Original Research Article

Exploring the Effects of *Asystasia vogeliana* Benth. on Liver Health, Kidney Function, and Lipid Profile in Wistar Rats

ABSTRACT

Asystasia vogeliana is employed in Nigeria traditional medicine to relieve pain and boost blood counts in inflammatory and anemic conditions. This study investigated the effects of long term consumption of *A. vogeliana* leaf on the liver health, kidney function, and lipid profile of female Wistar rats. Acute oral toxicity and LD_{50} were determined. Animals were randomized into three groups (n = 6) and treated as follow: Group 1 (normal control; distilled water), group 2 (250 mg/kg) and group 3 (500 mg/kg) for 21 days. Animals were sacrificed on 22^{nd} day and plasma was analyzed for liver-kidney biomarkers: aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, urea, and creatinine. Also the lipid biomarkers (total cholesterol TC), triglyceride TG, high-density lipoprotein HDL, and low-density lipoprotein-cholesterol LDL) and atherogenic indices were determined. Result showed LD_{50}> 2000 mg/kg. Non-significant difference was observed in ALT activity, total bilirubin, direct bilirubin, and urea levels. Significant elevation in AST and ALP, and a significant decrease in creatinine were observed in the treated group suggesting detrimental effect on the kidneys. TC, TG, and LDL were significantly high whereas HDL was significantly low in the treated group. Similarly, significant increase in non-HDL, atherogenic coefficient (Ac), coronary risk index-1 (CRI-1), and 2 (CRI-2), were observed in the treated groups. Suggesting likelihood for cardiovascular diseases. The study concluded that prolong administration of *A. vogeliana* leaf impacted the kidneys and induced elevated lipid profile sharp decrease in HDL. Therefore, prolonged use of *A. vogeliana* should be discouraged.

Keywords: *Asystasia vogeliana; toxicity;hepato-renal biomarkers; lipid profile; cardiovascular; atherogenic indices.*

1. INTRODUCTION

Medicinal plants are plants containing a substance or group of substances with therapeutic potentials or plants containing substances that can serve as lead molecules for synthesis of new drug(s) (Zhang *et al*., 2023). Different parts of medicinal plants may have different therapeutic purposes based on the phytochemical species they contain. Some of the plant parts used in traditional medicine include the leaves, roots, stems, barks, rhizomes, flowers, fruits, and seeds (Tahir *et al*., 2023). According to the World Health Organization (WHO), medicinal plants constitute a large portion of traditional and modern medicine; and over 80% of the world population rely heavily

on medicinal plants for healthcare maintenance (WHO 2022). In fact, 170 of the 194 WHO Member States have reported the use of traditional medicine. According to the Director General of WHO, traditional medicine is the first port of call to treat many diseases for many millions of people around the world (WHO 2022). Medicinal plants elicit strong bio-therapeutic activities because of numerousphytochemical constituents they containwith each having diverse bio-pharmacological activities (Pandita *et al*., 2021). This explains why they are employed as treatment agents, designer foods, dietary supplements and complementary therapies for reduction, reversal or prevention of metabolic diseases (Bari *et al*., 2021).

Despite notable progress in the manufacture of modern drugs in industrialized world, significant fraction of modern drugs are still being derived directly or indirectly from medicinal plant sources (Guo et al., 2022). Hence, making medicinal plants powerful agent in new drug development especially in the daily emergence of infectious diseases (Guo et al., 2022). There is no limit to the health application of medicinal plants or herbal extracts. For example, Lianhuaqingwen capsule derived directly from the traditional Chinese medicine (TCM) was shown in a multicenter, prospective, and randomized clinical controlled trial, to marginally inhibit SARS-CoV-2 replication (Hu *et al*., 2023).

The global production of medicinal plant natural products (PNPs) has become a multi-million investment (WHO 2022). Unfortunately, not many plants used in traditional medicine have been completely studied to ascertain their medicinal properties or potential toxicity to vital organs or tissues of the body (Agbodjento *et al*., 2023). Consequently, people consume herbal remedies indiscriminately without complete knowledge, understanding or regimentation. This attitude predisposes consumers to greater risks of toxicity, organ damage, and eventual death as a result of sub-lethal effects of plants' toxic principles (Heinrich *et al*., 2022).

Asystasia vogeliana (Acanthaceae) is a multipurpose medicinal plant used traditionally for treatments of several disease conditions such as hepatitis, malaria, gastric disorder, and reversal of female menstrual disorder (Ugwuanyi *et al*., 2020; Popoola *et al*., 2017; Gildas *et al*., 2017). In Nigeria, people with low blood count (due to anemia) often consume the leaf decoction of *Asystasia vogeliana* to restore their blood volume. This study was undertaken to investigate the acute and subacute toxicity potentials of *Asystasia vogeliana* leaf extract using female Wistar rat model.

MATERIALS AND METHODS

Plant Sample Collection and Identification

Fresh leaves of *Asystasia vogeliana* were collected at the Obafemi Awolowo University (OAU) Museum Garden, Ile-Ife, Nigeria. The leaves were identified and authenticated (IFE-17776) at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Ethical Clearance

The ethical approval (IPH/OAU/12/1627) on use of laboratory animals was processed at the Institute of Public Health (IPH), Obafemi Awolowo University, Ile-Ife, Nigeria.

Experimental Animals

Thirty female Wistar rats (100-150 g) were purchased from the Animal Breeding House of the Faculty of Pharmacy, OAU, Ile-Ife, Nigeria. The animals were acclimatized for 2 weeks at the Animal Housing Unit, Department of Biochemistry

and Molecular Biology, OAU, Ile-Ife. The animals were fed with standard rat chow and water *ad libitum*.

Preparation of *A. vogeliana* Crude Extract

Fresh leaves of *A. vogeliana* were air dried, ground, and weighed. The powdered sample (1.13 kg) was soaked in 80% (v/v) ethanol (4.5 L) for 72 h, and filtered. Filtrate was concentrated *in vacuo* on a rotary evaporator to produce crude ethanol leaf extract.

Acute Toxicity Test (ATT) Determination of *A. vogeliana* Crude Extract

ATT was carried out following the limit test method (Kamble and Swamy, 2022). Prior to the test, animals were fasted overnight and weighed. Wistar rats (n = 5) were orally administered a single dose of *A. vogeliana* leaf extract (2000 mg/kg body weight) using intubation cannula. Thereafter, animals were monitored for signs of toxicity or death for 24 h and daily for 14 days.

Sub-acute Toxicity Study of *A. vogeliana* Crude Extract (CE)

Sub-acute test of *A. vogeliana* was carried out using healthy female rats (Kamble and Swamy, 2022). Before the test, the experimental animals were fasted overnight but allowed free access to water. The following day, the animals were weighed on a scale and randomized into three groups (n = 6) and treated orally as follows: Group 1 (control; distilled water 1 ml/100 g), group 2 (250 mg/kg extract) and group 3 (500 mg/kg extract) for 21 days. The animals were given free access to feed and water ad libitum throughout the test period. After the last administration on the 21st day, the animals were fasted overnight but allowed free access to water. Then on the 22nd day, the animals were sacrificed.

Dissection of Animals and Collection of Blood Samples

According to Parasuraman *et al*. (2010), cardiac puncture is recommended for terminal stage experiment to collect a single, good quality, and large volume of blood from the experimental animals. Hence, on the 22nd day, the animals were weighed, and sacrificed via cardiac puncture in a least painful and stress manner, under terminal anaesthesia (Parasuraman *et al*., 2010). Using a 5 ml syringe, the blood sample was taken slowly from the heart ventricle to avoid collapsing of heart into heparinized vials. Blood samples were centrifuged at 3000 rpm (313 x g) for 10 min and the plasma supernatant was collected for biochemical analyses.

Liver and Kidney Function Tests

Aspartate Amino Transferase (AST) Assay

The AST assay was carried out based on colorimetric method (Reitman *et al*., 1957) using commercially available RandoxTM kit. Briefly, to 200 µl buffered substrate R1 (consisting of 100 mM phosphate buffer, pH 7.4, 200 mM L-aspartate, 2 mM α-ketoglutarate), 50 µl plasma were added and mixed. This was incubated at 37 ºC for 30 minutes and allowed to cool. Thereafter, 250 µl of reagent R2 (2 mM 2, 4-dinitrophenylhydrazine) were added and allowed to stand at room temperature for twenty minutes. The reaction was terminated by adding 2500 µl of NaOH solution (0.4 M). Absorbance was measured at 546 nanometer against the reagent blank. AST Activity was extrapolated from the calibration curve provided in the kit and expressed as U/ml.

Alanine Aminotransferase (ALT) Assay

Activity of ALT was determined based on colorimetric method (Reitman *et al*., 1957) using commercially available RandoxTM kit. Briefly, to 200 µl buffered substrate R1 (consisting of 100 mM phosphate buffer, pH 7.4, 200 mM L-alanine, 2 mM α-

ketoglutarate), 50 µl plasma were added and incubated at 37 ºC for thirty minutes and cooled. Then, 250 µl of reagent R2 (2 mM 2, 4-dinitrophenylhydrazine) were added and mixed. After allowing to stand at room temperature for twenty minutes, the reaction was terminated by adding 2500 µl of NaOH solution (0.4 M). Absorbance was read at 540 nanometer against the reagent blank. ALT Activity was extrapolated from the calibration curve provided by the manufacturer in the kit and expressed as U/ml.

Kinetic Determination of Alkaline Phosphatase (ALP)

The activity of ALP in the plasma was determined based on colorimetric method (Recommendations of the German Society for Clinical Chemistry, 1972) using commercially available RandoxTM kit. The working solution was prepared by adding 1 ml of R2 (p-Nitrophenyl phosphate 10 mmol/l) to 9 ml of R1 (Diethanolamine buffer pH 9.8, 1.0 mol/l; Magnesium chloride ions 0.6 mmol/l). This was mixed and protected from direct light. Thereafter, 1 ml of the working solution was added to 20 µl of plasma inside a cuvette and mixed. After 30 seconds of incubation at room temperature, absorbance was measured at 405 nm at 1, 2 and 3 minutes intervals. ALP activity was extrapolated from the standard calibration curve in the manufacturer's kit and expressed as U/l protein.

Determination of Total Bilirubin Concentration (TBC)

TBC was determined using assay kit method (Garber and Jendrassik-Grof, 1981). Exactly 0.2 ml of reagent 1 (sulphanilic acid, 21 mmol/l in 0.17 N hydrochloric acid) was added to both blank and sample test tubes. Then 0.05 ml of reagent 2 (sodium nitrite, 38.5 mmol/l) was added to the sample test tubes but not to the blank. This was followed by addition of 1.0 ml reagent 3 (caffeine, 0.26 mol/l and 0.52 mol/l sodium benzoate) and 0.2 ml of appropriately diluted plasma to blank and sample tubes. The reaction was mixed and allowed to stand for ten minutes at room temperature. Thereafter, 1.0 ml of reagent 4 (0.93 mol/l sodium tartarate and 1.9 N sodium hydroxide) was added to all the test tubes and incubated at room temperature for 20 minutes. Absorbance was read at 578 nanometer against the reagent blank and TBC was calculated as: $\text{Total bilirubin } \left(\frac{\mu\text{mol}}{\text{l}}\right) = 185 \text{ x Absorbance of sample}$

Estimation of Direct Bilirubin Concentration (DBC)

The assay kit method was used to determine the DBC Garber and Jendrassik--Grof, 1981). Two clean test tubes (blank and sample) were arranged in triplicates. Exactly 0.2 ml Reagent 1 (sulphanilic acid, 21 mmol/l in 0.17 N hydrochloric acid) was added to both tubes; and then 0.05 ml of Reagent 2 (sodium nitrite, 38.5 mmol/l) was added to the sample tube only. Then 1.0 ml Reagent 3 (caffeine, 0.26 mol/l and 0.52 mol/l sodium benzoate) and 0.2 ml of appropriately diluted plasma were added to blank and sample tubes. The reaction mixture was vortexed and allowed to stand for 10 minutes at room temperature. Then 1.0 ml of Reagent 4 (0.93 mol/l sodium tartarate and 1.9 N sodium hydroxide) was added to all the tubes, mixed and incubated at room temperature for 20 minutes. Absorbance was read at 578 nm against the blank DBC was calculated as: $\text{Direct bilirubin } \left(\frac{\mu\text{mol}}{\text{l}}\right) = 246 \text{ x Absorbance of sample}$

Determination of Creatinine Concentration (CC)

The creatinine concentration was determined using Randox kits (Bartels *et al*., 1972). Creatinine standard solution (0.1 ml) and plasma (0.1 ml) were dispensed into separate cuvettes labeled as standard and sample. Then 1.0 ml of the working reagent (picric acid and NaOH) was added and the absorbance was measured at 492 nm after 30 sec (A1) and after 2 minutes (A2). The ΔA of the sample or standard was calculated by subtracting A2 from A1 of sample or standard.

Plasma CC was calculated as:

$$\text{Creatinine concentration (mg/dl)} = \frac{\Delta\text{Absorbance of Sample}}{\Delta\text{Absorbance of Standard}} \times \text{Conc. of Standard}$$

Determination of Urea Concentration (UC)

The urea concentration was determined using Randox kits method (Fawcett and Scott, 1960). Exactly 100 µl of Reagent 1 (containing 6 mmol/L of sodium nitroprusside and 1 g/L of urease) were dispensed into three separately labeled test tubes: reagent blank, standard and sample containing 10 µl of distilled water, 10 µl urea standard and 10 µ test sample respectively. The mixture was incubated at 37 °C for 10 min in a water bath. Thereafter, 2.5 ml of Reagent 2 (120 mmol/L of phenol) and 2.5 ml of Reagent 3 (27 mmol/L of sodium hypochlorite) were added and incubated at 37 °C for 15 minutes. Absorbance was measured at 546 nanometer against blank. Plasma urea concentration was calculated as:

$$\text{Urea concentration (mg/dl)} = \frac{\Delta\text{Absorbance of Sample}}{\Delta\text{Absorbance of Standard}} \times \text{Conc. of Standard}$$

Lipid Profile Assays

Estimation of Plasma Triglycerides Concentration (PTC)

The triglyceride level was estimated using Randox kit method (Bassey *et al*., 2018). A working reagent was prepared by reconstituting 15 ml of buffer into one vial. The plasma (0.01 ml) and distilled water (0.01 ml) were pipetted into two separate test tubes (test sample and reagent blank). Working reagent (0.5 ml) was added and incubated at room temperature for 10 min. The same procedure was repeated with 0.01 ml of the standard solution. Absorbance was measured at 514 nanometer within 60 minutes and triglyceride concentration was estimated as:

Triglyceride concentration (mg/dl) = ASample/Astandard × Standard Concentration (mg/dl)

Estimation of Plasma High-Density Lipoprotein Cholesterol (HDL-c) Concentration

The plasma HDL-c concentration was estimated using Randox Diagnostic Kit method (Friedewald *et al*., 1972). The plasma (50 µl) and standard (50 µl) were precipitated using 125 µl of precipitating reagent (0.55 mmol/l phosphotungstic acid and manganese chloride 25 mmol/l). After allowing to stand at room temperature for ten minutes, the suspension was centrifuged at 4000 rpm for ten minutes and supernatant was collected for HDL-c estimation. The supernatant of the sample and standard (0.025 ml) were separately pipetted into clean microplate wells. Reagent solution (250 µl) was added and incubated at room temperature for ten minutes. Absorbance was read at 500 nanometer against reagent blank containing distilled water (250 µl) in place of reagent solution. The plasma HDL-c concentration was calculated as:

HDL-c Concentration (mg/dl) = ASample/Astandard × Standard Concentration (mg/dl)

Estimation of Plasma VLDL-Cholesterol Concentration

The plasma VLDL-c concentration was estimated using standard method (Friedewald *et al*., 1972).

VLDL-c = Triglycerides/5 (mg/dl)

Estimation of Plasma LDL Cholesterol Concentration

Plasma LDL-c was estimated using Friedelwald equation (Friedewald *et al*., 1972).

LDL-c = Total plasma cholesterol – (triglycerides/5) - HDL-c (mg/dl)

Estimation of Atherogenic Indices

Atherogenic Coefficient (AC)

AC, a biomarker for assessing cardiovascular risk, was estimated using mathematical expression (Friedewald *et al*., 1972; Nimmanapalli *et al*., 2016).
AC = (TC – HDL-c)/ HDL-c; Where non-HDL-c = TC – HDL-c.

Estimation of Atherogenic Index of Plasma (AIP)

AIP was estimated based on the arithmetic method (Friedewald *et al*., 1972).
AIP = Log (TG/ HDL-c

Estimation of Non-HDL-C

The non-HDL-c was calculated following standard procedure (Nimmanapalli *et al*., 2016). Non-HDL-c = TC – HDL-c

Estimation of Castelli Risk Index-1

Castelli Risk Index-1, also known as coronary risk index (CRI) or cardiac risk ratio (CRR), was estimated using standard procedure (Cai *et al*., 2017).
CRI-1 = TC/ (HDL-c).

Estimation of Castelli Risk Index-2 (CRI-2)

Castelli Risk Index-2 was also estimated (Cai *et al*., 2017). CRI-2 = LDL-c/HDL-c.

Data Analyses

Data were analyzed using Microsoft Excel 2013 package and GraphPad Prism 5.0. Differences between control and treated groups were determined by ANOVA; and considered significant at $p \leq 0.05$. The values were expressed as Mean ± SEM (n = 3).

RESULTS AND DISCUSSION

Median Lethal Dose (LD_{50}) of *A. vogeliana* Crude Extract

Asystasia vogeliana ethanol leaf extract showed no sign of toxicity or mortality at 2000 mg/kg. Therefore, the LD50 was estimated to be greater than 2000 mg/kg body weight. This suggests that *A. vogeliana* ethanol leaf extract did not produce lethal effect at the tested dose2000 mg/kg. This finding therefore corroborated earlier work using a methanol extract in which an $LD_{50} > 5000$ mg/kg body weight was reported (Ugwuanyi et al., 2020). Our earlier study on *A. vogeliana* showed that the oil isolated from the dichloromethane leaf extract had skin protection and antioxidant activities (Godwin et al., 2022). Ekanem *et al*. (2003) reported the embryonic effects of *A. vogeliana* and *Tephrosia vogeli* on zebra fish (*Danio rerio*); and concluded that the use of *A. vogeliana* and *Tephrosia vogeli* in water bodies could produce damaging effects on fish larvae survival. Acute toxicity is the harmful effect of an agent when administered in a single dose or more, over a period not exceeding twenty four hours (Abebe, 2023; Abebe *et al*., 2021). Subacute toxicity usually occurs as a result of repeated daily dosing of the test agent (Abebe et al, 2021).

Effects of *A. vogeliana* Ethanolic Leaf Extract on Hepato-renal Indices

The effects of *A. vogeliana* ethanol leaf extract on hepato-renal indices (AST, ALT, ALP, total bilirubin, direct bilirubin, urea and creatinine) were presented in Table 1. Groups administered with 250 and 500 mg/kg *A. vogeliana* leaf extract showed a significant increase in AST level (26.27 ± 1.01 and 34.10 ± 2.21 U/L) as compared with control group (18.43 ± 0.71 U/L). In contrast, only group 2 (250 mg/kg) showed significant reductions in ALT and ALP levels when compared with the control group. There was no significant difference in bilirubin and urea concentrations in the treated and control groups. However, group 3 (500 mg/kg extract) had a significant decrease in creatinine (-10.03 ± 1.94 mmol/l) concentration when compared with control group (10.62 ± 3.42 mmol/l).

Table 1: Effects of *A. vogeliana* ethanolic leaf extract on hepato-renal biomarkers

Biomarker	Group 1 (Control,)	Group 2 (250 mg/kg)	Group 3 (500 mg/kg)
AST (U/L)	18.43 ± 0.71	26.27 ± 1.01[a]	34.10 ± 2.21[a]
ALT (U/L)	118.20 ± 5.84	103.40 ± 4.74 [b]	115.2 ±3.21
ALP (U/L)	0.31 ± 0.01	0.12 ± 0.01 [b]	0.27 ± 0.015
Total bilirubin (µmol/l)	112.90 ± 8.15	104.40 ± 8.01	118.0 ± 14.80
Direct bilirubin (µmol/l)	77.10 ± 2.96	71.57 ± 3.83	79.19 ± 3.08
Urea (mmol/l)	14.27 ± 1.22	14.91 ±1.08	13.64 ±0.93
Creatinine (mmol/l)	10.62 ± 3.42	9.57 ± 2.62	-10.03 ± 1.94 [b]

(a): significant increase; (b): significant decrease; AST: Aspartate amino transferase; ALT: Alanine amino transferase; ALP: Alkaline amino phosphatase. Results were expressed as Mean ± SEM (n = 6). Values were significant at $p<0.05$.

The pathological profile (biomarker profile) of a tissue sample is generally considered as a proof of damage of a toxic agent (Nigussie *et al*., 2021). Considering the fundamental structure, high metabolic potential, and resilience in xenobiotics detoxification, the liver is unarguably the most exposed organ (in the human body) to chemical assaults (Ishfaq and Mishra, 2023). Measurement of liver function enzymes (ALT, AST, ALP) is a common clinical practice for ascertaining the liver function health (Lala *et al*., 2021). AST mediates the metabolism of aspartate to oxaloacetate and glutamate. ALT catalyzes the metabolism of alanine to pyruvate during cellular energy production. In healthy animals, both AST and ALT levels are expressed at a very low concentration for housekeeping functions. However, their increase in bloodstream is associated with hepatic or heart damage. In this study however, administration of 250 and 500 mg/kg extract caused a significant rise in plasma AST level; suggesting possible damage to the liver or heart tissues. Also bilirubin assay is employed clinically to monitor disease conditions such as hepatic jaundice, hepatitis, and even anemia (Kalakonda *et al*., 2022). The two main sources of bilirubin are the breakdown of hemoglobin derived from senescent red blood cells supplying 80% of bilirubin. The remainder come from the turnover of heme-containing proteins (e.g. myoglobin, cytochromes, catalase, peroxidase, and tryptophan pyrrolase) found especially in liver and muscle tissues (Hinds and Stec, 2018). This study observed a non-significant difference in bilirubin and urea concentrations between the control and treated groups; suggesting that administration of *A. vogeliana* did not produce destruction of erythrocytes or heme-containing proteins in rat muscles and livers (Kalakonda *et al*., 2022).

Group 3 (500 mg/kg extract) had significant reduction in creatinine level (-10.03 ± 1.94 mmol/l) when compared with the control group (10.62 ± 3.42 mmol/l). Creatinine is a byproduct of muscle metabolism routinely found in bloodstream and eliminated via glomerular filtration. Level of creatinine in the bloodstream as compared with the level in urine is clinically used to assess the glomerular (kidney) function (Gounden *et al*., 2022).

Effects of *A. vogeliana* Leaf Extract on Plasma Lipid Profile

The effect of ethanol leaf extract of *A. vogeliana* on plasma lipid profile was presented in Table 2. There was a dose-dependent increase in total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), and triglyceride. In contrast, there was a

significant decrease in high density lipoprotein cholesterol when compared with the control group. Also there was a non-significant increase in very low density lipoprotein cholesterol (VLDL-c. Suggesting that the ethanol leaf extract of *A. vogeliana* caused plasma lipid dyslipemia and could therefore promote cardiovascular disease when consumed for a long period.

Table 2: Effects of *A. vogeliana* Leaf Extract on Plasma Lipid Profile

Group	Parameters (mg/dl)				
	TC	HDL-c	LDL-c	TG	VLDL-c
Control	127.20±3.03	55.11±5.30	38.21±2.27	164.7±3.59	32.94±0.71
250 mg/kg	236.60±3.41 [a]	46.97±7.22	158.50±9.58 [a]	181.5±6.79	38.14±2.01
500 mg/kg)	274.1±15.40 [a]	35.54±9.07	244.60±32.64 [a]	171.5±5.75	34.30±1.15

a) Significantly higher than the control group; TC: Total cholesterol; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein; TG: Triglyceride; VLDL-c: Very low density lipoprotein

Elevated lipid levels has been implicated in cardiovascular disease, the number one cause of death globally (Morakinyo *et a*l., 2022). Other risk factors including lifestyle, environmental, genetic, dietary exposures could negatively affect cardiovascular health (Morakinyo *et a*l., 2022). In this study, administration of *A. vogeliana* leaf extract caused significant rise in plasma TC, TGs, LDL, and VLDL levels; and significant reduction in HDL-c level. Increase in lipid profile level could be attributed to the stimulation of lipogenesis by the extract. Among the lipid particles, HDL-c is associated with the removal of cholesterol deposits and their transportation to hepatocytes for complete metabolism and removal. High levels of HDL-c is associated with good cardiovascular health, while low HDL-c levels indicate cardiovascular risk (Morakinyo *et a*l., 2022). In this study, however, there was a significant reduction in HDL-c suggesting that recurrent use of *A. vogeliana* may impair cardiovascular health via lipid profile perturbation.

Effect of Ethanol Leaf Extract of *A. vogeliana* on Atherogenic Indices

The effect of ethanol leaf extract of *A. vogeliana* on atherogenic indices was presented in Table 3. Result showed a significant increase in non-high density lipoprotein cholesterol (non-HDL-c), atherogenic coefficient (Ac), coronary risk index-1 (CRI-1), and coronary risk index-2 (CRI-2), respectively levels between the treated and control groups. Suggesting likelihood to promote cardiovascular related diseases. Atherogenic index of plasma (AIP) is an important biomarker for assessing the risk of cardiovascular disease (Ranjit *et al*., 2015). Individuals with high AIP were reported with coronary artery disease (Shen *et al*., 2016; Kutkiene et al., 2018). In this study, there was no significant difference in AIP between *A. vogeliana*-treated groups and control. Whereas a large significant increase in non-HDL-c particles was obtained in the treated groups suggesting possible perturbations in cardiovascular health. Also atherogenic coefficient (AC) was used to measure the level of atherogenic potential of *A. vogeliana*. Increase in AC level is strongly correlated with development of cardiovascular disease. Significant increase in AC was obtained at higher dose (500 mg/kg) of *A. vogeliana* leaf extract. Increases in CRI-1 and CRI-2 in *A. vogeliana* treated rats compared to the control group could be linked to increase in TC and LDL-C. The risks of developing cardiovascular disorder correlate with increase in

plasma levels of TC, TG and LDL-C and decrease in HDL-C level (Wu *et al*., 2018).

Table 3: Effect of ethanol leaf extract of *A. vogeliana* on atherogenic indices

	Parameters (mg/dl)				
Group	Non-HDL-C	AC	AIP	CRI-1	CRI-2
Control	74.92±5.38	1.12±0.12	-0.65±0.13	2.12±0.11	0.81±0.15
250 mg/kg	197.0± 7.33 [a]	3.88±0.42	-0.68±0.10	4.88± 0.42	3.02± 0.33
500 mg/kg	277.8±32.32 [a]	9.13±1.47 [a]	-0.64±0.08	10.13±1.46 [a]	8.21±1.46 [a]

Non-HDL-C: Non high density lipoprotein-cholesterol; AC: Atherogenic Coefficient; AIP: Atherogenic Index of Plasma; CRI-1: Coronary risk index; CRI-2: Coronary risk index-2.

CONCLUSION

In conclusion, *A. vogeliana* ethanol leaf extract had median lethal dose (LD_{50}) greater than 2000 mg/kg body weight. Sub-acute oral administration of the extract for 21 days induced significant lipid profile elevation with a decrease in high density lipoprotein (HDL). This could be detrimental to cardiovascular health. Therefore, prolonged use of A. vogeliana should be discouraged.

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