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Acute toxicity study of *Sphaeranthus bullatus* used as herbal tea in Tanzania

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Sphaeranthus bullatus **aerial part is used by Chaga people in Northern Tanzania as herbal tea. It is usually drunk for the management of diarrhea. In this study acute toxicity of *S. bullatus* was evaluated in order to establish its safety to verify its use as herbal tea. Acute toxicity was performed per Organization for Economic co-operation and Development 423 (OECD) guideline. Six groups of female white albino mice were orally administered as a single dose of 1000, 2000 mg/kg body weight, 3000, 4000 and 5000 mg/kg body weight of *S. bullatus* dichloromethane extract. Internal organ weights, hematological and biochemical parameters of animal blood were determined after 14 days of the experiment. No animal died up to the 14th day of the experiment. Red blood cells, haemoglobin concentration, and hematocrit concentration of the treated animals were significantly higher than the control while white blood cells and platelets were found lower than the control animals. Level of biochemical parameters of the treated animals were not significantly different from the control animals except the level of bilurubin of treated animals which was found higher than control animals. *S. bullatus* dichloromethane extract is therefore established to have safety up to dose of 5000 mg/kg body weight which authenticates its use as herbal tea and for management of diarrhea. However a dose of more than 2000 mg/Kg body weight indicated to lower WBC.**

Key words: *Sphaentharus bullatus*, herbal tea, physical observation, hematology, biochemical.

INTRODUCTION

Herbs have been used to treat ailments for ages (Motaleb et al., 2011). Information on their use passed from generation to generation (Nwachukwu et al., 2010). Recently there has been a growing trend of using infusions or decoction of plant parts to make herbal tea; they are mainly used to treat ailments but also as a healthy alternative to caffeinated beverages such as coffee, tea and cocoa (Shaw et al., 2012; Mainteiga et al., 1997; Marete et al., 2011). One of these plants used in Tanzania for this purpose is *Sphaeranthus bullatus*

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(Asteraceae).

Herbal tea consisting of *S. bullatus* infusions is being used in Northern Tanzania to manage diarrhea; *S. bullatus* is an East African native plant found in wet areas along streams and swamp sites (Machumi et al., 2012). Apart from being used as herbal tea concoction of its leaves are used to treat stomach problems, astheniam and malaria in East Africa (Quantrocchi, 2006; Machumi et al., 2102). It is also used to remove retained placenta to livestock in Tanzania and Kenya (Quantrocchi, 2006). *S. bullatus* contain carvotacetone derivatives which usually have antibacterial activities (Machumi et al., 2012). Moreover, dichloromethane extracts of *S. bullatus* exhibited higher antibacterial activity in the study conducted by Ochanga and Chacha (unpublished paper). However, its acute toxicity has never been done.

In order to establish its safety, *S. bullatus* dichloromethane extract which exhibited higher antibacterial activity was evaluated for acute toxicity. Acute toxicity test has been established as the effective method of testing effects caused by exposure of harmful substances and the tested animals are usually monitored within 14 days of administration of the substance. However, acute toxicity occurs over a very short period of time, usually within 24 h (OECD, 2000).

MATERIALS AND METHODS

Aerial parts of *S. bullatus* were collected from Tengeru in Arumeru districts. The plant parts were identified by trained taxonomist working in the National herbarium, Tropical Pesticide Research Institute (TPRI), Arusha, Tanzania. Voucher specimen was deposited at Nelson Mandela African institution of Science and Technology (NM-AIST), Arusha Tanzania.

Preparation and extraction of plant material

Collected plant sample was air dried under the shade and pulverized. The pulverized material (250 g) was sequentially macerated using dichloromethane for 48 h. The mixtures was filtered through cotton wool then with filter paper No. 1 and dichloromethane was removed through vacuum using rotary evaporator and stored at -20°C until the time of testing.

Experimental animals

A total of 18 female Swiss albino mice (24 to 41 g) were obtained from the Department of Agriculture of the Tropical Pesticide Research Institute (TPRI) in Arusha, Tanzania. Female mice were selected because they are known to be slightly more sensitivity to phytochemical than male (OECD, 2001). Animals were housed in a cage in a well ventilated room and maintained with light for 12 h and darkness for 12 h. They were fed on diet prepared from chick Grower's mash (BCA Grain and Feed Company Limited) and were given water *ad libitum* throughout the study period. Acute toxicity was performed as per organization for Economic Co-operation and Development (OECD) guidelines 423 (OECD, 2001) and was in compliance with the procedure of National Health Research Ethics Sub-Committee of the National Institute for Medical Research of Tanzania (permit number No. NMR/HQ/R.8a/Vol. IX 2163).

Experimental design for acute toxicity

Mice were assigned to six groups of three mice. Group 1 mice were given 1000 mg/Kg body weight, group 2 were given 2000 mg/Kg body weight, group 3 were given 3000 mg/ Kg body weight, group 4 were given 4000 mg/Kg body weight and group 5 were given 5000 mg/Kg body weight of the extracts. Group six were set as control given only feeds and water. All experimental animals were assigned special identification and were exposed to feeds and water in an *adlibitum*. These extracts were orally given as a single dose after 6 days of adaptation to the room environments.

Mode of administration

The albino mice were fasted on water for 4 h, and then weighed. They were then administered a single dose consisting of 5 ml of plant extract dissolve/mixed in polysorbate -80(tween 80). The amount of extract in 5 ml was given in accordance to the weight of each mouse, calculated as follows:

$$\text{Administration volume} = \frac{5\text{mL} \times \text{weight of mouse (g)}}{1000\ \text{g}}$$

Food was given to the mice three hours after administering test extract.

Physical observation

Animals were observed at least after every 30 min during the first 24 h with special attention given to the first 10 h. Observation and recording of any sign of illness continued three times a day for 14 days. Observations of signs like eyelid closure, difference in breathing, change of skin and fur, eye muscle membranes, sleep, general weakness, loss of appetite, diarrhea, change of body weight and mortality.

Hematological and biochemical parameters

At the end of 14th day of the observation of physical parameters animals were sacrificed for hematological and biochemical parameters studies. Briefly animals were sacrificed by restraining them using chloroform in desiccators jar, blood was collected from the experimental animals by cardiac puncture using sterile needle and 5 ml syringe. Blood aliquot was immediately transferred into ethylene-diamine-tetra-acetic acid (EDTA) bottles to prevent coagulation factors in cell-cell and cell-matrix interactions for hematological determinations. Other portion of blood were left in the syringe without EDTA and allowed to clot for biochemical investigation. Clotted blood was centrifuged for 5000 revolution per minute for five minutes to obtain serum for biochemical studies. Red blood cell count (RBC), hemoglobin concentration, hemacrotic (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), absolute content of lymphocyte (LMP), absolute content of neutrophils (NEUT), proportion of whole blood occupied by platelets (PCT), the relative width of the distribution in volume index of the heterogeneity of platelets (PDW), relative distribution width of red blood cell by volume- standard deviation (RDW-SD), relative distribution width of red blood cell by volume, coefficient of variation (RDW-CV), ratio of large platelets (P-LRC), absolute content of monocyte (MONO), absolute content of eosinophils (EO), and absolute content of basophil (BASO) hematological parameters were analyzed. Moreover, biochemical parameters such as alanine

aminotrans liquid, aspartate aminotrans liquid, albumin, total protein, cholesterol, triglycerides liquid, and bilirubin were analyzed for biochemical blood investigation.

Observation of internal organs and calculation of organ - body weight index

Experimental animals were sacrificed using chloroform in the desiccators. Gross observation of the liver, heart, spleen, and kidney were done for treated and control animals to observe any sign of abnormalities. However the weight of animal at the 14^{th} day and weight of organs after dissection were taken and used to calculate organ-body weight index.

$$\text{Organ - body weight index} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100$$

Statistical analysis

Statistical analysis was performed using Statistical. Ink version 8 computer program. Result was presented as Mean ± Standard deviation of mean (SD). One way analysis of variance (ANOVA) was employed for between and within group variation. To compare two variable students T-test was employed. Post hoc (TURKEY HSD) analysis was used to show where the variation occurs. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Physical observation

S. bullatus dichloromethane extract (SBDE) was evaluated for acute toxicity study as per OECD guideline 423. Physical observation of the mice revealed that there was neither mortality nor signs of toxicity in all animals up to 14^{th} day after administering SBDE up to 5000 mg/Kg body weight. It was also observed that the body weights of both treated and control mice increased significantly (Table 1) from the day zero to day 14 ($P < 0.05$). Moreover, there was no significant difference in the change of weights in all mice given doses from 1000 mg/ Kg body weight to 5000 mg /Kg body weight and control mice between the first day of administration of plant extract and 14^{th} day ($P > 0.05$). Since none of the SBDE tested dosage induced mortality or any sign of toxicity, it is therefore suggestive that SBDE is non toxic to a dosage of 5000 mg/Kg body weight which is supported by Kabubii et al. (2015).

Macroscopic examination of internal organs

The macroscopic examination of the internal organs of the mice revealed no differences between the control and the treated mice. In toxicology studies internal organs such as livers, heart, lungs, spleen and kidney are primarily affected by metabolic reaction caused by toxicants (Akindele et al., 2014).

Organ body weight index

Difference in organ weights between treated and untreated mice has widely been accepted to assess toxic effects of foreign substance(s) in the organs (Michael et al., 2007; Nirogi et al., 2013; Piao et al., 2013). In this study, liver, heart, lung, kidney and spleen were considered as recommended for toxicological studies (Akindele et al., 2014). Although there was a variation in organ body weight index between most of organs of mice tested with that of control mice as shown in Table 2, variation was not significant except for the liver of mice given 5000 mg/Kg body weight of the extract ($P > 0.05$, Turkey HSD).

Hematological parameters

The hematological parameters test is used to reveal the adverse effect of the extract in the blood constituents of the animal or determine possible alteration in the level of enzymes, metabolic products and normal functioning of organs (Michael et al., 2007). Results obtained from this study indicated that white blood cells (WBC) count or leukocytes of both treated and untreated mice were in the normal health range ($3.20 - 8.20 \times 10^9$ /L). However WBC of mice given 3000 to 5000 mg/Kg body weight were significantly lower ($P < 0.05$) than the control mice (Table 3). In this study, neutrophils, lymphocytes, eosinophils, monocytes, basophils were evaluated to establish any variation between the tested and control groups. Among these significance variations were observed in neutrophil. The function of neutrophil in the body of animals is to digest foreign substances (Kaplan, 2013). Low level of WBC can be caused by number of conditions including presence of anticancer drugs in the body (Cancer Care, 2014). Anticancer activity of *S. bullatus* has been reported by Machumi et al. (2012). This study suggests that the gradual decrease of the level of WBC was probably contributed by presence of *S. bullatus* in the body of animal.

Blood sample from experimental and control mice were analyzed for the red blood cell count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) (Akindele et al., 2014). Results from this analysis are shown in Table 3. The RBC, HGB and HCT of the treated mice were significantly higher than that of the control mice ($P < 0.05$). However, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) of the treated animals were not significantly different from the control animals ($P > 0.05$) indicating signs of absence of anemia causing compounds in SBDE (Kabubii et al., 2015).

Platelets parameters namely platelets count (PLT),

Table 1. Effect of dichloromethane extract of *S. bullatus* on the body weight of white albino mice given as mean and standard deviation.

Groups	Dose (Mg/Kg bwt)	Day 0 wt (g)	Day 14 wt (g)
I	1000	23.67 ± 1.15	26.33± 1.53
II	2000	30.33 ± 2.08	32.67± 2.31
III	3000	27.67 ± 2.52	30.67± 1.52
IV	4000	31.33 ± 1.15	33.33± 0.58
V	5000	40.33 ± 1.53	41.67± 1.53
VI	Control	24.67 ± 0.58	27.33± 1.15

Bwt, Body weight, wt, weight.

Table 2. Effect of dichloromethane extracts of *S. bullatus* on organ - body weight index of white albino mice given as mean and standard deviation.

Organ	1000	2000	3000	4000	5000	Control
Liver	8.45 ± 1.18	8.02 ± 0.07	7.40 ± 0.43	7.71 ± 0.40	9.27 ± 0.27*	7.23 ± 0.46
Heart	0.57 ± 0.09	0.56 ± 0.12	0.50 ± 0.07	0.59 ± 0.00	0.62 ± 0.04	0.61 ± 0.02
Lung	0.91 ± 0.13	0.98 ± 0.11	0.95 ± 0.04	1.00 ± 0.04	0.91 ± 0.01	0.70 ± 0.03
Spleen	0.35 ± 0.02	0.67 ± 0.25	0.55 ± 0.04	0.50 ± 0.01	0.59 ± 0.05	0.49 ± 0.07
Kidney	0.70 ± 0.07	0.87 ± 0.06	0.66 ± 0.07	0.65 ± 0.03	0.79 ± 0.01	0.70 ± 0.03

Organ, body weight index = organ weight/body weight x 1000; * Significant different difference in row.

mean platelet volume (MPV), the ratio width of distribution of platelets in volume index of heterogeneity of platelets (PDW) and proportion of whole blood occupied by platelets (PCT) were analyzed. Platelets are important as thrombosis mediator which stop bleeding and to accelerate vascular inflammatory conditions in the body (Morrel et al., 2014). It was found that PLT of the treated mice was significantly lower ($P < 0.05$) than the control mice (Table 3). Other parameters of the platelets measurements such as mean platelet volume (MPV), the ratio width of distribution of platelets in volume index of heterogeneity of platelets (PDW) and proportion of whole blood occupied by platelets (PCT) of the treated animals were not significantly different from the control animals except platelets large cell ratio (P-LCR) of the treated mice was found to be significantly higher than the control mice ($P <0.05$).

Biochemical parameters

Enzymes that play major roles in the metabolism and detoxification of compounds in the liver namely alanine aminotranse (ALT) and aspartate Aminotranse (AST) were considered to establish if there was cellular damage in the liver (Shah and Sass, 2015). The increase in the concentration of ALT and AST in the blood indicates liver injury (Velayudam et al., 2013). In this study there was no significant difference in the level of ALT and AST in treated and untreated animals (Table 4), which indicates that SBDE is not harmful to the liver (Velayudam et al., 2013).

The total protein level was analyzed and the findings indicated that both treated and control mice had normal protein level of 75.0 g/L (Table 4). The normal protein level range from 63.0 to 82.0 g/L, thus the total protein level of the analyzed blood falls within the normal range (Agbaje et al., 2009). It is therefore conclusive that SBDE did not induce inflammation to the body of mice treated (Fakanya and Tothil, 2014). Erythrocyte sedimentation rate, C-reactive protein and plasma viscosity are blood tests that detect inflammation (Fakanya and Tothil, 2014). It is has been established that extra protein released in the blood stream usually from the site of inflammation is used as a marker of inflammation (Chan et al., 2002).

Cholesterol and triglycerides level in the blood of experimental and control mice were determined to establish if SBDE elevates level of aforementioned compounds. Level of cholesterol and triglyceride in the body of treated albino mice and untreated ones was not significantly different ($P < 0.05$) which suggests that SBDE doesn't elevate the level of cholesterol and fatty acids. High level of cholesterol and triglycerides emanating from diet has implicated to cause heart diseases and stroke (Sher et al., 2012).

In this study, SBDE was assessed if its use could lead to destruction of blood cells. It was monitored by the determination of the level of bilirubin in blood. The breakdown of blood cells is usually done in the liver and bilirubins are removed out of the body through defecation (Hansen, 2010). High level of bilirubin in the body is usually implicated by liver failure to remove them from the

Table 3. Effect of dichloromethane extracts of *S. bullatus* on hematological parameters of white albino mice.

Parameter	Control	1000 mg/Kgbwt	2000 mg/Kgbwt	3000 mg/Kgbwt	4000 mg/Kgbwt	5000 mg/kg bwt
RBC (10^9/L)	4.77 ± 0.11	5.17± 0.13	5.35 ± 0.08	5.63 ± 0.10	5.79 ± 0.05	5.92 ± 0.03
HGB (g/dL)	13.70 ± 0.26	13.91± 0.48	14.13 ± 0.1	14.13 ± 0.31	14.37 ± 0.22	14.40 ± 0.20
HCT (%)	39.87 ± 0.49	40.09 ± 0.71	41.98 ± 0.67	40.95 ± 0.87	42.11 ± 0. 65	42.53 ± 0.57
MCV (fL)	83.07 ± 0.46	83.74 ± 0.36	81.58 ± 0.77	81.49 ± 0.59	81.53 ± 0.17	81.46 ± 0.23
MCH (pg)	29.76 ± 0.47	29.12 ± 0.79	29.19 ± 0.14	29.19 ± 0.18	29.24 ± 0.73	29.27 ± 0.12
MCHC (g/dL)	35.10 ± 0.10	35.18 ± 0.17	35.70 ± 0.10	35.61 ± 0.14	35.53 ± 0.19	35.63 ± 0.12
RDW-SD (fL)	43.00 ± 0.1	43.01 ± 0.58	43.03 ± 0.82	42.45 ± 0.36	42.89 ± 0.97	42.73 ± 0.45
RDW-CV (%)	14.47 ± 0.12	15.13 ± 0.54	15.45 ± 0.29	15.32 ± 0.59	15.45 ± 0.63	15.53 ± 0.47
PLT (10/uL)	243.00 ± 2.0	193 ± 2.28	193 ± 2.15	193 ± 2.66	194.19 ± 2.14	194.33 ± 2.52
PDW (fL)	10.37 ± 1.01	9.87 ± 0.92	9.63 ± 0.03	9.63 ± 0.04	9.63 ± 0.02	9.63 ± 0.40
MPV (fL)	9.30 ± 0.26	9.30 ± 0.19	9.49 ± 0.20	9.83 ± 0.43	9.78 ± 0.38	9.97 ± 0.40
P-LCR (%)	18.97 ± 0.51	20.91 ± 0.32	21.18 ± 0.37	23.19 ± 0.54	23.63 ± 0.31	24.43 ± 1.36
PCT (%)	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.03	0.23 ± 0.04	0.23 ± 0.03	0.21 ± 0.02
WBC (10^9/L)	5.29 ± 0.03	5.27 ± 0.08	5.26 ± 0.11	4.76 ± 0.21	4.42 ± 0.13	4.47 ± 0.28
NEUT (10^9/L)	3.33 ± 0.12	3.31 ± 0.23	3.30 ± 0.12	2.27 ± 0.25	2.28 ± 0.37	2.26 ± 0.12
LYMP (10^9/L)	1.69 ± 0.12	1.72 ± 0.16	1.74 ± 0.19	1.78 ± 0.14	1.82 ± 0.25	1.87 ± 0.06
MONO (10^9/L)	0.43 ± 0.01	0.42 ± 0.77	0.42 ± 0.03	0.42 ± 0.06	0.42 ± 0.07	0.42 ± 0.02
OE (10^9/L)	0.10 ± 0.00	0.21 ± 0.06	0.22 ± 0.03	0.22 ± 0.54	0.21 ± 0.01	0.22 ± 0.03
BASO (10^9/L)	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01

Bwt, Body weight

Table 4. Biochemical parameters.

Parameter	Control	1000 mg/Kgbwt	2000 mg/kgbwt	3000 mg/Kgbwt	4000 mg/Kgbwt	5000 mg/kg bwt
ALT (U/L)	28.83 ± 0.35	29.17 ±0.52	29.61 ± 0.76	29.63 ± 0.49	30.41 ± 0.68	30.57 ± 1.36
AST (U/L)	42.00 ± 0.41	42.04 ± 0.35	42. 13 ± 0.33	42.35 ± 0.37	42. 35 ±0.33	41.37 ± 0.31
Albumin Gen 2. (g/L)	51.00 ± 1.91	52.37 ± 1.80	52.40 ± 1.87	52.81 ± 1.95	52.82 ± 1.97	52.83 ± 2.29
Total protein (g/L)	75.33 ± 0.31	75. 35 ± 0.32	75.35 ± 0.33	75.39 ± 0.33	75.40 ± 0.35	75.40 ± 0.35
Cholesterol (mmol/L)	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03
Triglycerides liquid (mmol/L)	1.49 ± 0.12	1.49 ± 0.19	1.49 ± 0.11	1.49 ± 0.21	1.49 ± 0.20	1.49 ± 0.07
Bilirubin (Umol/L)	1.26 ± 0.02	1.51 ± 0.01	1.51 ± 0.00	1.52 ± 0.03	1.52 ± 0.01	1.53 ± 0.01

Bwt, Body weight.

blood stream leads to health condition known as jaundice. The present study revealed that the treated animals have high significant level of bilirubin compared to untreated animal ($p < 0.05$). This is probably due to breakdown of cells caused by compounds in *S. bullatus* with anticancer activity.

Conclusion

S. bullatus dichloromethane extract has been established to have safety up to dose of 5000 mg/Kg body weight which authenticates its use as herbal tea and for management of diarrhea. However a dose more than 2000 mg/Kg body weight indicated to lower WBC.

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Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Agbaje EO, Adeneye AA, Daramola AO (2009). Biochemical and

toxicological studies of aqueous extract of *Syzigium aromaticum* (L.) Merr. & Perry (Myrtaceae) in rodents. Afr. J. Tradit. Complement. Altern. Med. 6(3):241-254.
Akindele AJ, Adeneye AA, Salau OS, Sofidiya MO, Benebo AS (2014). Dose and time-dependent sub-chronic toxicity study of hydroethanolic leaf extract of *Flabellaria paniculata* Cav. (Malpighiaceae) in rodents. Front Pharmacol. 5:78.
Cancer care (2014). Neutropia (low white blood cell) cancer care Nova Scotia. 1-866-599-2267).
Chan YL, Liao HC, Tsy PK, Chang SS, Chen JC, Liaw SJ (2002). C-reactive protein as indicator of bacterial infection of adult patients in the emergency department. Chang Gung Med. J. 25(7):437-445.
Fakanya WM, Tothil IE (2014). Detection of inflammation biomarkers C-creative protein in serum samples towards an optimal biosensor. Biosensors (Basel) 4(4):340-357.
Hansen TWR (2010). Core concept: bilirubin metabolism. Neo Review. 11(6):316-322.
Kabubii ZN, Mbaria J, Mathiu M (2015). Acute toxicity studies of Myrsine Africana aqueous seed extract in male Wistar rats on some hematological and biochemical parameters. Clin. Phytosci. 1:9.
Kaplan MJ (2013). Role of neutrophils in systemic autoimmune diseases. Arthritis Res. Ther. 15:219.
Machumi F, Yenesewa A, Midiwoa JO, Heydenreichb M, Kleinpeterb E, Tekwanic BL, Khan SI, Walker LA and Muhammad I (2012). Antiparasitic and antcancer carvotacetone derivatives of *Spheranthusbullatus.* Nat Prod. Commun. 7(99):1123-1126.
Marete EN, Jacquier JC, O'Riordan D (2011). Feverfew as a source of bioactive for functional foods: Storage stability in model beverages, J. Funct. Foods 3:38-43.
Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK, Schafer K (2007). Evaluation of organ weights for rodent and nonrodent toxicity studies: A review of Regulatory Guidelines and a Survey of Current Practices. Toxicol. Pathol. 35:742-750.
Morrel CN, Aggrey AA, Chapman LM, Modjeski KM (2014). Emerging roles for platelets as immune and inflammatory cells. Blood 123(18):2759-2767.
Nirogi R, Goyal VK, Jana S, Pandey SK, Goth A (2014). What suits best for organ weight analysis: Review of relationship between organ weight and body/brain weight for rodent toxicity studies. Int. J. Pharm. Sci. Res. 5(4):1525-1532.
Nwachukwu CU, Umeh CN, Kalu IG, Slyvester O, Magnus N (2010). Identification and Traditional uses of some common medicinal plants in Ezinihitte Mbaise LGA of Imo State, Nigeria. Report Opin. 2(6):1-8.
Organization for Economic Co-operation and Development (OECD) (2001). Guidelines for the Testing of Chemicals "Acute Oral Toxicity-Acute Toxic Class Method" No. 423
Organization for Economic Co-operation and Development (OECD) (2000). Guidance document on acute oral toxicity. Environmental health and safety monograph Series on testing and assessment: No 24.
Piao Y, Liu Y, Xie X (2013). Change Trends of Organ Weight Background Data in Sprague Dawley Rats at Different Ages. J. Toxicol Pathol. 26(1):29-34.
Quantrocchi U (2012). CRC world dictionary of medicinal and poisonus plants: common names, scientific names, eponyms, synonynis and entomology. CRC press. 5:3960p.
Shah SC, Sass DA (2015). Cardiac Hepatopathy: A review of liver dysfunction in heart failure. Liver Res. Open J. 1(1):1-10.
Shaw YAB, Salmon CNA, Green CE, Hibbert SL, Smith AM, and Williams LAD (2012). Evaluation of the Nutraceutical Potential of *Rytidophyllum tomentosum*(L.) Mart.: HPTLC Fingerprinting, Elemental Composition, Phenolic Content and *in vitro* Antioxidant Activity. Pharm. Crops. 3(47):2210-2906.
Sher A, Mahmood MFU, Shah SNH, Bukhsh S, Murtaza G (2012). Effect of garlic extract on blood glucose level and lipid profile in normal and alloxan diabetic rabbits. Adv. Clin. Exp. Med. 21(6):705-711.
VElayudam, Ilavarasan, Arul amuthan (2013). Acute and28-day subchronic oral toxicity study of kadukkai maathirai, an iron based siddha herbal formulation in wistar albino rats. Int. J. Pharm. Pharm. Sci. 5(4):186-191.