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TOXICOLOGICAL IMPLICATIONS OF ACUTE AND REPEATED DOSE ADMINISTRATIONS OF HYDROETHANOLIC EXTRACT OF *CITRULLUS VULGARIS* SEEDS IN WISTAR RATS

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ABSTRACT

In spite of the wide therapeutic usage of formulations of Citrullus vulgaris seeds, there is still paucity of enriched biochemical information on its safety profiles in animal. Hence, the present study evaluated the acute and sub-chronic toxicological implications of its hydroethanolic extract in Wistar rats using OECD guidelines. In the acute toxicity test, single oral administration of 5000 mg/kg body weight (b.w.) of the extract was given to the animals and observed for 14 days. The sub-chronic toxicity study was conducted by daily oral administration of graded doses (100, 500 and 1000 mg/kg b.w.) of the extract for 28 days. Clinical signs of toxicity, behavioral changes, hematological, and biochemical parameters were subsequently evaluated. The extract at 5000 mg/kg b.w. produced no treatment-related signs of toxicity, behavioral changes or mortality in the animals. Thus, its No Observed Adverse Effect Level dose was estimated to be greater than 5000 mg/kg b.w. In the sub-chronic study, except for the significantly altered serum activity of aspartate aminotransferase at 100 and 500 mg/ kg b.w., treatments with the extract revealed no significant difference in hematological and clinical biochemistry indices relative to the control. However, the extract dose-dependently and significantly increased the body weight of the treated animals compared to the control group. Cage side observations also recorded no treatment-induce signs of toxicity. In view of these, hydroethanolic seeds extract of C. vulgaris may be concluded to be non-toxic within the tested doses and period of investigation. Hence, it could be adjudged relative safe for consumption in rats.

KEY WORDS: *acute toxicity, erythropoiesis, hematopoietic, sub-chronic toxicity, watermelon.*

INTRODUCTION

The discovery since prehistoric era that products of plants, in addition to their nutritive values, could serve as therapeutic weapons against various human, animal and even plant diseases has made plants a sine qua non to lives (Ogbonnia *et al.*, 2009). Although, there are reports on the unflinching supports by WHO for the use of herbal medicines. Indiscriminate use and lack of standardization are issues consistent with their safety (WHO, 1985). Besides, only a

few of these preparations have been scientifically tested and evaluated for safety. Similarly, the increasing morbidity and mortality associated with the use of herbal or the so-called traditional medicines has also raised universal attention in the last few years (Bandaranayake, 2006). Therefore, there is the continuous need for the scientific evaluation of herbal medicines for safety.

Citrullus vulgaris (CV), commonly called watermelon, is a prostrate annual plant with herbaceous, firm and stout stems (3 m long). The plant belongs to Cucurbitaceae family and produces a fruit that is about 93% water (Baker *et al.*, 2012). It is Native to Southern Africa with wild presence and significant diversity. CV has been cultivated in Africa for over 4,000 years and has been widely distributed to other parts of the world including Nigeria. The young parts are densely wooly with yellowish to brownish hairs while the older parts are hairless. The leaves are herbaceous but rigid, becoming rough on both sides; 60-200 mm long and 40-150 mm broad. While the fruit in the wild form is subglobose, indehiscent and up to 200 mm in diameter (Maynard, 2001; Van der vossen *et al.*, 2004), the seeds are obovate to elliptical, flattened, 0.5-1.5 cm × 0.5-1 cm, smooth, yellow to brown or black, rarely white (Laghetti & Hammer, 2007). Every part of the fruit of CV including the rind and the seeds has been nutritionally valued (Wind, 2008). The flat brown seeds have a much higher food value (with significant amounts of vitamin C, minerals, fat, protein, starch and riboflavin) than the fleshy rind. The seeds of CV are usually either eaten dried, roasted or pulverized into flour to make bread. The seeds are also saponin-rich and with significantly high percentage of oil (which is similar to pumpkin seed oil) that is used in cooking and in the cosmetics and pharmaceutical industries (Maynard, 2001).

In view of the foregoing and coupled with no previous scientific reports on the toxicity profile of the seeds of CV, the present study was designed. Hence, the study evaluated the acute and sub-chronic toxicological implications of hydroethanol extract of *Citrullus vulgaris* seed on key metabolic markers of Wistar rats.

MATERIALS AND METHODS

Sample collection and authentication

Fruits of *C. vulgaris* were purchased in January 2016 from a vendor at Post office area, Ilorin, Kwara State, Nigeria. They were identified by Mr. Bolu of the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen (No. UILH/001/007) was prepared and deposited in the Herbarium. Thereafter, the fruits were cut into pieces and the seeds were diligently removed in preparation for further use.

Extract preparation

The seeds of the plant were thoroughly washed with distilled water, air-dried to constant weight and subsequently powdered with an electric blender. A portion (400 g) of the powder seed was suspended in 4 L of hydro-ethanol (50:50) with regular agitation for 72 hrs. The resulting solution was filtered (Whatman No. 1 filter paper) and thereafter concentrated to dryness over water bath maintained at 40°C. The thick brown pasty extract (CVE) obtained was kept air-tight and refrigerated prior to use.

Experimental animals

Thirty five (35) healthy adult female Wistar rats weighing between 117.0 to 153.0 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of

Ilorin, Ilorin, Nigeria. They were allowed to acclimatize to the laboratory conditions for ten days. During the acclimatization and experimental periods, the rats were provided drinking water and normal rat chow *ad libitum*.

Experimental protocols

Acute toxicity testing

The oral acute toxicity study was conducted according to Organization for Economic Co-operation and Development (OECD) guideline 423 (OECD, 2001). All the animals were uniquely identified based on the body weights and fasted overnight but had free access to water before the experiment. The ten rats used were randomized into two groups of 5 rats per cage. Animals in group 1 were given sterile distilled water and served as control, while group 2 comprised animals treated with 5000 mg/kg body weight single oral dose of CVE. Subsequent to this treatment, all the rats were pertinently observed (for general behavioral changes, symptoms of toxicity and mortality) for the first 4 h (critical hours), then over a period of 24 h and thereafter daily for 14 days. While the feed and water intakes of the rats were monitored and recorded on daily basis (Sabiu *et al.*, 2015), the changes in their body weights were recorded on weekly basis throughout the experimental period.

Blood collection, hematological analysis and biochemical analyses

Twenty four hours after the last treatments (i.e. on the 15th day), the animals were humanely anesthetized using diethyl ether and subsequently bled via cardiac puncture. Blood samples were collected in either sample bottles containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) or plain bottles and used for hematological or biochemical analyses respectively. Hematological analyses were performed using an Automated Hematological Analyzer (Sysmex KX21) and parameters including red blood cell (RBC) or erythrocyte count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) or leukocyte count and platelet count (PLT) were determined.

For the biochemical analysis, the blood samples were allowed to clot and thereafter centrifuged (3000 rpm, 15 min) prior to serum aspiration into new sample bottles and stored at –20°C. The serum samples were analyzed to determine the levels of blood urea nitrogen (BUN), creatinine (Crea) and albumin (Alb) as well as the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using an Automated Biochemistry Analyzer.

Sub-chronic toxicity testing

Twenty rats were used in this study. They were divided into 4 groups of 5 rats each; one control group and three treatment groups. While group 1, given sterile distilled water served as control, groups 2-4 were rats administered with 100, 500 and 1000 mg/kg body weight doses of CVE for 28 days. All administrations were done once daily via oral gavaging. The rats were observed individually and special attention was given to the treatment groups. The cage-side observations (changes in skin, and eyes; respiratory effects; autonomic effects, including salivation, diarrhea, and urination; central nervous system effects, including tremors and convulsions; changes in the level of activity, gait and posture; reactivity to handling or sensory stimuli; and altered strength) and mortality were monitored daily throughout the study period. The weekly body weight changes of all the animals were also taken and recorded.

On the 29th day, all the rats were similarly anesthetized as done in the acute toxicity testing and blood samples used for hematological and biochemical analyses.

Statistical analysis

The data were expressed as the mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) coupled with Duncan multiple range test was performed to compare the differences between the treatment means. A mean difference was considered significant at $p< 0.001$ and $p< 0.05$. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Acute toxicity evaluation

The acute oral administration of 5000 mg/kg b.w. of CVE neither produced any mortality nor clinical signs of toxicity in rats. Generally, when compared with the control group, the extract-treated animals elicited no treatment-induced changes in behavior and feeding patterns (Figures 1 and 2). Similarly, the data obtained with respect to weight gained, hematological and clinical biochemistry indices revealed that there were no significant differences ($p> 0.05$) between the 5000 mg/kg b.w. CVE-treated animals and the control (Tables 1, 2 and 3).

Sub-chronic toxicity testing

Cage side observations did not record any mortality and behavioral changes such as tremor, convulsion, salivation or diarrhea but urine output (data not shown) was found to be increased in the CVE-treated animals compared to the control. While the weight gained by all the extract-administered animals were dose-dependently and significantly ($p<0.05$) different from that of the control (Table 4), their haematological parameters compared favourably with those of the control group (Table 5). With the exception of the significantly altered activity of ALT at the lower doses (100 and 500 mg/kg b.w.) of the extract, all other clinical biochemistry parameters produced values that compared well with the control (Table 6).

Toxicity tests are most widely used to examine specific adverse events or specific end points. It is also helpful in determining the No Observed Adverse Effect Level (NOAEL) dose of a chemical agent and constitutes an integral part of clinical trial evaluation (Setzer *et al.*, 2006). Apart from the mortality rate, cage-side functional observations, such as tremor, convulsion, salivation or diarrhea as well as respiratory effects, autonomic effects and nervous system effects, are very crucial observatory parameters in toxicity studies (Ajani *et al.*, 2016). Eaton and Klaassen (1996) suggested that animals given high doses of plant extracts or chemicals might show slight changes in behavior as a consequence of the metabolism of the plant extracts or chemicals. Since no clinical signs of toxicity were observed subsequent to a single oral dose (5000 mg/kg b.w.) administration of CVE, it may be logically inferred that the extract is non-acutely toxic when administered via oral route and could be adjudged relatively safe for consumption. Furthermore, if a dose as high as 5000 mg/kg b.w. of an extract is found to be survivable, no further acute testing will be recommended (NRC, 2006). The similar patterns of feeding (food and water intakes) as well the non-significant difference in the hematological and biochemical indices between the CVE-administered rats and the control group in this study might be another justifiable reason supporting the non-acute toxic tendency of the extract. Also, the fact that the weight gained by the extract-treated rats compared

favourably with that of the control over the treatment period could suggest that CVE is endowed with nutrients that supported normal metabolism and stimulated growth and developmental mechanisms in the animals. This may be attributed to the nutritive nature of the phytoconstituents of the seeds of CV as earlier reported by ABC.

TABLE 1. Body weight changes of the animals following treatment with 5000 mg/kg body weight of *C. vulgaris* seeds hydroalcohol extract (n= 5, Mean ± SEM)

Treatments	Weekly weight changes (g)			
	0 (initial)	1	2 (final)	% WG
Control	126.00±1.87	143.00±2.20	148.50±3.23	15.15
5000 mg/kg	103.00±3.03	123.25±3.94	125.25±2.21	17.76

WG=weight gain

TABLE 2. Effect of 5000 mg/kg body weight single oral dose administration of *C. vulgaris* seeds hydroalcohol on hematopoietic systems of the animals (n = 5, mean ± SEM)

Parameters	Control	5000 mg/kg extract
RBC ($x10^{12}$/L)	7.74±9.00	7.27±2.07
HGB (g/dL)	13.08±0.13	13.58±0.12
HCT (%)	47.35±0.33	46.18±0.50
MCV (fl)	61.20±1.13	63.63±1.40
MCH (pg)	17.90±0.35	18.75±0.56
MCHC (g/dL)	28.63±0.08	29.43±0.31
WBC ($x10^9$/L)	2.5±2.44	1.98±1.61
Platelet ($x10^9$/L)	888.43±1.29	888.80±1.80

RBC= red blood cell, HGB= haemoglobin, HCT= hematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, RDW= red blood cell distribution width and WBC= white blood cell.

TABLE 3. Effect of 5000 mg/kg body weight single oral dose administration of *C. vulgaris* seeds hydroalcohol on some kidney and liver function parameters of rats (n = 5, mean ± SEM)

Parameter	Control	5000 mg/kg extract
Potassium(mmol/L)	8.65±0.42	7.85±2.74
Chloride (mmol/L)	1.30±2.38	1.25±33.50
HCO_3^- (mmol/L)	24.00±1.35	23.00±0.91
Urea (mg/dL)	1.14±2.52	1.62±3.73
Uric acid (mg/dL)	7.55±7.73	8.05±1.62
Total protein (g/dL)	18.75±0.48	17.35±0.8
Albumin (g/dL)	7.53±0.20	7.50±1.45
ALT (IU/L)	61.01±1.47	63.50±5.87
AST (IU/L)	31.38±4.02	32.00±1.47
ALP (IU/L)	63.08±4.00	63.25±1.92

ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, ALP=Alkaline phosphatase

TABLE 4. Mean body weight and percentage weight gain of rats following 28 days treatment with hydroethanol seeds extract of *Citrullus vulgaris*

Treatments	Weekly weight changes (g)					
	0 (initial)	1st	2nd	3rd	4th (final)	%WG
Control	130.75±2.29	130.17±2.29	134.05±3.53	138.75±3.50	140.75±3.74	7.10
100 mg/kg CVE	140.10±2.29	140.75±2.02	144.75±2.29	148.25±2.29	156.75±4.15	10.62*
500 mg/kg CVE	133.50±0.65	136.00±1.47	140.25±3.25	140.75±2.17	154.50±4.47	13.59*#
1000 mg/kg CVE	121.00±1.58	140.67±4.91	138.00±2.48	139.75±2.14	141.50±3.52	14.48*#

*Significantly different ($p<0.05$) from the control, #Significantly different ($p<0.001$) from the 100 mg/kg treated group. WG=weight gain, CVE= *C. vulgaris* hydroethanol seeds extract

TABLE 5. Effect of sub-chronic oral administration of *Citrullus vulgaris* seeds hydro-ethanol extract on hematological parameters of rats (n=5, Mean ± SEM)

Parameters	Control	Extract (mg/kg body weight)		
		100	500	1000
WBC ($x10^9$/L)	13.35±1.34	13.60±2.83	12.90±3.44	13.90±2.90
RBC ($x10^{12}$/L)	7.40±9.00	6.83±2.25	7.00±1.50	7.75±2.50
HGB (g/dL)	13.08±0.13	12.99±0.38	12.77±0.27	13.30±0.00
HCT (%)	47.35±0.33	46.93±0.97	47.13±2.03	47.70±0.40
MCV (fl)	61.200±1.13	62.38±1.03	61.53±3.76	62.05±0.75
MCH (pg)	16.90±0.35	17.02±0.41	17.23±0.38	17.35±0.05
MCHC (g/dL)	27.63±0.08	27.43±0.55	27.03±0.52	27.20±0.30
Platelet ($x10^9$/L)	884.28±2.29	884.80±2.84	882.26±3.13	889.40±2.00

RBC= red blood cell, HGB= haemoglobin, HCT= hematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, RDW= red blood cell distribution width and WBC= white blood cell.

TABLE 6. Effect of sub-chronic oral administration of *Citrullus vulgaris* seed hydro-ethanol extract on blood serum of rats (n=5, Mean ± SEM)

Parameters	Control	Extract (mg/kg body weight)		
		100	500	1000
ALP (IU/L)	63.75±3.99	62.50±1.93	63.67±3.65	63.00±1.60
AST (IU/L)	37.50±4.02	27.00±4.07*	29.67±9.19*	36.00±1.07
ALT (IU/L)	60.50±1.47	60.00±1.18	60.00±2.61	58.00±8.00
Urea (mg/dL)	1.14±2.52	1.56±1.46	1.68±2.12	1.20±1.20
Total protein (g/dL)	18.75±0.48	17.88±1.24	17.93±0.67	17.99±1.40
Uric acid (mg/dL)	7.55±7.73	7.80±6.99	7.07±1.27	7.80±2.00
Albumin (g/dL)	7.73±0.38	7.25±0.78	7.40±1.59	7.80±1.30

*Significantly different ($p<0.05$) from the control. ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, ALP=Alkaline phosphatase

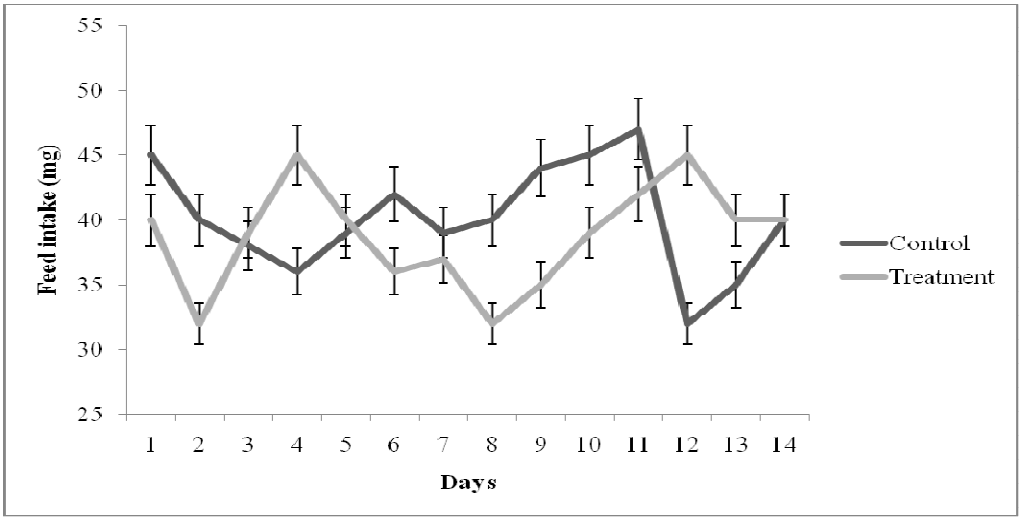

FIGURE 1. Effect of 5000 mg/kg body weight single oral dose administration of *Citrullus vulgaris* seeds hydroalcohol extract on feed intake of rats (n=5, Mean ± SEM)

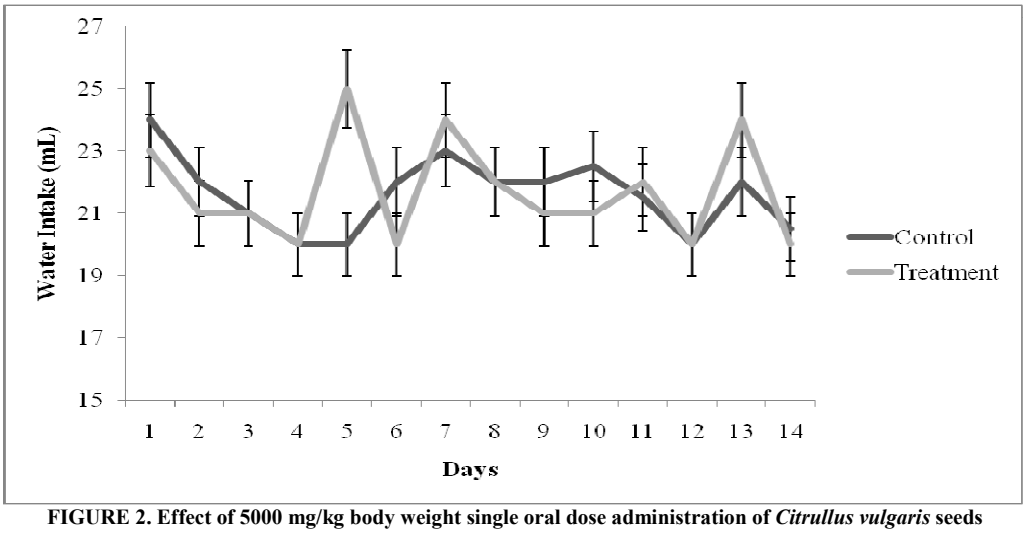

FIGURE 2. Effect of 5000 mg/kg body weight single oral dose administration of *Citrullus vulgaris* seeds hydroalcohol extract on water intake of rats (n=5, Mean ± SEM)

Subsequent to no evidence of clinical signs of toxicity in the acute toxicity testing, further investigation was performed to evaluate the sub-chronic toxicity implication of CVE over a 28-day experimental period. This was done with a view to providing enriched toxicological data on the extract. In this study, the fact that continuous 28 days daily dose treatment with CVE showed no signs for morbidity, toxicity or mortality across all the

treatment groups may be suggestive of its unlikely toxic tendency at the tested doses over the exposure period. A change in body weight is one of the first important signs of toxicity and may serve as sensitive indication of the overall health status of animals (Sireatawong *et al.*, 2008). While an increase in the body weight of animals may signify optimal body fat accumulation rather than the toxic effects of drugs or chemicals (Harizal *et al.* 2010), a reduction may suggest physiological adaptation responses to a pharmacological agent or extract, which may result in low appetite and, hence, lower caloric intake by the animal (Rhiouani *et al.* 2008). In this study, the body weight gained by the extract-administered animals relative to the control might imply that CVE contained nutrients and minerals that are closely associated with growth and developmental events in rats. In addition to indicating that the extract might have enhanced appetite of the treated rats, it could also further support the submission on the weight gain parameter in the acute toxicity testing in this study. This assertion is consistent with the report of Ajani *et al.* (2016), where body weight gain in Wistar rats was attributed to the phytonutrients of aqueous extract of *Cyperus esculentus*.

The blood is very sensitive to xenobiotics treatment and serves as an important system of physiological and pathological status of animals (Checiu *et al*., 2004; Filimon *et al.,* 2012; Ungurean, 2013; Adeneye, 2014; Toader, 2016). Its assessment subsequent to treatment with a pharmacological agent is imperative and the results thereof can be used to establish how safe an agent is on the well-being of humans (Ajani *et al.*, 2014). The non-significant differences in all the hematological parameters of the extract-treated animals compared to the control over the 28-day experimental period may be an indication that CVE might not be toxic to the blood. This suggests that the blood-related mechanistic events like erythropoiesis and thrombopoiesis were not adversely impacted in the extract-administered rats. This may also mean that the repeated dose administration of CVE does not predispose the animals to either developing or showing symptoms of toxicity. This submission is in agreement with previous reports on selected medical plant extract (Sabiu *et al.*, 2015; Ajani *et al.*, 2016; Sabiu & Ashafa, 2016).

Clinical biochemistry analyses were conducted to assess the probable alterations in the liver and kidney functions of the animals following administration of the extract. Such analyses are crucial in toxicological evaluation of plant extracts due to the involvement of the hepatic and renal systems biotransformation events. Kidney damage may be evaluated by concurrent measurements of urea, creatinine and electrolytes, and deviations from normal in their serum levels is a possible pointer to renal injury (Saheed & Omotayo 2016). In this study, the non-significant difference observed in the kidney function indices of the CVE-administered rats and those of the control group is indicative of preserved or normal renal function and further lent support to the non-toxic tendency of seeds hydroalcoholic extract of CV. Significantly altered serum activities of ALP, ALT, and AST are closely associated with liver damage (Sabiu *et al.*, 2015). Although, serum AST activity was decreased following treatment with the extract at the lower investigated doses, it was however normalized in the 1000 mg/kg b.w. treated rats. This alteration was not only inconsistent with all other liver function indices evaluated in this study, but also insufficient to undermine the non-hepatotoxic and therapeutic relevance of the repeated daily dose administration of CVE. These findings are consistent with the submission of Ajani *et al.* (2014), where ethanolic leaves extract of *Lagenaria brevifolia* L. (Cucurbitaceae) was reported to be non-hepatotoxic in Wistar rats.

CONCLUSIONS

In light of the findings from this study, it is evident that the NOAEL dose of CVE is in greater than 5000 mg/kg b.w. in Wistar rats. Following the daily oral dose administration of the extract for 28 days in the animals, it may also be inferred that it does not elicit any serious adverse sign of toxicity at the tested doses and thus, may be adjudged relatively safe for consumption. Overall, the data from the present study have lent scientific support to the safety and pharmacological significance of *C. vulgaris* in folkloric medicine.

REFERENCES

- Adeneye A. A. 2014. Sub-chronic and chronic toxicities of African medicinal plants. In: Victor K, (ed.). *Toxicological survey of African medicinal plants,* 1st ed., Elsevier, Pp 99-133.
- Ajani E. O., Sabiu S., Bamishaye F. A. 2016. A 4-week daily dose oral administration assessment of *Cyperus esculentus* L. aqueous extract on key metabolic markers of Wistar rats. *Pharmacologia*, 7: 125-133.
- Bandaranayake W.M. 2006. Modern Phytomedicine: Turning Medicinal Plants into Drugs. In:Ahmad, I., Aqil F. and M. Owais (Eds.), Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim. 102p
- Eaton D.L., Klaassen C.D. 1996. *Principles of toxicology.* In: Klaassen, C.D. (Ed.),
- Casarett and Doull's Toxicology: *The Basic Science of Poisons,* 5th ed. McGrawHill, P.13
- Checiu M., Checiu I., Iluţ I., Tuduce I., Checiu Hutanu D. 2004. The effect of acute maternal $CuCl_2$ intoxication upon mouse fetal skeleton. *Annals of West University of Timişoara, ser. Biology*, 7: 61- 66
- Filimon M.N., Sinitean A., Ianovici N., Borozan A., Bordean D.M., Dumitrescu G., Popescu R. 2012. The influence of deoxynivalenol (DON) on hemoleucogram components at rats**,** *Analele Universităţii din Oradea - Fascicula Biologie*, XIX (1): 23-28
- Harizal S.N., Mansor S.M., Hasnan J., Tharakan J.K.J., Abdullah J. 2010. Acute toxicity study of the standardized methanolic extract of *Mitragyna speciosa* Korth in Rodent. J. *Ethnopharm*. 131:404-409.
- Laghetti G., Hammer K. 2007. The Corsican citron melon [*Citrllus lanatus* (Thunb.) Matsumura & Nakai subsp. Lanatus var. citroides (Bailey) Mansf. Ex Greb.] a traditional and neglected crop. *Genetic Resources and Crop Evolution*, 54: 913-916.
- Maynard D.N. 2001. *Watermelons: characteristics, production and marketing*. American Society for Horticultural Science (ASHS) press. United states. 227pp.
- Maynard D.N. 2001. *Watermelons: characteristics, production and marketing*. American Society for Horticultural Science (ASHS) press. United states. 227pp
- National Research Council (NRC). 2006. *Toxicity testing for assessing environmental agents*, Interim Report, National Academies Press, Washington, DC, USA.
- OECD 2001. Organization of Economic Co-operation and Development (OECD). G*uideline for testing of chemicals, acute oral toxicity- acute toxicity class method*, 423. Adopted 17th December, 2001.
- Ogbonnia S.O., Nkemehule F.E., Anyika E.N. 2009. Evaluation of acute and subchronic toxicity of *Stachytarpheta angustifolia* (Mill) Vahl (Verbanaceae) extract in animals. *Afr. J. Biotech.*, 8(9): 1793-1799.
- Rhiouani H. R., Nazari P., Kamli-Nejad M., Lyoussi B. 2008. Acute and subchronic oral toxicity of an aqueous extract of leaves of *Herniaria glabra* in rodents. *J. Ethnopharm.* 118:378-386.
- Sabiu S.. Ashafa A. O. T. 2016. Evaluation of daily double dose administration of ethanolic root extract of *Morella serrata* (Lam.) Killick in rats. *J Exp Integr Med,* 6(3): 109-117.
- Sabiu S., Ajani E. O., Abubakar A. A., Sulyman A.O., Nurain I. .O., Irondi E. A., Abubakar A. Y., Quadri D. F. 2015. Toxicological evaluations of *Stigma maydis* aqueous extract on hematological and lipid parameters of Wistar rats. *Tox. Rep.* 2, 638-644
- Saheed S., Omotayo T. A. A. 2016. Toxicological implications and laxative potential of ethanol root extract of *Morella serrata* in loperamide-induced constipated Wistar rats. *Pharm Biol.* 54(12):2901-2908
- Sireatawong S., Lertprasertsuke N., Srisawat U. 2008. Acute and sub-chronic toxicity study of the water extract from *Tiliacora trianora* (Colebr.) Diels in rats. *Songklankarin J Sci Tech*, 30, 729-737.
- Toader O.R. 2014. Study of the effects of *Zingiber officinale* (ginger) on spermatogenesis in mice. *Annals of West University of Timişoara, ser. Biology*, 17 (2):.145-152
- Ungurean L. M. 2013. Aspects regarding the immunity of the laboratory mouse after chronic administration of „IMUNITATE CU 7 CIUPERCI". *Annals of West University of Timişoara, ser. Biology*, 16 (2): 107-114

- Van der Vossen H. A. M., Denton O. A., El Tahir I. M. 2004. *Citrullus lanatus,* htpp:/database.prota.org/search.htm. Accessed March 8, 2016
- WHO Expert Committee, 1985. In WHO Technical Report Series of Diabetes Mellitus,
- Wind D. 2008. Watermelon, *Citrullus lanatus*- Nutrition and growing tips. Dave's garden, ElSegundo, California.<http:/davesgarden.comguides/articlesview/1517/> Accessed July 1st,2016.