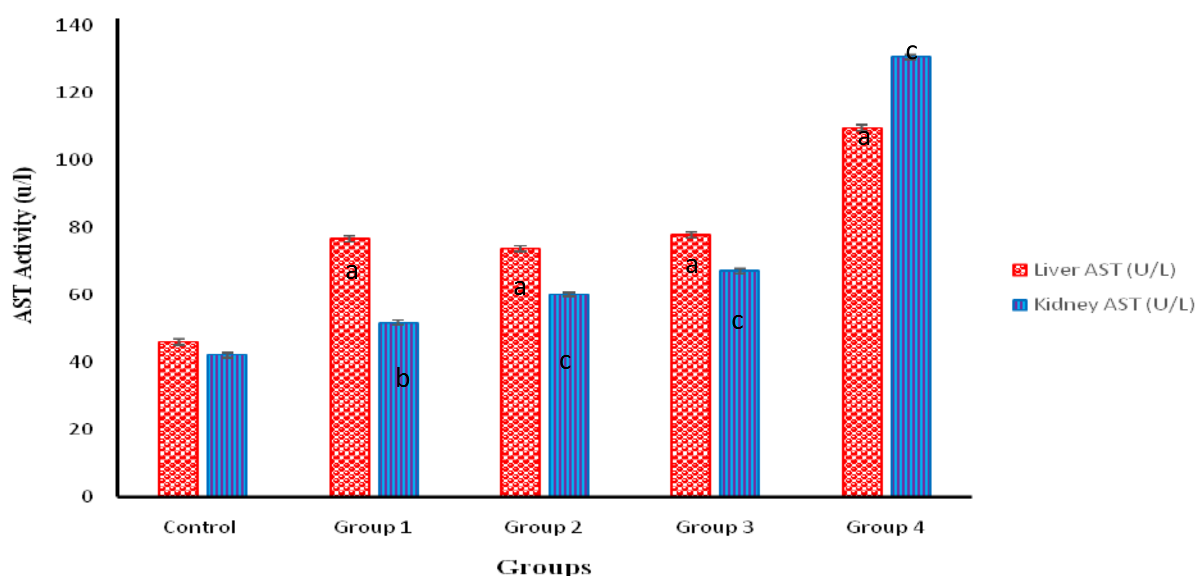

Figure 6: **Effect of *A. vogeliana* leaf extract on AST activity in liver and kidney.**

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control ($p<0.05$)

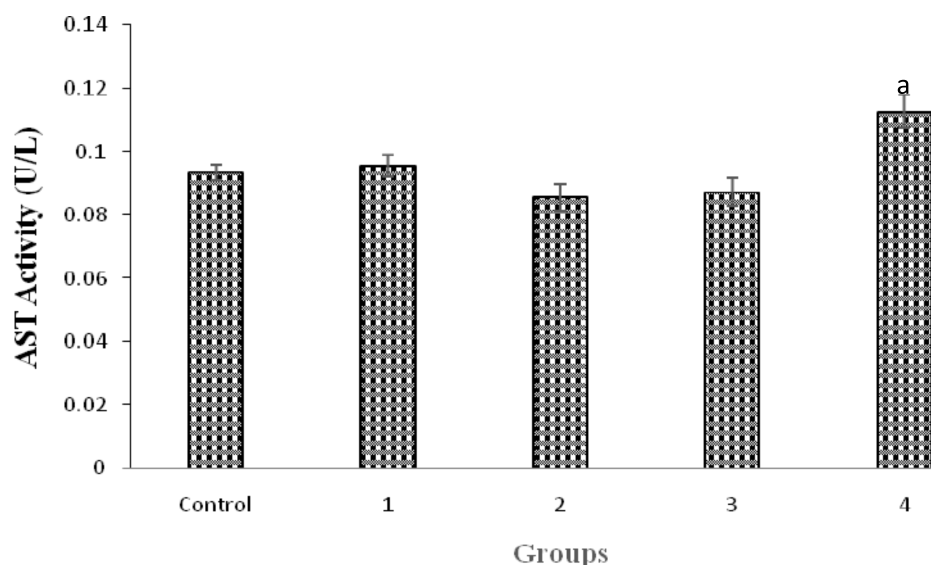

Figure 7: Effect of *A. vogeliana* leaf extract on the blood AST level

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control ($p<0.05$)

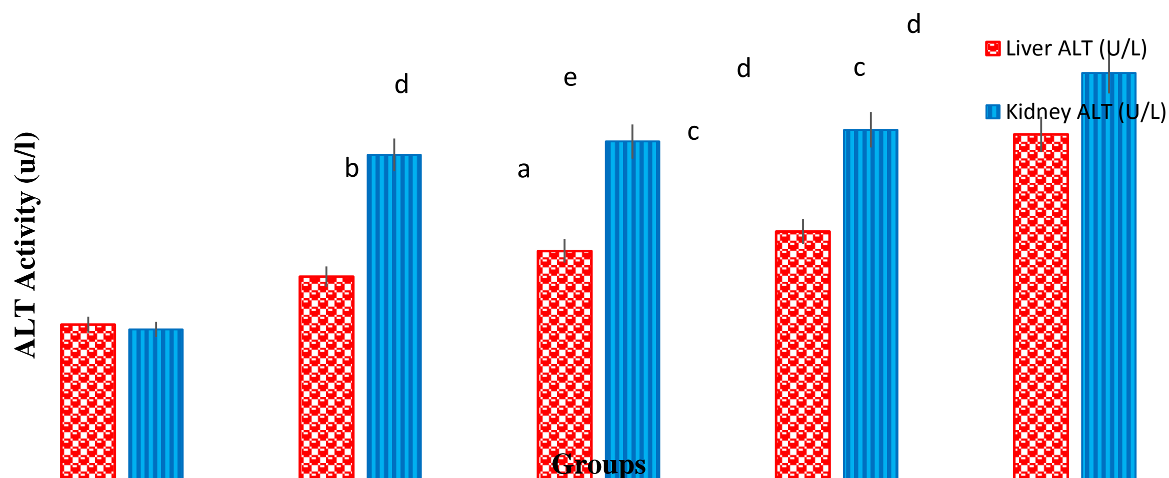

Figure 8: Effect of *A. vogeliana* leaf extract on ALT activity in liver and kidney

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control ($p<0.05$)

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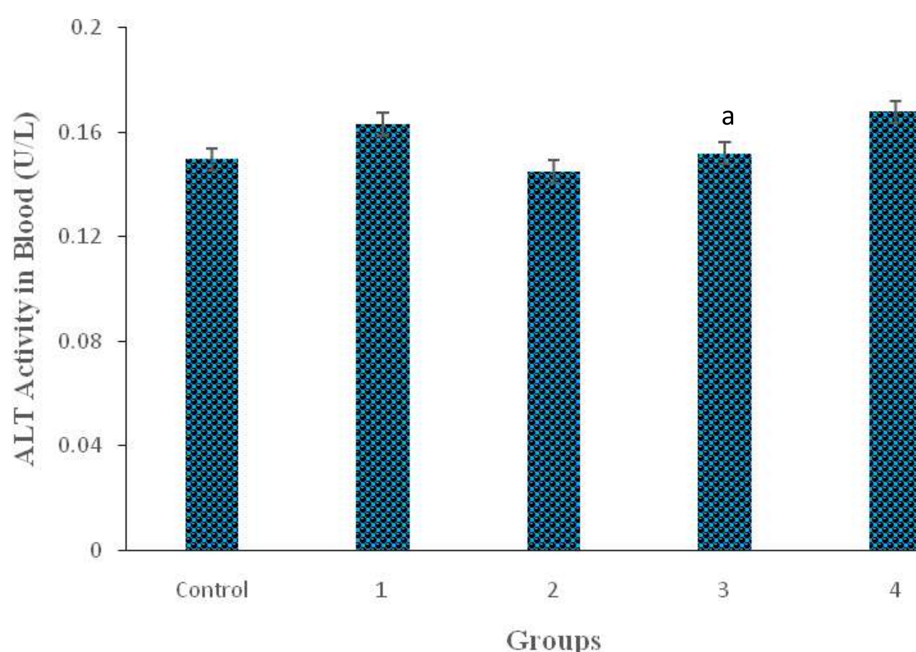

Figure 9: Effect of *A. vogeliana* leaf extract on the blood ALT level

Control:- dH_2O; Group 1:- 200 mg/kg; Group 2:- 400 mg/kg; Group 3:- 600 mg/kg; Group 4:- 800 mg/kg.Values are mean ± Standard deviation, n = 3. Experimental groups are compared with the control. Bars with alphabet are consider significantly different from the control ($p<0.05$)

Table 1 Phytochemical constituents of *A. vogeliana* leaf extract

Phytochemical	Result
Alkaloids	+++
Flavonoids	+
Cardiac Glycosides	-
Phenols	+
Saponins	+
Tannins	-
Triterpenes	+
Steroids	+

NOTATION: + shows presence, - shows absence

Table 2 Chemical constituent of n-hexane fraction

S/N	Compounds	Mol. Formula	RT (min)	Area%
1	Alkylbenzenes	$C_{11}H_{16}$	7.364	3.52
2	Alkylbenzenes	$C_{10}H_{14}$	8.621	4.40
3	N-BOC-trans-3-methylproline	$C_6H_{11}NO_3$	9.628	2.44
4	Venlaflaxine	$C_{17}H_{27}NO_2$	9.753	3.35
5	tetradecamethyl-1-cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	11.361	3.76
6	4-methyl-6-phenylpyrimidin-2-yl	$C_{11}H_{12}N_4$	12.08	1.62

7	2,4-dimethyl-2,3-heptadien-5-yne	C_9H_{12}	12.349	2.13
8	Alkylbenzene	C_9H_{12}	13.488	3.05
9	Octamethylcyclotetrasiloxane	$C_8H_{24}O_4Si_4$	13.650	2.42
10	L-leucine, N-acetyl-1-phenylalanine, butylester	$C_{10}H_{13}NO_2$	15.771	2.29
11	Alkylbenzene	$C_{10}H_{14}$	17.028	2.30
12	Alkylbenzene	$C_{10}H_{14}$	26.993	4.09
13	Alkylbenzene	$C_{10}H_{14}$	31.785	3.95
14	n-hexadecanioc acid	$C_{16}H_{32}O_2$	37.295	24.01
15	6-octadecenioc acid	$C_{18}H_{34}O_2$	38.497	36.68

Table 3 Chemical Constituents of DCM Fraction

S/N	Library Compounds	Mol. formula	RT (min)	Area%
1	4-chlorobenzylsulphonamide	$C_7H_9SO_2N$	4.700	1.59
2	Acetamide-methyl-N-(4-[2-Hydroxyethyl]-1- piperdyl)-2-butynl]	C_2H_5O	6.613	1.78
3	Iodomethane	CH_3I	9.464	1.61
4	2-amino-4-chlorobenzonitrile	$C_7H_5ClN_2$	10.377	1.19
5	1-Silacyclopenta-2,4-diene	$C_{14}H_{18}Si$	11.229	0.77
6	Oxypurinol	$C_5H_4N_4O_2$	13.717	15.68
7	7-dione-2,6- diazaspiro (4,4) nonane-3	$C_7H_{10}N_2O_2$	14.173	4.13
8	Biphenylene	$C_{12}H_8$	15.188	2.13
9	4-chlorophenylhydrazine	$C_6H_7ClN_2$	16.356	2.61
10	Acenaphthene	$C_{12}H_8$	16.762	0.97
11	Diethyl-2-acetylglutarate	$C_{11}H_{18}O_5$	17.929	0.64
12	1H-Indene-1-methanol	$C_{10}H_{10}O$	18.538	2.38
13	4-chlorophenylhydrazine	$C_6H_7ClN_2$	18.792	1.94
14	2,2-Dimethylcyclohexan-1,3-dione dioxime	$C_8H_{14}NO_2$	20.001	11.21
15	2,4,6-cycloheptatrien-1-one	$C_7H_6O_2$	20.619	4.96
16	n-octyl succinic anhydride	$C_{12}H_{20}O_3$	21.076	1.37
17	3,6-dibutyl-2,5-dimethylpyrazine	$C_{14}H_{24}N_2$	22.142	0.57
18	Silane,(E)-trimethyl[1-(1-methylethenyl)-1-butenyl]	$C_{10}H_{20}Si$	22.396	1.98
19	2-methoxyl-1,1’-bisphenyl	$C_{15}H_{13}NO$	23.208	0.78
20	4,4’-dimethyl-2,2’-bispyridine	$C_{12}H_{12}N_2$	23.817	2.33
21	1-(5-(1-oxoethyl) pyrazin-2-yl)-2,2-dimethypropan-1-one	$C_{11}H_{10}N_2O_2$	24.680	3.12
22	p-chlorophenylallyl ether	C_9H_9ClO	25.238	2.60
23	2-(methylthio)benzoic acid	$C_8H_8O_2S$	25.847	2.89
24	1H-Indene, 2,3,3a,4,7,7a-hexahydro-1H-isoindole	C_9H_7	26.304	1.85
25	2,6-bis(1,1-dimethylethyl) phenol	$C_{14}H_{22}O$	27.370	4.02
26	8-hydroxylnaphthoic acid, methyl ester	$C_{11}H_{12}O_3$	27.827	2.08
27	3-dibenzofuranamine	$C_{12}H_9NO$	28.639	0.62
28	5-(4-methylphenyl) furan-2-carboxylicacid	$C_{13}H_{12}O_3$	28.994	1.84
29	1,3 dinitrobenzene	$C_6H_4N_2O_2$	30.365	1.11
30	2-amino-5-bromothiazole	$C_3H_3BrN_2S$	31.177	0.84
31	Anthracene	$C_{14}H_{10}$	32.090	3.04
32	2-methyl-1,1’-biphenyl	$C_{13}H_{12}$	33.207	3.14
33	2,2’-ethanethiol	C_2H_5SH	33.664	2.01
34	2-(2-chlorophenylimino) thiazolidin-4-one	$C_{10}H_9SClN_2$	34.375	2.03
35	2-Exomethyl-3-methylene benzonorbornene	$C_{15}H_{15}$	35.288	0.86
36	2-amino-4-triflluoromethyl- [1,3] thiazine-6-thione	$C_8H_9NF_3$	36.709	4.15
37	5-methyl-1-phenyl-1,5-dihydropyrazolo[3,4-d] pyrimidin-4one	$C_{12}H_{10}N_4O$	38.486	0.90
38	1-chloromethyl-3-spirocyclohexyl-3,4-dihydroisoquinoline	$C_{12}H_{14}ClN$	39.953	0.94

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39	phenothiazine-10-propionitrile	$C_{15}H_{12}N_2S$	41.328	0.53
40	4-amino-2-methyl-6,7,8,9-tetrahydropyrrolo[3,4-c] quinoline-1,3-dion	$C_6H_{11}N_3O_7P_2$	46.150	0.73

DISCUSSION

Globally, the use of plants as source of extracts with medicinal and physiological potential is well documented. Various parts of plant have been implicated in the treatment or management of a particular illness or disease. Side effects are often not noticed with the use of plant extracts as against synthetic drugs. People depend on herbal products for healthcare needs and usually use them extensively. The chemical constituents of medicinal plants are closely related to their biological or therapeutic activities (Hashemi *et al.,*2008). The current study is on preliminary phytochemical screening of the leaf methanol extract of *A. vogeliana.* Secondary metabolites such as alkaloids, flavonoids, saponin, triterpene and steroids, with high pharmacological and therapeutic effects were also detected in the extract, while tannin and cardiac glycoside were not detected.

Alkaloids are major secondary metabolites present in various part of plant and with diverse structural types. They have been established to possess many biological activities like antioxidant, antimicrobial, anti-inflammatory, anti-viral, anti-parasitic among others (Wei *et al.,* 2021; Bridi 2018). According to the reports of Rui *et al.,* (2022), alkaloids found in Chinese herbs showed significant protection against Acute Kidney Injury (AKI). The alkaloids employed various mechanisms at improving AKI conditions. In a contrast report, Benbott *et al.,* (2018) established that crude alkaloid extract from *Peganum harmala* induced serious renal damage in rats at high dosage and concluded that the plant is not fit for human and animal consumption. In support of this, Xu *et al.,*(2020) raised concerns about some herbal medicine that possess renal toxicity due to the phytochemicals present especially the alkaloids.

Flavonoids have been reported to have antioxidant activity with high free radical scavenging capacity, coronary heart disease prevention (hepatoprotective), anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities (Pandey and Rizvi, 2009; Kumar and Pandey 2013). Several studies have demonstrated the effects of some flavonoids in mitigating oxidative stress, thwarting DNA damage, reducing inflammatory cytokines, and inhibiting apoptosis-mediated cell death, thereby preventing or improving liver and kidney injuries/diseases (Kumar and Pandey 2013; Vargas *et al.,* 2018; Cao *et al.,* 2022; Lin *et al.,* 2023). Moreover, flavonoids play essential roles in reducing chemical toxicity. This was demonstrated in this study. The level of toxicity that have been reported for this plant was greatly reduced in the current research. Recently, *A. vogeliana* leaf was reported to show toxicity effect in rats (Godwin et al., 2023). Its uses as fish poisoning and cytogenotoxicity activity have also been reported (Uno et al., 2018; Odekanyin et al., 2024).Saponins are naturally occurring surface-active glycosides with a distinctive foaming characteristic (Desai *et al.,* 2009). They are mainly produced by plants but also by lower marine animals and some bacteria. Saponins have been variously attributed with a diverse range of properties, some of which include both beneficial and detrimental effects on human health, pesticidal, insecticidal and molluscicidal activity, allelopathic action, antinutritional effects, sweetness and bitterness, and as phyto-protectants that defend plants against attack by microbes and herbivores (Desai *et al.,* 2009:

Faizal and Geelen, 2013). Saponins are usually not associated with renal damage, but when absorbed, their membrane-permeabilizing effect may possibly be detrimental to the renal epithelial cells (Soetan *et al.,* 2014; Brandao-Costa *et al.,* 2019). The presence of saponins in the *A. vogeliana* leaf extract may be responsible for the mild toxicity noticed in this study. It has been reported that saponins possess both hepato- and nephro-protective potential (Soetan *et al.,* 2014; Brandao-Costa *et al.,* 2019; Golmohammadi *et al.,* 2023).

Infrared spectroscopy and GC-MS studies revealed the presence of fatty acids esters and aromatics in both the n-hexane and DCM fractions. The n-hexane fraction contained more of fatty acids and their esters while DCM fraction containedmore compounds that are aromatic. N-Hexadecanoic acidcommonly known as palmitic acid with molecular formula -$CH_3(CH_2)_{14}COOH$,16:0, is one of the main constituents of the methanolic extract of *A. vogeliana.* Itis found in microorganisms, plants as well as animals (Gunstone *et al.,*2007).Saturated fatty acidprotects against microorganism infections. It also possesses pharmacological activities among other biological activities recorded by Ortega and Compos (2021). It has anti-inflammatory and antioxidant properties (Aparna *et al.,*2012). It is also used as hypocholesteromic nematicide, pesticide, anti-androgenic flavor, haemolytic, 5-Alpha reductase inhibitor (Kumar *et al.,*2010) and potent mosquito larvicide (Rahumann *et al.,*2000). Aside this, its role in cancer management and treatment are well documented in the literatures (Du *et al.,*2023; Zhu *et al.,*2021; Ortega and Compos 2021). Based on the its pharmacological properties, hexadecenoic acid might be responsible for alleviating the toxic effects of some compounds such as 4-chlorobenzylsulphonamide, iodomethane, anthracene and 3-dibenzofuran amine also present in the extract, that have been reported to have a potential to cause tissue damage, thereby causing the changes in the levels of enzymes in kidney and liver.Another fatty acid in n-hexane fraction is 6-Octadecenoicacid, a naturally occurring fatty acid, commonly found in fat and oil of vegetables and some animals (Dinh *et al*., 2021). It is known as petroselinic acid which cosmetic industries has employed as a moisturizing and anti-aging agent in the products. Besides, it also possesses antimicrobial potential as well as anti-inflammatory activity. Its inhibition of arachidonic acid synthesis counteracts the vasoconstrictive effects related to excessive production of arachidonic acid. Apart from its health benefits, 6-Octadecenoicacid was mentioned among the compounds present in the boiled whole wall gecko solution that was responsible for causing degeneration of kidney and liver cells of rats (Esseh *et al*., 2024).

Oxypurinol is a metabolite of allopurinol, and both are inhibitors of xanthine oxidase (Stocker *et al.,*2011). Acting as competitive inhibitor of xanthine oxidase, it blocks the conversion of purines like hypoxanthine, xanthine and oxypurine to uric acid therefore has therapeutic importance in the prevention and treatment of hyperuricemia. High level of urate in blood serum results in several ill-health condition like gout, kidney stone and others. The role of this phytochemical is not so reflected in our study because there was an increase though not significant in the urea concentration in the blood serum. It is also possible that the clearance of oxypurinol in the kidney (Elion *et al.,*1968) led to the increase in the uric acid level in the blood serum.

The toxicological effects of the methanol extract of *A. vogeliana* were investigated in Swiss Albino mice. Biochemical parameters such as the activities of enzymes in tissues and body fluids play major role in disease investigation, diagnosis and liver toxicity (Malomo, 2000; Awe and Banjoko, 2013). Two enzyme biomarkers (AST and ALT) and

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two non-enzyme biomarkers (urea and creatinine) were examined in blood serum, liver and kidney tissue.

Aspartate transaminase and ALT are enzyme biomarkers for liver damage or injury. Increased level of these enzymes in blood serum indicates a reversible or irreversible damage to the hepatocytes. ALT is more of cytoplasmic enzyme than AST which is also present in the mitochondria. ALT is liver specific in certain disease conditions that affects liver and it is easily release into the blood stream when the liver cell membrane is damaged. Mitochondrial AST is only released when there is irreversible damage to the liver cells. In the present study, there were no significant changes in the AST concentration of the blood serum of the mice in all the treated groups except group treated with 800 mg/kg bwt which is significantly difference from the control. Similarly, ALT level in the serum was found to be higher than control only in the group treated with 800 mg/kg bwt but the increase was not significant. These results suggest that the extract cause mild clinical damage to the hepatocytes at higher concentration of the extract.. The amount of AST and ALT in the liver and kidney tissue homogenates of the treated groups were significantly difference from the control but not directly proportional to the amount release to the blood.

Creatinine and urea are non-protein nitrogenous metabolites that are cleared by the body following the glomerular filtration (Nwankpa *et al.,* 2018). Therefore, determination of level of biochemical markers like serum urea, creatinine and other metabolites are vital and essential in the diagnosis of renal failure (Agbasi *et al.,*2010; Yakubu *et al.,*2003). Those metabolites are among the metabolic waste products excreted by the kidney. In this study, the blood urea concentration was observed to increase significantly from the lowest dose (200 mg/kg bwt) to the highest dose (800 mg/kg bwt) in relation to the control. The same results were obtained in the liver and kidney tissue. This is an indication that there is possibility of renal injury in the treated groups. The determination of urea concentration of serum was useful in the diagnosis of acute renal injury while elevation of blood urea level is linked to kidney disease/congenital heart failure and indicates the possibility of severe kidney damage (Gowda *et al.,*2010; Egbung *et al.,*2017). Egbung et al (2017) have reported similar effectof *Vernonia calviana* extract on rats. The level of urea increased drastically in an untreated group that received only *Vernonia calviana* extract. The use of serum creatinine level as an indicator of likely kidney dysfunction has been reported (Nwankpa *et al.,*2018; Egbung *et al.,*2017) and that the increase in the serum creatinine concentration could means a possible damage to the functioning nephrons of the kidney (Gross *et al.,*2005; Antangwho *et al.,*2013). Increase in the level of creatinine as the dose increases suggest that there is possible damage to the kidney of the mice. The possible mechanism as suggested by Nwankpa *et al.* (2018) may be due to glomerular inflammation or interstitial nephritis.

CONCLUSIONS

The study has shown that *A. vogeliana* leaf contained important bioactive constituents like alkaloids, flavonoids, phenolics, saponins, triterpenes and steroids. Administration of the leaf extract in mice caused increase in the AST and ALT levels in the blood serum, showing liver malfunction and creatinine and urea also increased significantly across the groups indicating impairment of kidney functions. The plant extract displayed mild toxicity but further research is needed to examine other biochemical markers of toxicity and histological studies is suggested to establish the extract toxicity.

Declaration of conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Abubakar, A. R., and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *J. Pharm. Bioallied Sci.,* 12(1): 1-10. DOI: 10.4103/jpbs.JPBS_175_19

Agbasi, P. U., Unekwe, P. C., Nweke, I. N., Okechi, O. O, Onyiaroah, I. V., *and*Emerole C. O. (2010). Toxic effects of Nimesulide on kidney of young albino rats. *Res. J. Health Sci.* 1: 17-29.

Anisuzzaman, A.S.M., Sugimoto, N., Sadik, G. and Gafur, M.A. (2001). Sub-acute toxicity study of 5-Hydroxy-2(Hydroxy-Methyl) 4H-pyran-4 One, isolated from *Aspergillus fumigatus*. *Pak. J. Biol. Sci.,* 4: 1012-1015.

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M. (2012). "Anti-inflammatory property of n-Hexadecanioc acid: Structural evidence and kinetic assessment". *Chem. Biol. Drug. Des,*80: 434-439

Atangwho, I. J., Edet, E. E., Egbung, G. E., Uti, D. E., and Ebong, P. E. (2013). Effect of *Vernonia amygdalina* supplemented diet on selected tissues function in diet-induced obese rats. *J. Med. Plants Res.,* 7: 1825-1832.

Awe, E. O. and Banjoko, S. O. (2013) Biochemical and haematological assessment of toxic effects of the leaf ethanol extract of *Petroselinum crispum* (Mill) Nyman ex A.W. Hill (Parsley) in rats. *BMC Complement Altern Med,* 13: 75. doi: 10.1186/1472-6882-13-75.

Bartels, H. and Bohmer, M. (1972). Micro-determination of creatinine. *Clinica Chimica Acta,* 32: 81-85.

Benbott, A., Mahdi, D., Derouiche, K., Karouche, S., and Zellagui, A. (2018) Nephrotoxicity of crude alkaloids extract of Peganum Harmala seeds in rats. *World J Environ Biosci,* 7(3):63- 66

Bribi N. (2018). Pharmacological activity of Alkaloids: A Review. *Asian J. Botany,* 1: 1-6. doi:10.63019/ajb.v1i2.467.

Desai, S. D, Desai, D. *and* Kaur, H. (2009) Saponins and their biological activities. *Pharma Times,* 41: 13–16.

Dinh, T. T., To, K. V. and Schilling, M. W., (2021). Fatty Acid Composition of Meat Animals as Flavor Precursors, *Meat Mus. Biol.* 5(1): 1 – 16. https://doi.org/10.22175/mmb.12251

Du, A., Wang, Z., Huang, T., Xue, S., Jiang, C., Qiu, G., and Yuan, K. (2023). Fatty acids in cancer: Metabolic functions and potential treatment. *MedComm–Oncol,* 2(1): e25. https://doi.org/10.1002/mog2.25

Egbung, G. E., Odey, O. D., and Atangwho, I. J. (2017). Effect of *Vernonia calvoana* extract on selected serum kidney function biomarkers of acetaminophen treated wistar rats. *Asian J. Biochem.,* 12: 99-104.

Ekanem, A. P., Meinelt, T., Kloas, W. and Knopf, K. (2003). Effects of the extracts of two African fish poison plants, *Asystasia vogeliana* and *Tephrosia vogeli*on embryos of zebra fish (*Danio rerio*). *Bull. Environ. Contam. Toxicol.* 71: 551-556.

Elion, G. B., Yu, T. F., Gutman, A. B., and Hitchings G. H. (1968). Renal clearance of oxipurinol, the chief metabolite of allopurinol. *Am. J. Med*., 45: 69– 77.

Esseh, J. N., Ujowundu, F. N., Ibegbulem, C. O., and Okereke, D. C. (2024). Chemical Evaluations of Boiled *Hemidactylus frenatus* and *Scolopendridae cataracta* Solutions and their Histopathological Effects on Livers and Kidneys of Rats. *Inter. J. Biochem. Res. Rev.* 33(5): 116-127.

Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 361-378
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https://doi.org/10.9734/ijbcrr/2024/v33i5882.

Faizal, A. and Geelen, D. (2013) Saponins and their role in biological processes in plants. *Phytochem Rev.* 12: 877–893. https://doi.org/10.1007/s11101-013-9322-4

Fawcett, J. K. and Scott, J. E. (1960). A rapid and precise method for the determination of urea. *J. Clin. Pathol.*13: 156-9.

Gowda, S., Desai, P. B. Kulkarni, S. S. Hull, V. V., Math, A. A. and Vernekar, S. N. (2010). Markers of renal function tests. *N. Am. J. Med. Sci.,* 2: 170-173

Gross, J. L., de Azevedo, M. J., Silveiro, S. P., Canani, L. H., Caramori, M. L.,and Zelmanovitz, T. (2005). Diabetic nephropathy: Diagnosis, prevention and treatment. *Diabetes Care,* 28: 164-176.

Gunstone, F. D., Harwood, J. L., and Dijkstra, A. J. (2007). The Lipid Handbook, 3rd ed. Boca Raton: CRC Press, ISBN 0849396883 | ISBN 978-0849396885

Hashemi, S. R., Zulkifli, I., Hair Bejo, M., Farida A. and Somchit, M. N. (2008). Acute Toxicity Study and Phytochemical Screening of Selected Herbal Aqueous Extract in Broiler Chickens. *Inter. J. Pharmacol.* 4: 352-360.**DOI:** 10.3923/ijp.2008.352.360

Kumar P. P., Kumaravel S., and Lalitha, C. (2010) Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo.Afr. J. Biochem. Res.*4(7): 191-195

Kumar, S. and Pandey, A.K. (2013) Chemistry and Biological Activities of Flavonoids: An Overview. *Sci. World J.* Article ID: 162750. http://dx.doi.org/10.1155/2013/162750

Malomo, S.O. (2000). Toxicological implications of ceftriaxone administration in rats. *Nig. J.* Biochem. Mol. Biol., 15(1): 33 - 38.

Nwankpa, P., Ekweogu, C. N., Egwurugwu, J. N., Chukwuemeka, O. G., Etteh, C. C., Ugwuezumba, P. C. and Emengagha, F. C. (2018) Assessment of Kidney Function Indices in Male Albino Wistar Rats Administered Ethanol Stem Extract of *Dennettia tripetala* (Pepper fruit). *Biochem Pharmacol (Los Angel),* 7: 242. doi: 10.4172/2167-0501.1000242.

Ortega, A. M., and Campos, M. S. (2021) Macronutrients and micronutrients in cancer prevention and treatment. In Oncological Functional Nutrition Jan 1 (pp. 99-124). Academic Press. https://doi.org/10.1016/B978-0-12-819828-5.00003-6

Pandey, K. B. and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* 2: 270-278. DOI:10.4161/oxim.2.5.9498

Popoola, J. O., Adebayo, A. H., Taiwo, O. S., Ayepola, O. O. and Okosodo, E. F. (2017). Studies on Local Knowledge and *In vitro* Cytotoxicity of *Moringa oleifera* L., *Andrographis paniculata* N. and *Asystasia vogeliana* B. Extracts. *Res. J. Appl. Sci.,*12 (2): 180-190.

Rahuman A. A., Gopalakrishman, G., Ghouse, B. S. and Himalayan, B. (2000). "Effect of *Fernonia limonia* on mosquito larva". *Fiteterapia*,71: 553-555.

Reitman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamate oxaloactetate and glutamic pyruvate transaminase. *Am. J. Clin. Pathol*., 28: 56-58.

Rui, Y., Li, S., Luan, F., Li, D., Liu, R. and Zeng, N. (2022) Several Alkaloids in Chinese Herbal Medicine Exert Protection in Acute Kidney Injury: Focus on Mechanism and Target Analysis. *Oxid Med Cell Longev.*, 2022(6):2427802. https://doi.org/10.1155/2022/2427802.

Sofowora, A. (2008) Medicinal Plants and Traditional Medicine in Africa. 3rd edn. Spectrum Books, Ibadan.

Stocker, S. L., Graham, G. G., McLachlan, A. J., Williams, K. M.and Day, R. O. (2011). Pharmacokinetic and pharmacodynamic interaction between allopurinol and probenecid in patients with gout. *J. Rheumatol.,* 38: 904 – 10.

Trease, G. and Evans, S. M. (2002). *Pharmacognosy*. 5th Edition, Bailer Tindal London. pp. 23- 67.

Uno, U. U., Ekpo1, P. B., Onwudiwe, C. F. and Agu, R. C. (2018). Comparative acute toxicity of ichthyotoxic plants (*Tephrosia vogelii, Adenia cissampeloides* and *Asystasia vogeliana*) on farmed African Catfish (*Clarias gariepinus*). *Asian J. Biol.,*5(4): 1-7.

Vaghasiya, Y. K., Shukla, V.J. and Chanda, S.V. (2011). Acute Oral Toxicity Study of *Pluchea arguta* Boiss Extract in Mice. *J. Pharm. Toxicol,*6: 113-123.

Wei, W, J., Chen, X, H., Guo, T., Liu, X, Q., Zhao, Y., Wang, L. L., Lan, J. X., Li, H. W., Si, Y. P. and Wang, Z. M. (2021). A Review on Classification and Biological Activities of Alkaloids from the Genus *Zanthoxylum* Species, *Mini-Rev. Med. Chem.,* ,21(3): 257-294. https://doi.org/10.2174/1389557520666200910091905.

Xu, X., Zhu, R., Ying, J., Zhao, M., Wu, X., Cao, G. andWang, K. (2020) Nephrotoxicity of herbal medicine and its prevention. *Front Pharmacol,* 11: 569551 DOI:10.3389/fphar.2020.569551

Xulu, J. H., Ndongwe, T., Ezealisiji, K. M., Tembu, V. J., Mncwangi, N. P., Witika, B. A., and Siwe-Noundou, X. (2022). The use of medicinal plant-derived metallic nanoparticles in theranostics. *Pharmaceutics*, 14(11): 2437. https://doi.org/10.3390/pharmaceutics14112437

Yakubu, M. T., Bilbis, L. B., Lawal, M. and Akanji, M. A. (2003) Evaluation of selected parameters of rat Liver and kidney function following repeated administration of yohimbine. *Biochemistry,*15: 50-56.Zhu, S., Jiao, W., Xu, Y., Hou, L., Li, H., Shao, J., Zhang, X., Wang, R. and Kong, D. (2021). Palmitic acid inhibits prostate cancer cell proliferation and metastasis by suppressing the PI3K/Akt pathway. *Life Sciences,* 286:120046. https://doi.org/10.1016/j.lfs.2021.120046