Original Research Article

Acute and sub-acute toxicity studies of methanol leaf extract of *Asystasia vogeliana* in Albino Wistar rats

ABSTRACT

Aim: To investigate the phytoconstituents of methanol and petroleum leaf extracts of *Asystasia vogeliana* (MLEAV and PLEAV), the median lethal dose (LD_{50}), and the effects of MLEAV on body weight, organosomatic indices in vital organs and erythrocyte membrane of Albino Wistar rats during sub-acute administration.
Place and Duration of Study: Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, in 2017.
Methodology: The crude extracts of MLEAV and PLEAV were used in determining the qualitative and quantitative analyses. The rats were assigned into four groups and dosed orally with distilled water (0.5ml/100g) as Group I, 62.5 mg/kg MLEAV (Group II), 125 mg/kg MLEAV (Group III) and 250 mg/kg MLEAV (Group IV) once daily for 28 days. Blood samples were collected from all the rats via the medial canthus into EDTA bottles for erythrocyte osmotic fragility (EOF) study on day 29. Relative organ-body weight indices of vital organs (spleen, heart, liver and kidney) were also evaluated.
Results: MLEAV and PLEAV showed the presence of saponins, flavonoids, phenols, alkaloids, anthraquinones and steroids. The total phenolic contents of MLEAV (3704.30 ± 44.00) significantly increase ($P = 0.000$) when compared with PLEAV (1349.46 ± 35.25). The LD_{50} of MLEAV is above 5000 mg/kg. There were significant ($P < 0.05$) decreases in the body weights of rats in Groups III and IV from 3rd to 4th week when compared with their baseline weights. There were significant ($P < 0.05$) increases in the relative spleen-body weight in Group IV when compared with other groups. There was no significant change ($P > 0.05$) in the hemolysis of rats in Group II when compared with control group at 0.9% NaCl concentration.
Conclusions: The findings reveal that MLEAV showed better antioxidant capacity than PLEAV, and that 62.5 mg/kg of MLEAV is safe during the sub-acute administration.

Keywords: *Asystasia vogeliana*, acute toxicity, sub-acute toxicity, erythrocyte osmotic fragility, phytoconstituents, rats.

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1. Introduction

Medicinal plants have been the mainstay of traditional herbs amongst rural dwellers worldwide since antiquity to date [1]. The therapeutic use of plants certainly goes back to the Sumerian and the Acadian civilizations in about the third millennium BC [2].
Herbal medicines consist of portions of plants or unpurified plant extracts containing several constituents which sometimes work together synergistically [3]. About 3.4 billion people in the developing world depend on plant-based traditional medicines [1,4].
The plant *Asystasia vogeliana* (Benth) belongs to the family *Acanthaceae*. *Asystasia vogeliana* is a straggling under shrubs. The leaves are up to 7½ by 2½ inches, narrowed at either end. When mature they are nearly globular, with rows of minute cystilitis along the midrib on the upper surface, and petiole 0-½ inch long. The plant has inflorence terminal compound, straggling 6-18 inches long, with ultimate spikes or racemes 1-sides, and bract which is ⅕ inch long [5].
The plant is used medicinally against hepatitis in Nsukka, Enugu State, Nigeria. The plant is fondly called by the people 'Ogwu iba ocha n' anya' translating literarily for a drug used for hepatitis [6].
Osmotic fragility (OF) refers to the degree of hemolysis that occurs when samples of red blood cells are subjected to osmotic stress by being placed in a hypotonic solution. Osmotic fragility is affected by various factors, including membrane composition and integrity as well as the cells' sizes or surface-area to volume ratio [7,8,9]. The osmotic fragility test is common in hematology and is usually carried out to aid diagnosis of diseases associated with red blood cells membrane abnormalities. Some diseases linked to increase in hemolysis include hereditary spherocytosis and hypernatremia, while some linked to decrease in hemolysis include chronic liver disease, iron deficiency anemia, thalassemia, hyponatremia, polycythemia vera and sickle cell anemia [10]. Osmotic fragility assay is a classical, rapid, useful and easy technique that has been used to obtain vital information about the interactions of both natural and synthetic drugs with cellular membrane [11].
There is dearth of information on the phytoconstituents of the leaf, LD_{50} of MLEAV and its effect on body weight, organosomatic indices and erythrocyte membrane of Albino Wistar rats. This study is therefore aimed at investigating the phytoconstituents of the leaf, the LD_{50}, the sub-acute toxicity profile and its effect on erythrocyte membrane.

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2. Materials and methods

2.1 Experimental animals

Inbred Albino female Wistar rats weighing between 100-120 g were obtained from Diamond Research Farm, Nsukka. The animals were housed in the laboratory animal units of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The environmental temperature varied between 18°C and 30°C, and the lighting period was between 15 and 17 hours daily. The animals were given feed and water *ad libitum*. The study was

compliance the guidelines of Animal Experimentation Ethics Committee (AEEC), University of Nigeria, Nsukka (UNEC/ 15 / 78756).

2.2 Plant collection

The leaves of *Asystasia vogeliana* (Benth) were collected from wild in Nsukka, Enugu State, Nigeria. The leaves were authenticated by a plant taxonomist in the person of Mr. Felix Okafor of the Herbarium Unit, Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The voucher specimen number (Px UNN / 0015) was deposited in the department's herbarium for reference purposes.

2.3 Extraction of plant material

Hundred grams (100g) of the powdered plant material were soaked in 500 ml (80 % v/v) petroleum ether for 48 h with intermittent shaking every 2 hours for de fattening. The extract was filtered first by white muslin cloth, and then later filtered with No.1 Whatman filter paper. It was concentrated *in vacuo* and kept at 4°C in the refrigerator. The marc was air dried, reweighed and soaked in 500 ml (80% v/v) methanol for 72 hours at room temperature (26^{0}C - 28^{0}C). The extract was obtained following the same procedure as in petroleum ether. The dried extract was reconstituted in tween-80 to make up test solutions of known concentration.

2.4 Phytochemical screening

The crude extracts MLEAV and PLEAV were dissolved in their respective solvents for complete dissolution and were subjected to qualitative analysis as described by [12,13,14].

2.5 Quantitative phytochemical analyses

2.5.1 Determination of total phenolic contents

Total phenolic contents of MLEAV and PLEAV was estimated using 100 mg of each extract which was later dissolved in 100 ml of distilled water. After then 1 ml of each solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalten reagent and 1.5 ml 20% Na_2CO_3 solution were added to each test tube after then the volume was made up to 8 ml with distilled water. Each test tube was vigorously shaken and left in dark for 2 hours. The absorbance of each sample was read at 765 nm [15]. Estimation the total phenolic content was done using a standard curve obtained from various diluted concentrations of gallic acid.

2.5.2 Estimation of total flavonoids contents

Total flavonoid contents of MLEAV and PLEAV was estimated by the method [16]. An aliquot of each extract 0.1 ml was taken into a test tube and made up to 5 ml with distilled water. After then 0.3 ml of 5% $NaNO_2$ was added to each test tube. Three milliliters (3 ml) of 10% $AlCl_3$ was added to each test tube after 6 minutes, and the absorbance was read at 510 nm. The results were expressed as mg quercetin / g of the plant tissue.

2.5.3 Estimation of tannin content

One hundred microliter (100 µl) of each extract was taken into a test tube and added up to 7 ml with distilled water. To each test tube, 8 mM potassium ferric cyanide and 20 mM ferric chloride prepared in 0.1 M hydrochloric acid were added respectively. The tannin content was read at absorbance 700 nm. Tannic content was expressed as mg of tannin per gram of plant tissue [17].

2.5.4 Determination of total alkaloid content (TAC)

Total alkaloid content MLEAV and PLEAV was quantified by spectrophotometric method [18,19]. Each extract was concentrated to 1mg /ml. One milliliter (1 ml) of each extract was dissolved in 2N HCl and then filtered. Again 1 ml of each solution was transferred to a separating funnel and washed with 10 ml chloroform. The pH of each of these solutions were adjusted to neutral with 0.1N NaOH. Then 5ml BCG solution and 5 ml phosphate buffer were added also to each of these solutions. The mixture was shaken, and the yellow complex formed

was extracted with chloroform by vigorous shaking in each solution. Then each solution was collected in a 10 ml - volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

2.5.5 Determination of total saponins

Total saponin content in crude extract was analyzed spectrophotometrically following the method [20,21]. Ten milligrams of each crude extract were dissolved in 5 ml of 50 % aqueous methanol. Then, 250 µl of aliquot from each crude extract was transferred to a test tube into which an equal volume of vanillin reagent (8%) was added followed by 72 % (v / v) sulphuric acid. Each test tube was shaken vigorously and placed in a water bath adjusted at 60 ^{0}C for 10 mins. Then each test tube was cooled on an ice-cold water bath for 3 to 4 mins and the absorbance was read at 544 nm. The saponin concentration was expressed as mg diosgenin equivalents (DE) per g crude extract.

2.6 Acute toxicity test

Oral acute toxicity and median lethal dose (LD_{50}) of MLEAV was done using OECD Acute Toxic Class Method [22]. MLEAV was used in determining the acute toxicity test based on the results of both qualitative and quantitative phytochemical analyses. The Annex 2b OECD test procedure with a starting dose of 50 mg/kg was adopted. Twelve female Albino Wistar rats (nulliparous) were used, three rats each for testing at 50, 300, 2000, and the limit dose of 5000 mg/kg, respectively. The rats were fasted for 12 hours before the commencement of the test, but drinking water was provided *ad libitium* [22]. The varied doses of the extract were each dissolved in 1 ml of water and administered orally using intubation cannula. The rats were observed for 14 days for signs of toxicity.

2.7 Sub-acute toxicity study

Twenty-eight female nulliparous Albino Wistar rats weighing between 100-120 g were used for the study. The rats were also acclimatized for two weeks. They were assigned into four groups of seven (7) rats per group. The four groups were given the following doses orally once a day for 28 days as described below:

Group 1: Received 0.5 ml / 100 g body weight of distilled water as control
Group 2: Received 62.5 mg / kg of MLEAV
Group 3: Received 125 mg / kg of MLEAV
Group 4: Received 250 mg / kg of MLEAV

The body weights (g) were taken with digital weighing balance (Metler, Germany) a day prior to administration of the extract and recorded as their baseline weight values. Their weights were later taken weekly as the extract administration progressed. Signs of toxicity such as death, depression, loss of appetite were also observed and recorded.

Twenty-four hours after the last dosing of all the groups, blood samples were collected from all the rats through the medial cantus with capillary tubes into sodium ethylene diamine tetra acetic acid (EDTA) bottles for erythrocyte osmotic fragility test.

2.7.1 Relative organ-body weight (ROW) indices

After 28 days of oral administration of MLEAV, the rats were anesthetized under mild ether (diethyl ether, 1.9%) administered by exposure in a closed container by the ''open-drop'' method. Thereafter, the rats were euthanized by cervical dislocation. Vital organs such as spleens, hearts, livers and kidneys were excised, weighed and observed for gross changes.

ROW = Weight of organ ÷ weight of animal X 100 %

2.7.2 The effect of MLEAV on erythrocyte membrane

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The erythrocyte osmotic test of the rats was carried out using the method as described by [23]. One percent (1%) sodium chloride (NaCl) solution was buffered with phosphate solution, Na_2HPO_4 (1.3mg/mg) and $NaH_2PO_4 . 2H_2O$ (0.24mg/mg). Briefly, freshly obtained blood (1ml) from each rat was pipetted into a set of test tubes containing 0.0, 0.1, 0.3, 0.5, 0.7, 0.9g/l of NaCl (pH 7.4), and thereafter carefully mixed and incubated for 30 minutes at room temperature (26°C-28°C). The test tubes were then centrifuged at 3000 rpm for ten minutes using a centrifuge model (Heraeus Christ, USA). The solution containing 0.0 g/L NaCl was regarded as the standard solution.

The supernatant was carefully transferred into a glass cuvette and the absorbance of the supernatant was read with a spectrophotometer at wavelength of 540 nm, distilled water as blank. The degree of hemolysis in the distilled water test tube was taken as 100% and others were read in relation to it. The percentage hemolysis for each sample was calculated using the formula below by [24].

$$\text{Percentage hemolysis} = \frac{\text{Optical density of test solution}}{\text{Optical density of standard solution.}} \times 100\%.$$

A cumulative erythrocyte osmotic curve was obtained by plotting the mean percentage hemolysis for the four groups against the concentrations of the NaCl solution.

2.8 Data Analysis

The data generated were analyzed using One-Way Analysis of Variance (ANOVA) with SPSS software version 25. The variant means were separated with least significant difference (LSD) post hoc test. Probability value less than 0.05 were considered significant. The results were presented as mean ± SEM.

3. Results and discussion

3.1 Results

3.1.1 Phytochemical screening of *Asystasia vogeliana* leaf extracts

Results of the phytochemical screening to identify the various constituents of extracts (MLEAV and PLEAV) are presented in Table 1. MLEAV and PLEAV both showed the presence of the secondary metabolites such as saponins, flavonoids, phenols, alkaloids, steroids and anthraquinones. PLEAV showed the presence of cardiac glycosides, and terpenoids while MLEAV showed the presence of starch. MLEAV percentage yield gave 11.51 % (w / w), while the percentage yield of PLEAV was 10.10 % (w /w).

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Table 1. Phytochemical screening for MLEAV and PLEAV

Phytoconstituents	Inference / color	MLEAV	PLEAV
Saponins	Stable foam was observed	+	+
Flavonoids	Color changed from light yellow to deep yellow	+	-
Tannins	Yellow precipitate was observed	+	+
Phenol	White precipitate was observed	+	+
Alkaloids	Orange precipitate was seen	+	+
Anthraquinones	A rose-pink coloration was observed	+	+
Cardiac glycosides	A brown ring formation at the interphase was seen	-	+
Steroids	Greenish coloration was observed	+	+
Terpenoids	Formation of reddish-violet color was observed	+	+
Starch	A blue-black coloration was observed at the	+	-

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interphase

+ indicated present; - indicated absent

(MLEAV = methanol leaf extract of *A. vogeliana*; PLEAV = petroleum ether leaf of *A. vogeliana*)

3.1.2 Quantitative phytochemical analyses of MLEAV and PLEAV

The quantitative contents of total alkaloids, total flavonoids, total phenolics, saponins and tannins of MLEAV and PLEAV are presented in Figure 1. There were no significant changes ($P > 0.05$) in the saponin and tannins contents of MLEAV when compared with PLEAV. The total phenolic contents of MLEAV (3704.30 ± 44.00) significantly increased ($P = 0.000$) when compared with PLEAV (1349.46 ± 35.25) which is about three times higher in activity. The total alkaloids and flavonoids contents of PLEAV (1015.56 ± 61.17; 165.50 ± 17.54) increased significantly ($P = 0.001$) when compared with MLEAV (443.33 ± 18.36; 21.25 ± 0.25) respectively.

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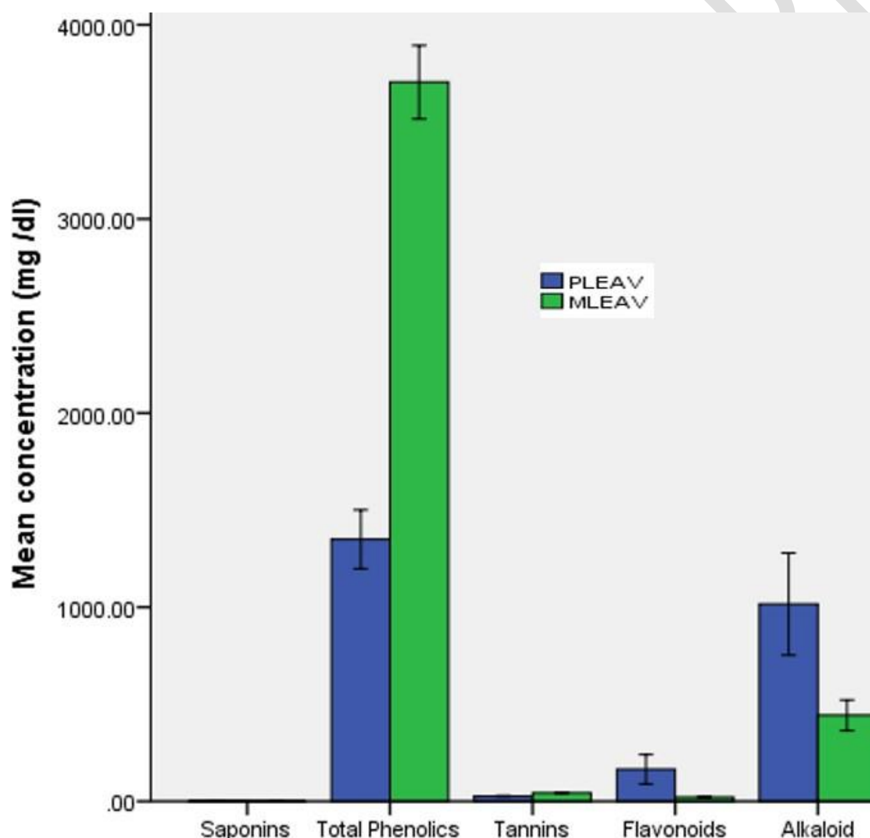

Figure 1. Quantitative determination of total flavonoids, total alkaloids, total phenolics, tannins and saponins contents of MLEAV and PLEAV(n=3) .

3.1.3 Acute toxicity test

The varying doses of MLEAV used for the acute toxicity test showed no adverse clinical effect on the behavioral responses of the tested rats. Physical observation also indicated that all the tested rats showed no signs of changes in the skin, fur, eyes, mucous membrane, and behavioral patterns. There was no mortality in all the tested rats. The rats tolerated the extract up to 5, 000 mg/kg. Thus, the LD_{50} of MLEAV is above 5,000 mg/kg (‘‘Category 5 – Unclassified’’ of the Global Harmonized Classification system [22].

3.1.4 Sub-acute toxicity

3.1.4.1 Body weight

The effects of MLEAV on body weights of the experimental groups are presented in Table 2. Group I (control) showed significant increase ($P < 0.05$) in their body weights from 3rd - 4th week when compared with their body weights in 1st and 2nd weeks and baseline weights. Group II (62.5 mg/kg MLEAV) showed gradual decrease in their body weights though not statistically significant ($P > 0.05$) when compared with their baseline weights. Groups III (125 mg /kg MLEAV) and IV (250 mg / kg MLEAV) showed significant ($P > 0.05$) decreases in their body weights at 4th week which were 48 % and 56 % lower respectively when compared with their baseline weights.

3.1.4.2 Observable clinical signs

The clinical signs of varying doses of MLEAV used for sub-acute toxicity test for 28 days are presented in Table 3. There were no signs of toxicity in Groups I and II. Rats in Groups III and IV showed decreased in body weight, loss of appetite.

3.1.4.3 Relative organ-body weight indices

The effects of MLEAV on the relative organ (liver, spleens, kidneys and heart) body weights of the experimental groups are presented in Figure 2. There was significant ($P < 0.05$) increase in the relative spleen-body weight of Group IV (250 mg / kg) when compared with other groups. However, there were no significant changes ($P > 0.05$) in the relative organ-body weights of kidneys, heart and liver of MLEAV-treated groups when compared with the control group.

3.1.4.4 The effects of MLEAV on erythrocyte membrane

The results of erythrocyte osmotic fragility of albino rats exposed to varying doses of MLEAV for 28 days are shown in Figure 3.

At 0.50% NaCl, there were no significant changes ($P > 0.05$) in the hemolysis of 250 mg/kg when compared with 125 mg/kg and 62.5 mg/kg. The hemolysis of the extract-treated groups significantly decreased ($P < 0.05$) when compared with the control group.

At physiological saline (0.90% NaCl), there was no significant changes ($P > 0.05$) in the degree of hemolysis in rats treated with 62.5 mg/kg MLEAV when compared with control group. The degree of hemolysis in rats treated with 125 mg/kg and 250 mg/kg MLEAV increased significantly ($P < 0.05$) when compared with control group and rats dosed with 62.5 mg/kg MLEAV in 0.9 % NaCl concentration.

3.2 Discussion

MLEAV and PLEAV both showed the presence of the secondary metabolites such as saponins, flavonoids, phenols, alkaloids, steroids and anthraquinones as shown in Table 1. MLEAV and PLEAV could be sources of antioxidants and anti-inflammatory agents due to the presence of flavonoids and phenol [25,26]. MLEAV and PLEAV could also be used therapeutically in managing atherosclerosis, obesity and cancer, as saponins have been shown to have hypolipidemic and anti-cancer activities [27,28]. The high significant increase ($P = 0.000$) in the

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total phenolic contents of MLEAV when compared with the PLEAV as shown in Figure 1 indicates that the methanolic leaf extract fraction of the plant could be used as strong antioxidant and anti-inflammatory agents. This agrees with the findings that phenolics essentially constitute a host of natural antioxidants and anti-inflammatory agents that are used in the management of oxidative damage leading to various degenerative diseases, such as cardiovascular disease, inflammation and cancer [29,30,31]. The MLEAV could also be used in the management of cytotoxic, antitumor, antispasmodic and antidepressant [32]. MLEAV was considered for further investigations in this study due to its high total phenolic contents which comprise flavonoids, phenolic acids and polyphenols.
The LD_{50} of MLEAV is above 5000 mg/kg (''Category 5 – Unclassified'' of the Global Harmonized Classification system [22].
The sub-acute toxicity study of the methanol leaf extract of *Asystasia vogeliana* (MLEAV) was carried out in order to evaluate its effects on body weight, organosomatic indices in vital organs, erythrocyte membrane and to determine the safe dosage with minimum side effects. The results as presented in Table 2 revealed that there were significant ($P < 0.05$) decreases in the body weights of rats in Groups III and IV from 3rd to 4th week post administration of the extract when compared with their baseline weights. However, at the dose of 62.5 mg/kg (Group II), the MLEAV did not have any significant ($P > 0.05$) effect on the body weights of the rats for 28 days oral administration when compared with their baseline weights.

Table 2. Weekly body weight (g) of rats exposed to varying doses of MLEAV for 28 days

Week	Group I (Control, 0.5 ml /100 g)	Group II (62.5 mg / kg, MLEAV)	Group III (125 mg / kg, MLEAV)	Group IV (250 mg / kg, MLEAV)
day zero	98.48 ± 4.000^{b}	132.06 ± 12.791^{a}	108.34 ± 3.757	103.62 ± 2.564
1st	85.74 ± 4.438^{a}	136.60 ± 9.007^{a}	90.42 ± 4.774^{a}	92.42 ± 3.779^{a}
2nd	90.66 ± 4.833^{a}	148.62 ± 11.642^{a}	83.18 ± 21.172^{a}	94.26 ± 6.600^{a}
3rd	98.48 ± 4.090^{b}	131.80 ± 12.804^{a}	65.22 ± 26.884^{a}	43.24 ± 26.532^{a}
4th	114.10 ± 10.888^{b}	128.40 ± 12.524^{a}	56.60 ± 23.494^{a}	45.16 ± 27.736^{a}

Results are presented as means ± standard error of mean, (n = 5). Different superscripts within the same column indicated significance difference between the designated means across the groups ($P < 0.05$). MLEAV= Methanol leaf extract of *Asystasia vogeliana*

These significant ($P < 0.05$) decreases in the body weights of the rats in groups III and IV could be as result of the increases in the bioavailability of saponins and alkaloids in the extract as the

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doses increase. This supports the findings that long-term administration of alkaloids and saponins rich extract significantly decrease food and water intakes in rats [34,35,36].
These negative effects have been ascribed to several properties of saponins such as decrease in feed intake caused by the astringent and irritating taste of saponins [37], reduction in intestinal motility [38], delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity [39], reduction in protein digestibility [40], and damage to the intestinal membrane and inhibition of nutrient transport [41]. Saponins reduce protein digestibility by formation of sparingly digestible saponin-protein complexes [41,42].
The result presented in Table 3 revealed that there were no signs of toxicity in Groups I and II, but signs of toxicity were observed in groups III and IV during the sub-acute oral administration of MLEAV. This showed that 62.5mg/kg of MLEAV demonstrated safety due to low amount of saponins and alkaloids in the extract, while the marked signs of toxicity in groups III and IV was due to high content of saponins and alkaloids which correlated to the significant increase in their bioavailability. The signs of toxicity seen in Groups III and IV were associated with high doses of MLEAV which could also be as a result of high content of alkaloids and saponin in the extract [36].

Table 3. Signs of toxicity of rats exposed to varying doses of MLEAV for 28 days

Week	**Group I (0.5 ml/100g)**	**Group II (62.5mg/kg)**	**Group III (125mg/kg)**	**Group IV (250mg/kg)**
1st	-	-	+	+
2nd	-	-	++	++
3rd	-	-	++	++
4th	-	-	++	++

- No sign of toxicity.
+ Indicated loss of appetite.
++ Indicated loss of appetite, decreased body weight

Relative organ-body weight index is an important requirement in toxicological experiments due to its ability to assess the effects of the xenobiotics on specific organs [43]. Figure 2 revealed that there were significant increases ($P < 0.05$) in the relative spleen-body weight index of Group IV when compared with Groups I and II. This could be as result of splenomegally which may have resulted from extensive degradation of hemoglobin indicative of hemolytic action of the MEAV. Spleen weight has been found to be significantly ($P < 0.05$) correlated with body weight [44,45].

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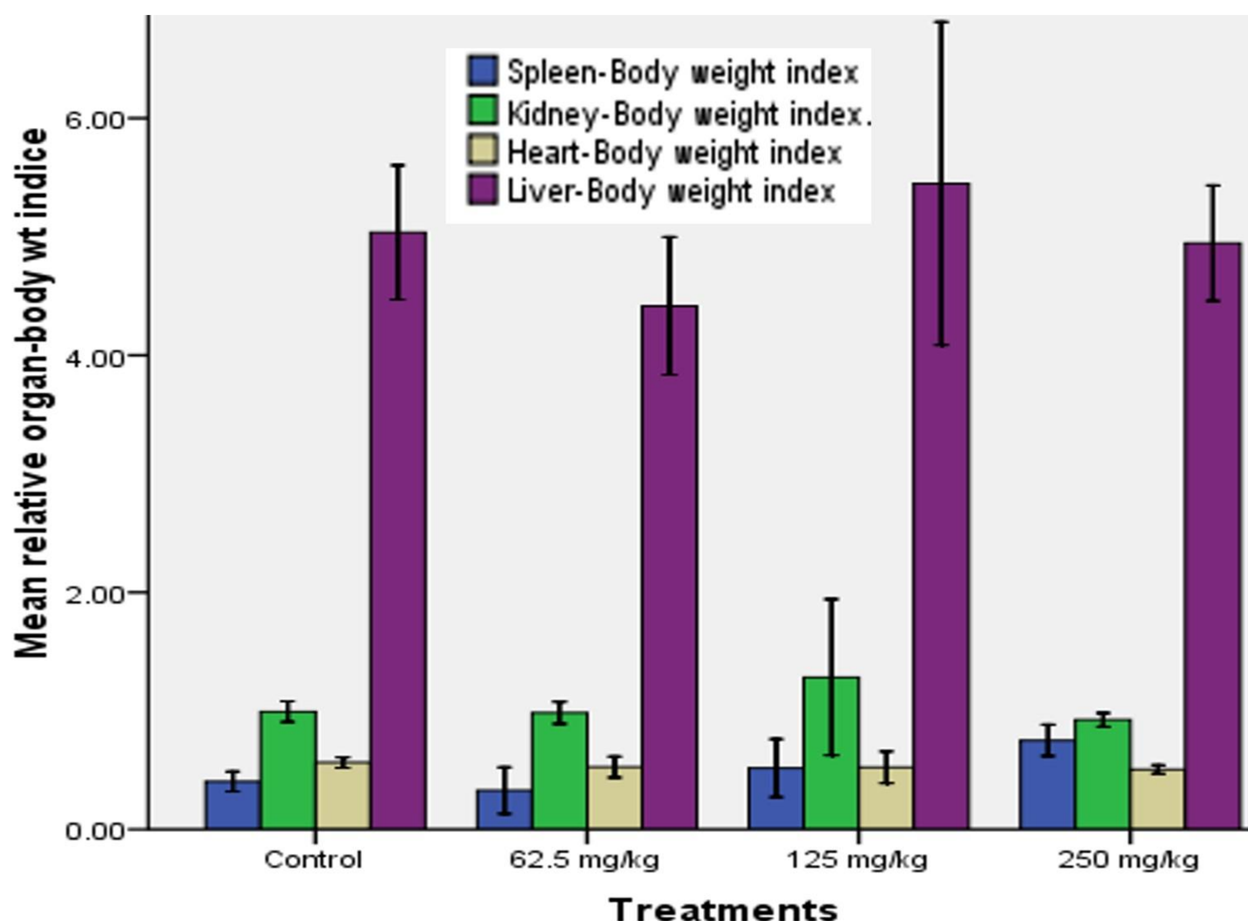

Figure 2. A graph of relative organ-body weight indices of various vital organs of rats exposed to varying doses of methanol leaf extract of *Asystasia vogeliana* (MLEAV) for 28 days. Results are shown as mean ± SEM (n = 3).

The result presented in Figure 3 showed that MLEAV at varying doses produced significant ($P < 0.05$) reductions in erythrocyte osmotic fragility at various NaCl concentrations after 28 days of administration. At physiological saline (0.90% NaCl), there were no significant differences ($P > 0.05$) in the hemolysis of red blood cells between control group and 62.5mg/kg MLEAV-treated group, while there were significant increases ($P < 0.05$) in the hemolysis of red blood cells of 125 mg/kg and 250 mg/kg MLEAV-treated groups when compared with control group.

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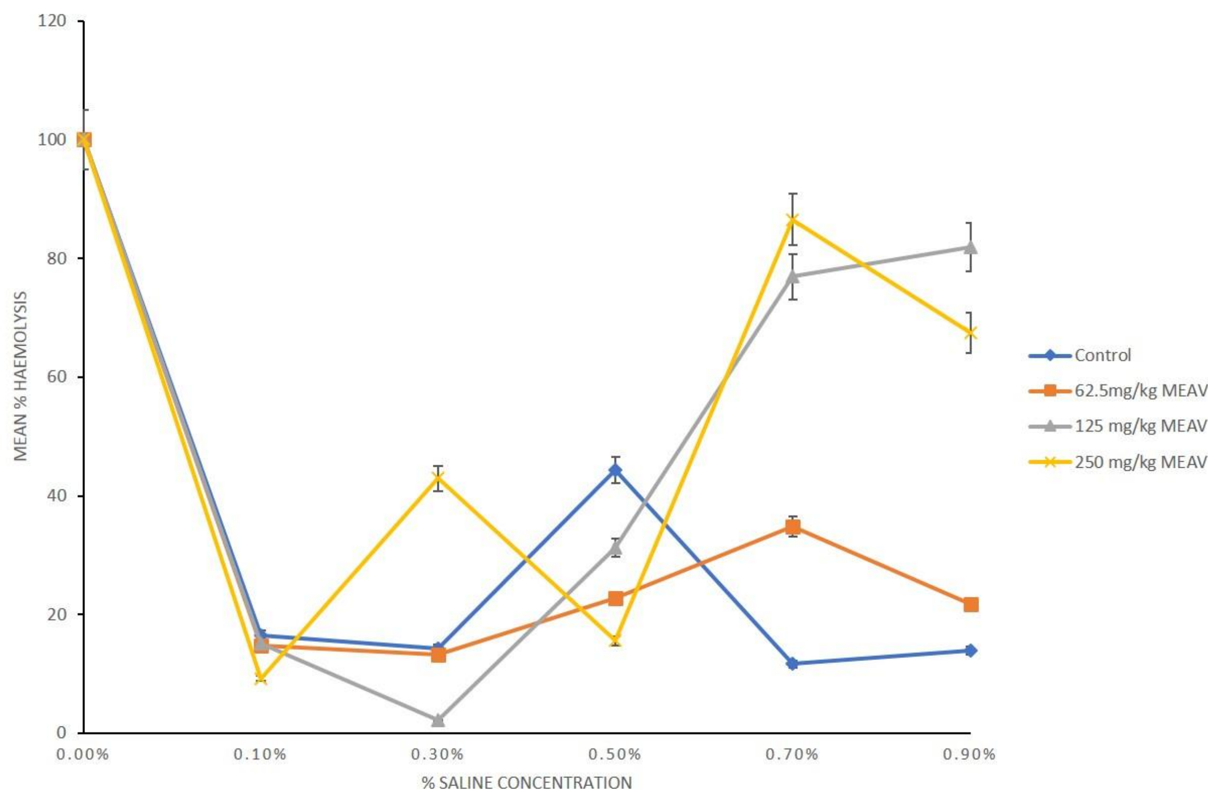

Figure 3. A cumulative erythrocyte osmotic curve of methanol leaf extract of *Asystasia vogeliana* (MLEAV) showing mean percentage hemolysis of the groups against saline concentrations. Results are shown as mean ± SEM (n = 4)

This could be probably attributed to the hemolytic action of saponins and alkaloids of the MLEAV on the red blood cells which hemolyzes more in higher doses of the extract. This supported the reports that saponins and alkaloid rich extract cause hemolysis of red blood cells perhaps by increasing the permeability of the plasma membrane and inhibits smooth muscle activity [41,46,47]. It has been reported that saponin-lysed erythrocytes do not reseal which indicated that saponins damage to the lipid bilayer is irreversible [48].

4. Conclusion

MLEAV at 62.5 mg/kg could be used in stabilizing the erythrocyte membrane in some diseases such as chronic liver disease, iron deficiency anemia, hyponatremia and sickle cell anemia.

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