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Original Research Article

Evaluation of The Effects of *Anthocleista vogelii* Leaf Extracts on Some Biochemical and Hematological Indices of Wistar Albino Rats: A Toxicological Study

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ABSTRACT

The continuous toxicological appraisal of *Anthocleista vogelii* is necessary to provide an up-to-date insight for its safe use in the management of various diseases. This study hereby examined the effects of *A. vogelii* on some biochemical and physiological indices in the serum of male Wistar rats. It included four experimental groups, labeled I-IV, each with six animals. The group I rats (control) were administered 1.0 mL of 1% tween-80 and groups II-IV rats were administered 200-800 mg/kg (body weights) of methanol extract of *A. vogelii* leaves (MEAL) daily for four weeks. MEAL caused a significant increase ($p<0.05$) in HDL-C (55.16 ± 6.41 mg/dL) of group IV rats relative to the control (31.33 ± 5.94 mg/dL), and a significant reduction ($p < 0.05$) in PCV (33.18 ± 2.01%), RBC (5.35 ± 0.31×10^6/µL) and Hb (11.06 ± 0.67 g/dL) levels at 800 mg/kg for group IV rats relative to the controls. However, non-significant alterations ($p>0.05$) were observed in some lipid and haematological parameters assessed in groups 1-III rats at 200 and 400 mg/kg doses. Although there were no significant effects on serum K^+ and Cl^- ions among treatment groups, oral administration of 800 mg MEAL/kg body weight of rats caused significant decrease in serum Na^+ (140.22 ± 1.32 mg/dL) and HCO_3^- (14.86 ± 0.42 mg/dL) levels of group IV rats relative to the control values. Evidently, some hematoxicological effects of the plant were found in the study though with positive benefits in the management of hypertension and obesity at high dose.

Keywords: *Anthocleista vogelii*, Biochemical, Hypertension, Obesity, Physiological.

Introduction

The use of herbal drugs as, "Alternative Medicine" is presently witnessing acceptability in developing countries because they are cheap, readily available and reasonably potent. The potency of some herbal drugs for preventive and therapeutic purposes is not in doubt, but the concern is whether they are safe or unsafe for human use. Toxicological studies across the fields of biochemistry, physiology molecular biology and other allied sciences cannot be neglected in this regard. To this end, the systematic uses of anthocleista species in the management of various disease conditions were reported.[1-4] The genus "Anthocleista" which is commonly called "Cabbage Tree", has unbranched shoot system with cluster of broad and elongated leaves at the posterior ends of the stems.[5] A vast population of this plant species, particularly *A. vogelii*, are found in Nigeria with diverse names; as "Apa oro or Sapo" (Yoruba), as "Kwari" (Hausa), as "Mpoto" (Ibos) and as "Urhe'upho" (Urhobos).[6-8]

The anthocleista species are sources of food for animals, and are well-known plants in Africa with both traditional and medicinal applications.[9,10] In Ghana forest region, anthocleista plants are reported to provide support for epiphytic plants, owing to their bumpy and roughly-coarse shoot system.[11] The domestic importance of *A. vogelii* was realized when the wood-ash obtained from it was found to be useful for the production of inks, dyes and tattoos. The dry wood parts were used in joineries. The aerial parts of the plant, especially the branches were used for fishing, hunting, forestry and farming purposes, and the leaves were of utmost importance in tobacco and snuff productions.[12]

Presently, the use of the aerial parts of anthocleista species for medicinal purposes is still widespread since they are lectured to be safe, effortlessly accessible, less costly and effective use. Thus, people living in less developed countries, freely used these plants for medicinal purposes.[4] It has been reported that various species of anthocleista are traditionally employed in the management of different types of pathologies, ranging from injuries, inflammatory disorders, stomach upsets, constipation, malaria fever, sexually transmitted infections and a host of other diseases too numerous to mention.[1,2,4] Specifically, pharmacological reports indicate that *A. vogelii* exhibits antidiabetic, antiplasmodial, antimicrobial, anti-obesity and antioxidant properties respectively, which are currently attributed to intrinsic bioactive compounds.[13-16] Oladimeji *et al* [3] described the effective use of *A. vogelii* by herbal medical professionals in the treatment of male and female infertility problems by the use of a blend of aerial parts of the plant. In Nigeria today, the mixture of aerial parts of *A. vogelii* and other medicinal plants are used by female folks as contraceptives to prevent the development of pregnancy after sexual intercourse.[17] With reference to the antidiabetic property of the plant, a study conducted by Olubemehin *et al.*[18] showed that 250 mg methanolic extract per kilogram body weight caused the inhibition of α-amylase activity; the effect was more pronounced when it was combined with other anthocleista species: *A. djalonensis, A. schweinfurthii and A. nobilis*, respectively.

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Despite the potency of the aerial parts of the plant in the management of various diseases in humans, there is still dearth of information on the toxicity of *A. vogelii*. Hence, acute toxicity assessment using various animal models and diverse parts of anthocleista forms have been proofread with variable LD_{50} values. Mbianctha *et al.*[19] reported a 14-day toxicity investigation using aqueous extract of *A. vogelii* stem in which lethality was found at a dose of 20000 mg/kg body weight in mice model. The predictable LD_{50} of 3162 mg/kg body weight has been reported for ethanol extract of *A. vogelii* stem administered orally to mice for 14 days.[20] In acute toxicity study in which petroleum ether extract of *A. vogelii* leaf was administered orally, no death was recorded when 2000 mg/kg body weight was used for mice. Hence, it was concluded that the LD_{50} value for the plant leaf extract was greater than 2000 mg/kg body weight.[21] Similarly; Apiamu *et al.*[22] showed that the methanolic extract of *A. vogelii* leaf produced no lethality among male Wistar albino rats when 400-3200 mg/kg body weights were administered orally. This suggested that the LD_{50} of the leaf extract was way beyond the maximum dose of 3200 mg/kg body weight used in the study. Comparatively, the LD_{50} value for ethanol extract for *A. vogelii* root was found to be greater than 5.0 g/kg body weight in one study, while LD_{50} value in another study was found to be greater than 6.4 g/kg body weight on the basis of oral route of administration in rats.[7,23] In continuation of the toxicological reporting,[19-23] the present study hereby evaluates the effect of *A. vogelii* leaf extract on some biochemical and physiological parameters in the serum of Wistar albino rats.

Materials and Methods

Collection, Authentication and Preparation of Plant Sample

The plant was collected from the premises of Western Delta University, Oghara and validated as *A. vogelii* by Dr. H.A. Akinnibosun of Plant Biology and Biotechnology, University of Benin, Benin City. The plant specimen with serial number UBHa0258 was deposited in his herbarium. A large quantity of the leaves of the plant were harvested and dried at room temperature (27 ± 2ºC) for two weeks in the laboratory. The leaves were pulverized by means of a warring blender into fine powdery form (913.0 g) and subjected to extraction using 70% methanol by cold maceration technique. It was further concentrated by a rotary evaporator (Buchi Rotavator-R, China) to brownish-green slurry at 40ºC. This was then dried in an open water bath at 40ºC, and weighed to determine the yield. It was kept in a tight container and refrigerated at 4ºC till required.

Experimental Animals

Twenty-four (24) male Wistar rats (130-160 g) were selected for the study. They were procured from the animal unit of Biochemistry Department, University of Benin, Benin City and kept in cages for six weeks in which they were allowed to acclimatize to the new environment for two (2) weeks and subjected to four (4) weeks of treatment with free access to pelleted livestock feed, and water ad libitum with 12-12 h light/dark cycle respectively. The handling and treatment of the rats were done based on the approval ethics for the use and care of animals in experiment of this sort.

Experimental Design

The empirical survey employed OECD guideline for toxicity study. The doses were carefully chosen based on an earlier report by Apiamu *et al.*[22] on the toxicological profile of methanolic extract of *A. vogelii* leaf (MEAL). A total of twenty-four (24) male Wistar rats were randomly divided into four (4) experimental groups of six rats each, labeled I-IV. Group I, which served as the control, received 1% tween-80 while groups II, III and IV received 200, 400 and 800 mg/kg body weight (p.o) of MEAL dispersed in 1% tween-80 solvent. The oral administration was done daily for four weeks by means of a gavage. Thereafter, animals were fasted overnight, sacrificed and blood samples were collected.

Treatment of Blood Samples

Blood samples were obtained by heart puncture by means of a hypodermal syringe and needle and put in EDTA containers. The blood samples collected into EDTA bottles were used for lipid, haematological and electrolyte analyses by automated processes.

Biomonitoring/Experimental Assays

Lipid parameters; total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in serum were determined by means of autoanalyzer COBAS 3. Serum electrolyte levels, (Na^+, K^+, Cl^- and HCO_3^- ions), were determined by means of SFRI-Electrolyte analyzer with ISE-4000 model. Haematological profile comprising RBC, WBC, PCV, Hb, MCV, MHC and MCHC parameters were assessed in whole blood of Wistar rats using ERMA autoanalyzer (PCE- 210N).

Statistical Analysis

Data generated in the present study were expressed as mean ± SEM for the six determinations of each experimental group. They were statistically analyzed using one-way analysis of variance (ANOVA) and turkey multiple comparison (TMC) test. The mean results with probability less than 0.05 ($p < 0.05$) were considered significant.

Results and Discussion

The over-dependence on the use of herbal drugs in the management of various diseases may be necessary for toxicologists to provide safe margins for the use of different medicinal plants. Among the treatment groups, the results for crude MEAL showed no significant difference ($p > 0.05$) on TC, LDL-C and TAG relative to control group, but a marked significant increase ($p < 0.05$) was observed for HDL-C at a peak dose of 800 mg/kg for Group IV rats in relation to the control group (Table 1). This development may be helpful in the therapeutic use of the plant for coronary diseases. Studies have shown that elevated serum levels of TC, TAG and LDL-C are conceivable indications of atherosclerosis and coronary heart diseases, while serum HDL-C levels comparatively function in the circumvention of bad cholesterol accretion in the arterial walls of biological systems thereby improving the risk of coronary heart disease.[24,25] The impact of oral administration of methanolic leaf extract of *A. vogelii* on lipid profile, (TC, HDL-C, LDL-C and TG), of Wistar rats revealed clearly that 800 mg/kg of the plant extract enhances HDL-C levels, as significantly compared with the control group (Table 4.2e). However, the non-significant alterations of TC, LDL-C and TAG levels regardless of the doses of the plant during treatment of rats indicated that there may be no potential risk associated with it. The present study agrees with previous report that *A. vogelii* shows no significant influence on serum TC, LDL-C and TAG levels respectively.[7, 26] Therefore, the present study highlighted that *A. vogelii* may be potentially described as plant with anti-antherogenic property.

The results presented in Table 2 showed that the crude MEAL produced no significant effect ($p>0.05$) on WBC, MCH, MCHC and MCV among the treatment groups relative to their control groups. However, there was marked significant effect ($p<0.05$) on packed cell volume (PCV), red blood cell (RBC) count and Hb content at 800 mg/kg treatment groups exposed to MEAL, as compared with the control group. Therefore, at this dose (800 mg/kg), the physiological function of RBC in the transport of oxygen and carbon (iv) oxide may be negatively affected. Haematological parameters such as RBC, WBC, MCV, MCH, MCHC and a host of others were reported to be of toxicological relevance in the field of clinical biochemistry for diagnosis of diseases, and monitoring of foreign substances, especially drugs and plant extracts, on how they affect the state of health of animals.[27-30] It was further reiterated in scientific reports that the evaluation of haematological indicators were very useful in the determination of degree of harmful impacts of plant extracts on blood

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constituents as well as providing useful clues of blood-related functions of plant extracts under investigation.[30,31] The present study observed a significant decrease ($p<0.05$) on PCV, RBC and Hb in a dose- dependent manner of treatment group in relation to their control groups at 800 mg/kg dose rate, and a non-significant difference, ($p > 0.05$), at 200 and 400 mg/kg dose rates for PCV, RBC, Hb, WBC, MCV, MCH and MCHC was also observed respectively (Table 2).

In accordance with the reports presented by Isaac *et al.*[32] and Etim et al.[33], RBCs, which are carriers of haemoglobin pigments, are physiologically employed in the transportation of gases like oxygen (O_2) and carbon (iv) oxide to tissues of complex biological systems during respiration. Consequently, a marked reduction of RBC reflects the proportionate unavailability of O_2 to tissues and a reduction in the amount of CO_2 transported to the lungs s.[32,34] However, it was promoted in literature that a significant reduction in PCV, Hb, MCH and MCHC are useful indicators of anaemic condition.[33, 35,36] Since there was no significant relationship of these variables, it may be suggested that 800 mg/kg of MEAL may not necessarily cause the animals to be anaemic for the period of subacute treatment, though much work still need to be done. In view of the novelty associated with the present study, there was discrepancy in the data obtained in the leaf extract in relation to the root extract of *A. vogelii*.[37] Therefore, the findings of the present study hereby posited that oral administration of MEAL to Wistar albino rats resulted in no physiologic alterations of haematological parameters at minimal dose of 200 and 400 mg/kg. However, there were harmful effects on the PCV, RBC and Hb levels at 800 mg/kg plant extract, which may require care when used for therapeutic purposes.

Among the electrolytes assessed, it was observed that rats administered 200 mg/kg body weight (group II) and 400 mg/kg body weight (group III) of MEAL showed no significant difference ($p > 0.05$) on serum K^+, Na^+, Cl^- and HCO_3^- ions for the treatment groups as compared with the control (Table3). However, 800 mg/kg of the leaf extract administered orally to Group IV rats indicated a marked significant decrease ($p<0.05$) on serum Na^+ and HCO_3^- ions relative to the control group.

The use of serum electrolytes like Na^+, K^+, Cl^- and HCO_3^- for the evaluation of drug safety is a measurable biochemical parameter to toxicologists.[38] In the present study, these parameters were assessed, and results obtained revealed that there was no significant difference ($p>0.05$) among treatment groups relative to control, in each case, for serum Na^+, K^+, Cl^- and HCO_3^- levels at 200 and 400 mg/kg respectively. At the peak dose (800 mg/kg), however, both serum Na^+ and HCO_3^- ion concentrations showed marked significant reductions ($p < 0.05$) as compared with the control group (Table 4.2g). Serum Na^+ concentration is the chief extracellular electrolyte functionally associated with high blood pressure, designated as "hypertension", through monitoring of the extent of contraction of blood vessels.[39-41] The physiological levels of serum Na^+ and Cl^- ions levels in the body are dependent factors of blood pressure in arteries of complex biological systems.[42] Reasonably, depleted serum Na^+ ion levels stimulates the secretion of renin, which in turn stimulates the expression of angiotensin II, a powerful hormone known to reduce the blood pressure exerted by blood vessels.[42] It was further proven that a significantly increased serum levels of this electrolyte inhibits the secretion of renin in the juxtaglomerular cells and subsequently prevent the expression of angiotensin II in the renal tubules.[43] Consequently, it was reiterated that striking a balance between serum levels of Na^+ and Cl^- may help in the avoidance of life-threatening state of hypo- or hypertension because high levels of Na^+ ions were reported to cause cell dehydration and death.[43,44] Although scientific reports regarding the anti- hypertensive property of *A. vogelii* are still lacking, but the reduced concentration of Na^+ ions in serum may validate the use of the plant in the management of hypertensive patients in a dose-dependent fashion.[4] Therefore, the leaves of *A. vogelii* may be considered to show anti-hypertensive property in the present study as a result of reduced serum Na^+ ion level. Bicarbonate (HCO_3^-), an important buffer system, was reported to physiologically function in the maintenance of acid-base balance of body fluids, especially the pH of blood, where a reduction in serum HCO_3^- levels reflects a lowered pH of the blood, and its occurrence may be due to increased renal excretion or hyperventilation.[45,46] Therefore, the intake of *A. vogelii* extract above 800 mg/kg for the treatment of diseases may be associated with metabolic acidosis.

Table 1: Effect of A. vogelii leaf extracts on serum lipid parameters in male Wistar rats

Parameter	Group I (control)	Group II 200 mg/kg	Group III 400 mg/kg	Group IV 800 mg/kg
TC (mg/dL)	81.72 ± 8.08^a	85.35 ± 6.84^a	62.50 ± 7.91^a	86.82 ± 4.90^a
HDL-C(mg/dL)	31.33 ± 5.94^a	46.70 ± 2.57^a	48.16 ± 3.29^a	55.16 ± 6.41^b
LDL-C (mg/dL)	20.58 ± 6.31^b	10.74 ± 1.24^b	16.51 ± 3.09^b	11.70 ± 2.24^b
TAG (mg/dL)	99.44 ± 7.38^c	91.98 ± 1.92^c	93.40 ± 5.79^c	94.80 ± 5.67^c

*****Experimental results are expressed as mean ± standard error of mean (SEM of six determinations (n=6) per group. The lowercase lettering of different types on the mean values across each row were considered significant at $p < 0.05$.

Table 2: Effect of *A. vogelii* leaf extracts on haematological parameters in male Wistar rats

Parameter	Group I (control)	Group II 200 mg/kg	Group III 400 mg/kg	Group IV 800 mg/kg
PCV (%)	38.18 ± 0.83^a	38.10 ± 1.19^a	34.44 ± 0.85^a	33.18 ± 2.01^b
RBC ($*10^6$/µL)	6.61 ± 0.37^a	6.32 ± 0.25^a	5.48 ± 0.24^a	5.35 ± 0.31^b
Hb (g/dL)	12.73 ± 0.28^a	12.70 ± 0.40^a	11.48 ± 0.28^a	11.06 ± 0.67^b
MCV (fL)	47.28 ± 0.53^a	47.40 ± 1.05^a	48.10 ± 1.39^a	47.30 ± 1.09^a
MCH (pg)	21.15 ± 0.56^b	21.62 ± 0.46^b	22.70 ± 0.42^b	20.84 ± 0.57^b
MCHC (g/dL)	43.95 ± 0.42^c	43.35 ± 0.46^c	43.92 ± 1.06^c	43.68 ± 0.95^c
WBC ($*10^3$/µL)	8.40 ± 0.19^d	9.37 ± 1.25^d	8.98 ± 1.02^d	11.51 ± 3.47^d

*****Experimental results are expressed as mean ± standard error of mean (SEM of six determinations (n = 6) per group. The lowercase lettering of different types on the mean values across each row were marked significant at $p < 0.05$.

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Table 3: Effect of *A. vogelii* leaf extracts on serum electrolytes in male Wistar rats

Parameters	Group I (control)	Group II 200 mg/kg	Group III 400 mg/kg	Group IV 800 mg/kg
K^+	3.90 ± 0.27[a]	4.43 ± 0.20[a]	4.15 ± 0.30[a]	3.49 ± 0.02[a]
Na^+	147.40 ± 1.78[a]	144.60 ± 1.96[a]	141.50 ± 1.84[a]	140.22 ± 1.32[b]
Cl^-	100.52 ± 1.36[b]	98.80 ± 0.86[b]	107.20± 3.25[b]	98.48 ± 0.83[b]
HCO_3^-	18.54 ± 0.79[a]	17.22± 0.88[a]	19.72± 0.72[a]	14.86± 0.42[b]

*****Experimental results are expressed as mean ± standard error of mean (SEM of six determinations (n=6) per group. The lowercase lettering of different types on the mean values across each row were marked significant at $p < 0.05$.

Conclusion

The study was a step forward to express concerns about the positive and negative effects of *A. vogelli* leaves on some biochemical and physiological parameters in male Wistar rats during treatment at high concentrations. The present study revealed that *A. vogelii* leaf possesses anti-antherogenic and anti-hypertensive properties as a result of the assessed lipid and electrolyte parameters and this may be beneficial to obesed individuals. However, increased consumption of the plant may be detrimental to health, as indicated by the haematological parameters assessed. These two-edged developments hereby require further studies to exercise extreme caution in the use of the plant for the management of different diseases highlighted in this study.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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