The Effects of *Asystasiavogeliana*Benth.onWistar Rat Liver Health, Kidney Function, and Lipid Profile

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ABSTRACT

Aims: This study investigated the effects of long term consumption of *A. vogeliana* leaf on the liver health, kidney function, and lipid profile of female Wistarrats. This was with a view to assessing the safety and potential health effects of the plant extract.
Study design: The study employed *in vivo* animal model in Wistar rats.
Place and Duration of Study: Department of Biochemistry and Molecular Biology, ObafemiAwolowo University, Ile-Ife, Nigeria, between June 2019 and February 2020.
Methodology: Acute oral toxicity and LD_{50} were determined. Animals were randomized into three groups (n = 6) and treated as follows: Group 1 (normal control; distilled water), Group 2 (250 mg/kg), and Group 3 (500 mg/kg) for 21 days. Animals were sacrificed on 22nd 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.
Results: The result showed LD_{50}> 2000 mg/kg. A non-significant difference was observed in ALT activity, total bilirubin, direct bilirubin, and urea levels. Significant ($p<0.05$) elevations in AST and ALP, and a significant decrease in creatinine were observed in the treated group, suggesting detrimental effect on the kidney health. TC, TG, and LDL were significantly high, whereas HDL was significantly low in the treated group. Similarly, significant increases in non-HDL, atherogenic coefficient (Ac), coronary risk index-1 (CRI-1), and 2 (CRI-2), were observed in the treated groups, suggesting a likelihood for cardiovascular diseases.

Conclusion: The study concluded that prolonged administration of *A. vogeliana* leaf impacted the kidneys and induced an elevated lipid profile and a sharp decrease in HDL. Therefore, prolonged use of *A. vogeliana* should be discouraged.

Keywords: Asystasiavogeliana; toxicity;hepato-renal biomarkers; lipid profile; cardiovascular;atherogenic indices

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Medicinal plants are plants containing a substance or group of substances with therapeutic potential or plants containing substances that can serve as lead molecules for the synthesis of new drug(s) [1]. 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 [2]. 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 [3]. In fact, 170 of the 194 WHO Member States have reported the use of traditional medicine. According to the Director

26 General of WHO, traditional medicine is the first port of call to treat many diseases for many
27 millions of people around the world [3]. Medicinal plants elicit strong bio-therapeutic activities
28 because of the numerous phytochemical constituents they contain, with each having diverse
29 bio-pharmacological activities [4]. This explains why they are employed as treatment agents,
30 designer foods, dietary supplements, and complementary therapies for reduction, reversal,
31 or prevention of metabolic diseases [5].
32 Despite notable progress in the manufacture of modern drugs in industrialized world, a
33 significant fraction of modern drugs are still being derived directly or indirectly from medicinal
34 plant sources [6]. Hence, medicinal plants are powerful agents in new drug development,
35 especially in the daily emergence of infectious diseases [6]. There is no limit to the health
36 applications of medicinal plants or herbal extracts. For example, a Lianhuaqingwen capsule
37 derived directly from traditional Chinese medicine (TCM) was shown in a multicenter,
38 prospective, and randomized clinical controlled trial, to marginally inhibit SARS-CoV-2
39 replication [7].
40 The global production of medicinal plant natural products (PNPs) has become a multi-
41 million investment. Unfortunately, not many plants used in traditional medicine have been
42 completely studied to ascertain their medicinal properties or potential toxicity to vital organs
43 or tissues of the body [8]. Consequently, people consume herbal remedies indiscriminately
44 without complete knowledge, understanding, or regimentation. This attitude predisposes
45 consumers to greater risks of toxicity, organ damage, and eventual death as a result of the
46 sub-lethal effects of plants' toxic principles [9].
47 *Asystasiavogeliana* (Acanthaceae) is a multipurpose medicinal plant used traditionally
48 for treatments of several disease conditions such as hepatitis, malaria, gastric disorder, and
49 reversal of female menstrual disorder [10-12]. In Nigeria, people with low blood counts (due
50 to anaemia) often consume the leaf decoction of *Asystasiavogeliana*to restore their blood
51 volume. This study was undertaken to investigate the acute and subacute toxicity potentials
52 of *Asystasiavogeliana* leaf extract using a female Wistar rat model.
53
54 ## 2. MATERIAL AND METHODS
55
56 **Plant Sample Collection and Identification**
57 Fresh leaves of *Asystasiavogeliana*were collected at the ObafemiAwolowo University (OAU)
58 Museum Garden, Ile-Ife, Nigeria. The leaves were identified and authenticated (IFE-17776)
59 at the IFE Herbarium, Department of Botany, ObafemiAwolowo University, Ile-Ife, Nigeria.
60 **Ethical Clearance**
61 The ethical approval (IPH/OAU/12/1627) on the use of laboratory animals was processed at
62 the Institute of Public Health (IPH), ObafemiAwolowo University, Ile-Ife, Nigeria.
63 **Experimental Animals**
64 Thirty female Wistar rats (100-150 g) were purchased from the Animal Breeding House of
65 the Faculty of Pharmacy, OAU, Ile-Ife, Nigeria. The animals were acclimatized for 2 weeks at
66 the Animal Housing Unit, Department of Biochemistry and Molecular Biology, OAU, Ile-Ife.
67 The animals were fed with standard rat chow and water *ad libitum*.
68 **Preparation of *A. vogeliana* Crude Extract**
69 Fresh leaves of *A. vogeliana* were air dried, ground, and weighed. The powdered sample
70 (1.13 kg) was soaked in 80% (v/v) ethanol (4.5 L) for 72 hours, and filtered. Filtrate was
71 concentrated *in vacuo* on a rotary evaporator to produce crude ethanol leaf extract.
72 **Acute Toxicity Test (ATT) Determination of *A. vogeliana* Crude Extract**
73 ATT was carried out following the limit test method [13]. Prior to the test, animals were
74 fasted overnight and weighed. Wistar rats (n = 5) were orally administered a single dose of
75 *A. vogeliana* leaf extract (2000 mg/kg body weight) using an intubation cannula. Thereafter,
76 animals were monitored for signs of toxicity or death for 24 h and daily for 14 days.
77
78 **Sub-acute Toxicity Study of *A. vogeliana* Crude Extract (CE)**

79 A sub-acute test of *A. vogeliana* was carried out using healthy female rats [13]. Before the
80 test, the experimental animals were fasted overnight but allowed free access to water. The
81 following day, the animals were weighed on a scale and randomized into three groups (n =
82 6) and treated orally as follows: Group 1 (control; distilled water 1 ml/100 g), Group 2 (250
83 mg/kg extract) and Group 3 (500 mg/kg extract) for 21 days. The animals were given free
84 access to feed and water ad libitum throughout the test period. After the last administration
85 on the 21st day, the animals were fasted overnight but allowed free access to water. Then,
86 on the 22nd day, the animals were sacrificed.

87 **Dissection of Animals and Collection of Blood Samples**

88 According to Parasuraman*et al*. [14](2010), cardiac puncture is recommended for a terminal
89 stage experiment to collect a single, good quality, and large volume of blood from the
90 experimental animals. Hence, on the 22nd day, the animals were weighed, and sacrificed via
91 cardiac puncture in a least painful and stressful manner under terminal anaesthesia. Using a
92 5 ml syringe, the blood sample was taken slowly from the heart ventricle to avoid collapsing
93 the heart into heparinized vials. Blood samples were centrifuged at 3000 rpm (313 x g) for 10
94 minutes, and the plasma supernatant was collected for biochemical analyses.
95

96 **Liver and Kidney Function Tests**

97 **Aspartate Amino Transferase (AST) Assay**

98 The AST assay was carried out based on the colorimetric method [15] using a commercially
99 available RandoxTM kit. Briefly, to 200 µl buffered substrate R1 (consisting of 100 mM
100 phosphate buffer, pH 7.4, 200 mM L-aspartate, 2 mM α-ketoglutarate), 50 µl of plasma were
101 added and mixed. This was incubated at 37 ºC for 30 minutes and allowed to cool.
102 Thereafter, 250 µl of reagent R2 (2 mM 2, 4-dinitrophenylhydrazine) were added and
103 allowed to stand at room temperature for twenty minutes. The reaction was terminated by
104 adding 2500 µl of NaOH solution (0.4 M). Absorbance was measured at 546 nanometer
105 against the reagent blank. AST activity was extrapolated from the calibration curve provided
106 in the kit and expressed as U/ml.

107 **Alanine Aminotransferase (ALT) Assay**

108 The activity of ALT was determined based on the colorimetric method [15] using a
109 commercially available RandoxTM kit. Briefly, to 200 µl buffered substrate R1 (consisting of
110 100 mM phosphate buffer, pH 7.4, 200 mM L-alanine, and 2 mM α-ketoglutarate), 50 µl of
111 plasma were added and incubated at 37 ºC for thirty minutes and cooled. Then, 250 µl of
112 reagent R2 (2 mM 2, 4-dinitrophenylhydrazine) were added and mixed. After allowing it to
113 stand at room temperature for twenty minutes, the reaction was terminated by adding 2500
114 µl of NaOH solution (0.4 M). Absorbance was measured at 540 nanometer against the
115 reagent blank. ALT activity was extrapolated from the calibration curve provided by the
116 manufacturer in the kit and expressed as U/ml.

117 **Kinetic Determination of Alkaline Phosphatase (ALP)**

118 The activity of ALP in the plasma was determined based on the colorimetric method [16]
119 using a commercially available RandoxTM kit. The working solution was prepared by adding
120 1 ml of R2 (p-Nitrophenyl phosphate, 10 mmol/l) to 9 ml of R1 (Diethanolamine buffer pH
121 9.8, 1.0 mol/l; Magnesium chloride ions, 0.6 mmol/l). This was mixed and protected from
122 direct light. Thereafter, 1 ml of the working solution was added to 20 µl of plasma inside a
123 cuvette and mixed. After 30 seconds of incubation at room temperature, absorbance was
124 measured at 405 nm at 1, 2, and 3 minutes intervals. ALP activity was extrapolated from the
125 standard calibration curve in the manufacturer's kit and expressed as U/l protein.

126 **Determination of Total Bilirubin Concentration (TBC)**

127 TBC was determined using the assay kit method [17]. Exactly 0.2 ml of reagent 1
128 (sulphanilic acid, 21 mmol/l in 0.17 N hydrochloric acid) was added to both blank and sample
129 test tubes. Then 0.05 ml of reagent 2 (sodium nitrite, 38.5 mmol/l) was added to the sample
130 test tubes but not to the blank. This was followed by the addition of 1.0 ml reagent 3
131 (caffeine, 0.26 mol/l, and 0.52 mol/l sodium benzoate) and 0.2 ml of appropriately diluted

plasma to the 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 [17]. Two clean test tubes (blank and sample) were arranged in triplicate. Exactly 0.2 ml of 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 of 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 the 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 measured 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 [18]. Creatinine standard solution (0.1 ml) and plasma (0.1 ml) were dispensed into separate cuvettes labelled 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 seconds (A1) and after 2 minutes (A2). The ΔA of the sample or standard was calculated by subtracting A2 from A1 of the 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 the Randox kits method [19]. Exactly 100 μl of Reagent 1 (containing 6 mmol/L of sodium nitroprusside and 1 g/L of urease) were dispensed into three separately labelled 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 minutes 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 a 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 the Randox kit method [20]. 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). A working reagent (0.5 ml) was added and incubated at room temperature for 10 minutes. 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 the Randox Diagnostic Kit method [21]. 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

180 allowing it to stand at room temperature for ten minutes, the suspension was centrifuged at
181 4000 rpm for ten minutes, and the supernatant was collected for HDL-c estimation. The
182 supernatant of the sample and standard (0.025 ml) were separately pipetted into clean
183 microplate wells. A reagent solution (250 µl) was added and incubated at room temperature
184 for ten minutes. Absorbance was measured at 500 nanometer against a reagent blank
185 containing distilled water (250 µl) in place of the reagent solution. The plasma HDL-c
186 concentration was calculated as follows:
187 HDL-c Concentration (mg/dl) =ASample/Astandard × Standard Concentration (mg/dl)
188 **Estimation of Plasma VLDL-Cholesterol Concentration**
189 The plasma VLDL-c concentration was estimated using standard method [21].
190 VLDL-c = Triglycerides/5 (mg/dl)
191 **Estimation of Plasma LDL Cholesterol Concentration**
192 Plasma LDL-c was estimated using Friedelwald equation [21].
193 LDL-c = Total plasma cholesterol – (triglycerides/5) - HDL-c (mg/dl)
194 **Estimation of Atherogenic Indices**
195 Atherogenic Coefficient (AC)
196 AC, a biomarker for assessing cardiovascular risk, was estimated using mathematical
197 expression [21-22].
198 AC = (TC – HDL-c)/ HDL-c; Where non-HDL-c = TC – HDL-c.
199 **Estimation of Atherogenic Index of Plasma (AIP)**
200 AIP was estimated based on the arithmetic method [21].
201 AIP = Log (TG/ HDL-c
202 **Estimation of Non-HDL-C**
203 The non-HDL-c was calculated following standard procedure [22]. Non-HDL-c = TC – HDL-c
204 **Estimation of Castelli Risk Index-1**
205 Castelli Risk Index-1, also known as coronary risk index (CRI) or cardiac risk ratio (CRR),
206 was estimated using standard procedure [23].
207 CRI-1 = TC/ (HDL-c).
208 **Estimation of Castelli Risk Index-2 (CRI-2)**
209 Castelli Risk Index-2 was also estimated [23]. CRI-2 = LDL-c/HDL-c.
210
211 **Data Analyses**
212 The data were analyzed using the Microsoft Excel 2013 package and GraphPad Prism 5.0.
213 Differences between control and treated groups were determined by ANOVA; and
214 considered significant at $P < 0.05$. The values were expressed as mean ± SEM (n = 3).
215

216 3. RESULTS AND DISCUSSION

217
218 **Median Lethal Dose (LD_{50}) of *A. vogeliana* Crude Extract**
219 *Asystasiavogeliana* ethanol leaf extract showed no sign of toxicity or mortality at 2000
220 mg/kg. Therefore, the LD_{50} was estimated to be greater than 2000 mg/kg body weight. This
221 suggests that *A. vogeliana*ethanol leaf extract did not produce a lethal effect at the tested
222 dose of2000 mg/kg. This finding therefore corroborated earlier work using a methanol extract
223 in which an LD_{50} > 5000 mg/kg body weight was reported [10]. Our earlier study on *A.*
224 *vogeliana* showed that the oil isolated from the dichloromethane leaf extract had skin
225 protection and antioxidant activities [24]. Ekanem*et al.* [25] reported the embryonic effects of
226 *A. vogeliana*and *Tephrosiavogeli*on zebra fish (*Daniorerio*); and concluded that the use of *A.*
227 *vogeliana*and *Tephrosiavogeli*in water bodies could produce damaging effects on fish larvae
228 survival. Acute toxicity is the harmful effect of an agent when administered in a single dose
229 or more over a period not exceeding twenty four hours [26-27]. Subacute toxicity usually
230 occurs as a result of repeated daily dosing of the test agent [27].
231 **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 the 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 the 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 $p<0.05$. (#): Normal control Group

The pathological profile (biomarker profile) of a tissue sample is generally considered as a proof of the damage of a toxic agent [28]. Considering the fundamental structure, high metabolic potential, and resilience of xenobiotic detoxification, the liver is unarguably the most exposed organ (in the human body) to chemical assaults [29]. Measurement of liver function enzymes (ALT, AST, and ALP) is a common clinical practise for ascertaining the liver function health [30]. AST mediates the metabolism of aspartate into 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 the bloodstream is associated with hepatic or heart damage. In this study, however, administration of 250 and 500 mg/kg of extract caused a significant rise in plasma AST levels; 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 anaemia [31]. The two main sources of bilirubin are the breakdown of hemoglobin derived from senescent red blood cells, which supply 80% of bilirubin. The remainder comes from the turnover of heme-containing proteins (e.g., myoglobin, cytochromes, catalase, peroxidase, and tryptophan pyrrolase) found especially in liver and muscle tissues [32]. 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 [31].

Group 3 (500 mg/kg extract) had a 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 the bloodstream and eliminated via glomerular filtration. The level of creatinine in the bloodstream as compared with the level in urine is clinically used to assess glomerular (kidney) function [33].

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

275 Effect of the ethanol leaf extract of *A. vogeliana*on the plasma lipid profile is presented in
276 Table 2. There was a dose-dependent increase in total cholesterol (TC), low density
277 lipoprotein cholesterol (LDL-c), and triglyceride. In contrast, there was a significant decrease
278 in high density lipoprotein cholesterol when compared with the control group. Also, there was
279 a non-significant increase in very low density lipoprotein cholesterol (VLDL-c). Suggesting
280 that the ethanol leaf extract of *A. vogeliana* causes plasma lipid dyslipemia and could
281 therefore promote cardiovascular disease when consumed for a long period.
282
283 **Table 2: Effects of *A. vogeliana* Leaf Extract on Plasma Lipid Profile**
284

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

285 a) Significantly higher than the control group; TC: Total cholesterol; HDL-c: high density
286 lipoprotein; LDL-c: low density lipoprotein; TG: Triglyceride; VLDL-c: Very low density
287 lipoprotein
288
289 Elevated lipid levels have been implicated in cardiovascular disease, the number
290 one cause of death globally [34]. Other risk factors, including lifestyle, environmental,
291 genetic, and dietary exposures, could negatively affect cardiovascular health [34]. In this
292 study, administration of *A. vogeliana* leaf extract caused a significant rise in plasma TC,
293 TGs, LDL, and VLDL levels and a significant reduction in HDL-c levels. The increase in lipid
294 profile level could be attributed to the stimulation of lipogenesis by the extract. Among the
295 lipid particles, HDL-c is associated with the removal of cholesterol deposits and their
296 transportation to hepatocytes for complete metabolism and removal. High levels of HDL-c
297 are associated with good cardiovascular health, while low HDL-c levels indicate
298 cardiovascular risk [34]. In this study, however, there was a significant reduction in HDL-c,
299 suggesting that recurrent use of *A. vogeliana*may impair cardiovascular health via lipid
300 profile perturbation.
301 **Effect of Ethanol Leaf Extract of *A. vogeliana* on Atherogenic Indices**
302 The effect of the ethanol leaf extract of *A. vogeliana* on atherogenic indices is presented in
303 Table 3. The result showed a significant increase in non-high density lipoprotein cholesterol
304 (non-HDL-c), atherogenic coefficient (Ac), coronary risk index-1 (CRI-1), and coronary risk
305 index-2 (CRI-2), respectively, levels between the treated and control groups, suggesting the
306 likelihood of promoting cardiovascular related diseases. The atherogenic index of plasma
307 (AIP) is an important biomarker for assessing the risk of cardiovascular disease [35].
308 Individuals with high AIP were reported to have coronary artery disease [36-37]. In this
309 study, there was no significant difference in AIP between *A. vogeliana*-treated groups and
310 controls. Whereas a significant increase in non-HDL-c particles was obtained in the treated
311 groups, suggesting possible perturbations in cardiovascular health. Also, theatherogenic
312 coefficient (AC) was used to measure the level of atherogenic potential of *A. vogeliana*.
313 Increase in AC level is strongly correlated with the development of cardiovascular disease. A
314 significant increase in AC was obtained at higher dose (500 mg/kg) of *A. vogeliana*leaf
315 extract. Increases in CRI-1 and CRI-2 in *A. vogeliana* treated rats compared to the control
316 group could be linked to an increase in TC and LDL-c. The risks of developing
317 cardiovascular disorder correlate with increase in plasma levels of TC, TG and LDL-C
318 and a decrease in HDL-C level [38].

319
320 **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]

321
322 Non-HDL-C: Non high density lipoprotein-cholesterol; AC: Atherogenic Coefficient; AIP:
323 Atherogenic Index of Plasma; CRI-1: Coronary risk index; CRI-2: Coronary risk index-2.

324 CONCLUSION

325 In conclusion, *A. vogeliana* ethanol leaf extract had a median lethal dose (LD_{50}) greater than
326 2000 mg/kg body weight. Sub-acute oral administration of the extract for 21 days induced
327 significant lipid profile elevation with a decrease in high density lipoprotein (HDL). This could
328 be detrimental to cardiovascular health. Therefore, prolonged use of *A. vogeliana* should be
329 discouraged.

330 FUTURE SCOPE OF THE STUDY

331 We strongly recommend further investigations into the mechanisms underlying the alteration
332 of the lipid profile; and correctly establish the exact impact of the lipid profile changes on the
333 cardiovascular health. In addition, a longer study should be conducted to assess the
334 cumulative effects of repeated administration of *A. vogeliana* leaf extract. Also, isolation and
335 identification of the specific bioactive compounds present in the *A. vogeliana* leaf extract
336 should be conducted to have an understanding of their chemical nature. Future studies
337 should also explore other administration routes (such as topical application or inhalation) to
338 determine if the effects on lipid profiles are consistent across different methods of delivery.
339 *A. vogeliana* ethanol leaf extract may also be combined with other compounds or
340 medications known to affect lipid metabolism to study their effects.

341
342

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347
348

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

GA, ABA, MAE = Conceptualization and design; AOO, AMO = Analyses; GA and BRE = manuscript writing.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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