REVIEW ARTICLE

Repeated Systemic Toxicity Tests: A Call for Proper Understanding of Tests Durations Nomenclature

EARNEST OGHENESUVWE ERHIRHIE*[1] and GOODIES EMUESIRI MOKE[2]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Abstract

Background: Testing of substances such as drugs, food, cosmetics, and chemicals meant for human utilization requires necessary guidelines to be followed. It was recently observed that the proposed duration protocols for subacute, subchronic, and chronic toxicity tests are miscomprehended and misapplied by some researchers.
Methods: This short evaluation, revealed areas where terminologies related to systemic toxicity test durations were misapplied and also properly applied. Data from recently published articles from peer reviewed journals were explored via Pubmed, Google Scholars, and Web of Science database using specific keywords such as "guideline on subacute, subchronic, chronic toxicity testing", "subacute toxicity study", "subchronic toxicity studies", and "repeated toxicity studies on plant extracts", and "6 months chronic toxicity test". The articles that deviated form or complied with the standard test duration protocol were selected for scrutiny in the present study. The need for proper adoption of appropriate terms when developing topics for repeated toxicity test results was also discussed in this study.
Results: This study indicated that although some scholars conducted repeated dosing for 14 or 28 days, they incorrectly used the term "subchronic" instead of "subacute" in the titles of their studies. Also, the term "chronic" was used instead of "subchronic" in the titles of some studies conducted for 90 days.
Conclusion: This study would enable researchers and reviewers of manuscripts in peer review toxicology journals to be acquainted with the laid down test duration protocols for subacute, subchronic, and chronic toxicity tests to ensure that previous errors are not repeated.

Keywords: Toxicity Tests; Subacute; Subchronic; Chronic; Nomenclature.

How to cite this article: ErhirhieEO, and Moke GE. Repeated Systemic Toxicity Tests: A Call for Proper Understanding of Tests Durations Nomenclature. ***Asia Pac J Med Toxicol*** **2022; 11(2):72-76.**

INTRODUCTION

Safety evaluations of herbal medicine, pharmaceuticals, food, chemicals, and cosmetics provide a vital justification for approval for human utilization [1].

While *in-vitro* and *in silico* approaches seem to be the leading edge of repeated toxicity testing, animal model cannot be completely overlooked because data generated from such studies could easily be translated (extrapolated) to humans [2]. Based on the postulation that animals respond to drugs in a similar way as humans when a test substance is administered using a similar route, the involvement of experimental animals in toxicity testing have been initiated long ago by Trevan J.W. For instance, asbestos produces lung cancer, while plastic solvent causes liver cancer in both human and animal species [2].

Besides acute toxicity testing, repeated toxicity testing is vital for substances that are used over time [3]. Thus repeated systemic toxicity test is an aspect of toxicity which assesses the potential of substances to produce deleterious effects on an organism following repeated exposure. Routes of exposure include oral, intraperitoneal, intravenous, subcutaneous, and implantation. Among these, the oral route is the most common and the route of testing is usually based on the therapeutic use or intended route of exposure of such test substance in human [2].

Laid down testing guidelines have been established for repeated systemic safety assessment of herbal products, pharmaceuticals, chemicals, food, and cosmetics [1, 2]. Terminologies such as "subacute", "subchronic", and "chronic" in relation to the duration of exposure of test substance in the course of repeated toxicity tests have led to debates in conferences, meetings, workshops, and seminars. To corroborate this fact, the titles of repeated toxicity studies published in several journals are not consistent with the exposure duration protocols. For instance, some repeated toxicity studies that lasted for 28 days were wrongly entitled as "chronic or subchronic", while some that lasted for 90 days were wrongly titled "chronic" (Table 1). These misconstructions were proved wrongs by highlighting some studies that followed the proposed guidelines (Table 2).

*Correspondence to: Earnest Oghenesuvwe Erhirhie, Ph.D., Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria.
Tel: +234-7060434974, Email: erhirhieochuko@yahoo.com; eo.erhirhie@coou.edu.ng

These inconsistencies and deviations from the established guideline have fueled this article to reaffirm the appropriate duration terminology, pinpoint errors that have been made and also bring to light the need for researchers to realize their errors and adhere strictly to the standard duration guidelines.

METHODS

Recently published articles on repeated toxicity studies

Table 1. Some studies that deviate from the duration guideline

S/No	Title of some research articles with flaws in duration terminology	Misconstrued duration	Reference
1	*Subchronic* toxicity evaluation of top three commercial herbal antimalarial preparations in the Kumasi metropolis.	*30 days*	[20]
2	Acute and *subchronic* oral toxicity assessments of *Combretum micranthum* (Combretaceae) in Wistar rats.	*28 days*	[21]
3	Acute and *subchronic* oral toxicity assessment of the aqueous extract leaves of *Ficus glumosa* Del. (Moraceae) in rodents.	*42 days*	[13]
4	Acute and *subchronic* Toxicity Studies of Aqueous Extract of *Desmodium adscendens* (Sw) DC.	*42 days*	[14]
5	Acute and *subchronic* oral toxicity profiles of the aqueous extract of *Cortex Dictamni* in mice and rats.	*28 days*	[12]
6	Toxicological investigation of acute and *chronic* treatment with *Gnidia stenophylla* Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice.	*91 days*	[22]
7	Genotoxicity, acute and *subchronic* toxicity studies of nano liposomes of *Orthosiphon stamineus* ethanolic extract in Sprague Dawley rats.	*28 days*	[23]
8	Acute and *subchronic* toxicity of *Cajanus cajan* leaf extracts.	*28 days*	[24]
9	*Subchronic* Administration of Methanolic Whole Fruit Extract of *Lagenaria breviflora* (Benth.) Roberty Induces Mild Toxicity in Rats.	*28 days*	[25]
10	Evaluation of cytotoxic effects and acute and *chronic* toxicity of aqueous extract of the seeds of *Calycotome villosa* (Poiret) Link (subsp. *intermedia*) in rodents.	*90 days*	[26]
11	Non-clinical acute and *chronic* toxicity evaluations of *Cissus sicyoides* L. (*Vitaceae*) hydroalcoholic leaf extract.	*90 days*	[27]
12	*Subchronic* Toxicity of the Hydroethanolic Leaf Extract of *Telfairia occidentalis* Hook. f. (Cucurbitaceae) in Male Rats.	*60 days*	[28]

Table 2. Studies that adhere to the duration guideline

S/No	Title of some research articles that adhere to the duration terminology	Approved duration	Reference
1	Acute and *subchronic* Oral Toxicity Evaluation of Aqueous Root Extract of *Dicoma anomala* Sond. in Wistar Rats.	*90 days*	[15]
2	*Subacute* toxicity study of methanol extract of *Tetrorchidium didymostemon* leaves using biochemical analyses and gene expression in Wistar rats.	*28 days*	[7]
3	Acute and *subacute* Toxicity Studies of the Ethyl Acetate Soluble Proanthocyanidins of the Immature Inflorescence of *Cocos nucifera* L. in Female Wistar Rats.	*28 days*	[6]
4	Acute and *subchronic* toxicity studies of the aqueous extract from leaves of *Cistus ladaniferus* L. in mice and rats.	*90 days*	[8]
5	*Subacute* and *subchronic* oral toxicity assessments of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root in Wistar rats.	*28* and *90 days*	[10]
6	Acute and *chronic* toxicity studies of the water extract from dried fruits of Terminalia bellerica (Gaertn.) Roxb. In Spargue-Dawley rats.	*270 days* (*9 months*)	[4]
7	Acute and *subchronic* oral toxicity study of black tea in rodents.	*90 days*	[5]
8	Acute/*subacute* and *subchronic* Oral Toxicity of a Hidroxytyrosol-Rich Virgin Olive Oil Extract.	*28* and *90 days*	[9]
9	Acute and *subacute* Toxicity Profiles of the Methanol Extract of *Lycopersicon esculentum* L. Leaves (Tomato), a Botanical with Promising *In Vitro* Anticancer Potential.	*28 days*	[29]
10	Acute and *subacute* toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats.	*28 days*	[16]
11	Acute and *subacute* toxicity of *Echinops kebericho* decoction in rats.	*28 days*	[30]
12	Evaluation of the acute and *chronic* toxicity of the jiangu capsules.	*6 months*	[31]
13	*Chronic* exposure to toluene and heavy metals and changes in indices of liver function, inflammation and oxidative DNA damage among automobile workers"	*1 year and above*	[17]

were searched using Pubmed, Google Scholars, and Scopus database. Keywords used include "guideline on subacute, subchronic, and chronic toxicity testing", "subacute toxicity study", "subchronic toxicity studies", "repeated toxicity studies on plant extracts", and "6 months chronic toxicity test". Among the obtained results, a number of articles whose titles harmonized with the standard duration guideline were selected (Table 2). Similar selection was done for articles whose titles deviated from the laid down study duration guideline (Table 1).

RESULTS

Definition of Repeated Dose Toxicity Testing

As the name implies, test substances are administered repeatedly or continuously via a known route over a long period of time [2, 4]. Repeated toxicity test incorporates subacute, subchronic, and chronic toxicity tests [5].

Subacute Toxicity Test

In this test, experimental animals are subjected to graded doses (at least three doses) of the test substance for a duration of 14 – 28 days, 2 - 4 weeks [6]. The term "subacute" does not really mean that the exposure duration will be less than acute (24- hours), but it connotes that the exposure doses have to be below the estimated LD_{50} value. This accounts for the selection of doses below the LD_{50} for repeated toxicity test. This study helps to evaluate the systemic side effects of substances on targets organs based on repeated administration of doses below the LD_{50}. The results of this study serve as basis for classification and labeling. It also provides information on the mode of toxic action of a substance. Furthermore, it offers a guideline for designing subsequent studies for longer durations. Hence, subacute toxicity test helps to establish doses for subchronic studies [2]. The exposure duration of 14 – 28 days is consistent with international regulatory guidelines and is considered to represent a reasonable approach [7].

Subchronic Toxicity Test

The goal of this test is to determine the effects that may occur following repeated exposures of animal species to a test substance for a period of three months (90-days) according to the OECD No 408 guidelines for testing of chemicals [8, 9]. This is also referred to as 90-day repeated dose toxicity test [10]. It helps to predict a rational and suitable dose for chronic exposure studies. At least three doses are employed: a high dose that produces toxicity but does not cause more than 10% fatalities, a low dose that produces no apparent toxic effect and an intermediate dose [11].

Chronic Toxicity Test

This test provides insights about the long-term (cumulative) effect of a test substance on experimental animals, usually lasting between 6 months and two years in rodents according to the OECD No 452 test guideline for testing chemicals [3, 4]. It is also applicable in assessing carcinogenic potential of test substances as well as drugs used in the management of terminal diseases such as diabetes, hypertension, arthritis, and rheumatism among others. Study durations of 6 months for rodents and 9 months for non-rodents were considered acceptable for chronic toxicity by the regulatory authorities [3].

The outcome of chronic toxicity test is useful in the establishment of no observed adverse effect levels (NOAEL), the highest dose where no toxicity effect occurs. It also helps in the establishment of safety criteria for human exposure to new drug entities undergoing clinical trials [3]. The major difference between chronic toxicity testing and subacute or subchronic is the duration of exposure [2]. The exposure period usually cover post-weaning maturation and development into adulthood of animals. A group is usually included to monitor reversibility in toxicity for a period of four weeks (28 –days) [11].

Biomarkers for Repeated Toxicity Test

Having considered the duration of exposure for subacute, subchronic, and chronic toxicity tests, relevant parameters including body weight, hematological, biochemical, cardiovascular, as well as behavioral parameters could be assessed when necessary before (pre-treatment/pre-exposures/baseline), during (treatment), and after exposure (post-treatment/recovery) of animals to the test substance [2]. Body weight is measured weekly and at the end of the study, samples are collected for biochemical, hematological, and histopathological evaluations [2, 3].

In the course of exposure, during subchronic and chronic toxicity tests, it is essential to carry out periodic (monthly) sample collection and monitoring of hematological, biochemical, cardiovascular, histopathological, and body weight parameters. This would help in observing the onset of toxicity (i.e. to understand whether the toxic effect occurred in the 1^{st}, 2^{nd}, 3^{rd} month for subchronic test as well as 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} months for chronic test) [4].

Results, Discussion, and Future Directions

The titles of studies in Table 1 revealed a discrepancy in the standard testing duration of exposure. For example, Wang *et al.* [12] conducted a 28 –day study and titled it "subchronic", Ntchapda *et al.* [13] carried out a study that lasted for 42 days and titled it "subchronic" while Quaye *et al.* [14] did a study that lasted for 42 days and titled it "subchronic". Following the approved protocol, the titles of typical studies presented in Table 1 did not cohere to the laid down duration guidelines.

On the other hand, the titles of studies depicted in Table 2 are succinctly in consonance with the laid down study duration guidelines. As a case in point, Balogun and Ashafa [15] administered aqueous root extract of *Dicoma anomala* Sond to Wistar rats for 90 days and titled the study "subchronic". Ugwah-Oguejiofor *et al.* [16] administered extract for 28 days and titled their study "subacute". Sireeratawong *et al.* [4] did their study for 270 days and titled the study "chronic". Also, Nsonwu-Anyanwu *at al.,* [17], ran a study on "Chronic exposure to toluene and heavy metals and changes in indices of liver function, inflammation and oxidative DNA damage among automobile workers" which was restricted to participants within the vicinity of an

automobile workshop or exposed to paint in their environment for a minimum of 1 year before the study. These are reflections of well-structured titles that followed the study duration protocol.

In light of the above, it is necessary for researchers to provide titles that are in consonance with the duration of exposure that such studies are meant for.

For the avoidance of non-conformity between the title of studies and duration of exposure, duration of exposure could be removed from the title such that testing duration is defined in the method section of such studies, but without mentioning any of the terms "subacute, subchronic, and chronic". For example, Wattanathorn et al. [18] administered their extract for a period of 90 days in a study they titled "Toxicity Evaluation of *Anacardium occidentale*, the Potential Aphrodisiac Herb*"*. Similarly, a study titled *"*Toxicopathological Evaluation of Hydroethanol Extract of *Dianthus basuticus* in Wistar Rats*"* by Ashafa, and Kazeem [19] lasted for 28-days, yet subacute, subchronic and chronic toxicity did not appear in the title.

CONCLUSION

With respect to duration of exposure, there are flaws in the titles of some repeated toxicity articles published in high-ranking journals. This study highlighted the relevance of using the right terminology in repeated toxicity tests. In a nutshell, it is suggested that repeated toxicity studies lasting for 14 – 28 days should have the keyword "subacute" in their titles. Those lasting for 90 days should have the keyword "subchronic" in their titles, while those lasting for 6 months and above should have the keyword "chronic" in their titles. Also, articles without keywords; "subacute, subchronic, and chronic" in their titles, could state such keywords as subtitles in their method section and ensure that they comply with the exposure duration guidelines provided by the regulatory bodies. Whenever the duration of exposure does not match with subacute, subchronic, and chronic, the term "repeated toxicity evaluation or assessment can be used". For instance, a study can be titled "42-day repeated dose toxicological evaluation".

ACKNOWLEDGMENTS

This goes to authors of references cited in this manuscript.

Conflict of interest: None to be declared.
Funding and support: None

REFERENCES

1. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in Acute Toxicity Testing: Strengths, Weaknesses and Regulatory acceptance. Interdiscip Toxicol. 2018*;* 11, 5–12. doi: 10.2478/intox-2018-0001. PMCID: PMC6117820; PMID: 30181707.
2. Parasuraman S. Toxicological screening, J Pharmacol Pharmacother. 2011; 2, 74–79. doi: 10.4103/0976-500X.81895. PMCID: PMC3127354; PMID: 21772764.
3. Jacobs AC, Hatfield KP. History of chronic toxicity and animal carcinogenicity studies for pharmaceuticals. *Vet Pathol.* 2012; 50, 324-333. doi: 10.1177/0300985812450727.
4. Sireeratawong S, Jaijoy K, Khonsung P, Lertprasertsuk N, Ingkaninan K. Acute and chronic toxicities of *Bacopa monnieri* extract in Sprague-Dawley rats, BMC Complement Altern Med. 2016; 16, 249. https://doi.org/10.1186/s12906-016-1236-4. PMCID: PMC4962406; PMID: 27460904.
5. Sur TK, Chatterjee S, Hazra AK, Pradhan R, Chowdhury S. Acute and sub-chronic oral toxicity study of black tea in rodents. *Indian J Pharmacol.* 2015; 47. 167–172. https://doi.org/10.4103/0253-7613.153423. PMCID: PMC4386124; PMID: 25878375.
6. Ekanayake CP, Thammitiyagodage MG, Padumadasa S, Seneviratne B, Padumadasa C, Abeysekera AM. Acute and Subacute Toxicity Studies of the Ethyl Acetate Soluble Proanthocyanidins of the Immature Inflorescence of *Cocos nucifera* L. in Female Wistar Rats, Biomed Res Int. 2019; 8428304. https://doi.org/10.1155/2019/8428304. PMCID: PMC6926421; PMID: 31886260.
7. Ebohon O, Irabor F, Omoregie ES. Sub-acute toxicity study of methanol extract of *Tetrorchidium didymostemon* leaves using biochemical analyses and gene expression in Wistar rats. Heliyon. 2020; 6; e04313. https://doi.org/10.1016/j.heliyon.2020.e04313. PMCID: PMC7327260; PMID: 32637701.
8. El Kabbaoui M, Chda A, El-Akhal J, Azdad Q, Mejrhit M, Aarab L, et al. Acute and sub-chronic toxicity studies of the aqueous extract from leaves of *Cistus ladaniferus* L. in mice and rats, J Ethnopharmacol. 2017; 209; 147-156. doi:10.1016/j.jep.2017.07.029. PMID: 28750941.
9. Rodríguez-Lara A, Mesa MD, Aragón-Vela J, Casuso RA, Vázquez CC, Zúñiga JM, et al. Acute/Subacute and Sub-Chronic Oral Toxicity of a Hidroxytyrosol-Rich Virgin Olive Oil Extract. *Nutrients*, 2019; 11; 2133. https://doi.org/10.3390/nu11092133. PMCID: PMC6770357; PMID: 31500145.
10. Kale OE, Awodele O, Akindele AJ. Subacute and subchronic oral toxicity assessments of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root in Wistar rats, *Toxicol. Rep*. 2019; 6; 161–175. doi: 10.1016/j.toxrep.2019.01.005. PMCID: PMC6360914; PMID: 30766799.
11. Aneeshkumar AL, Suja S, Vilash V, Nair RR, Siril EA, Rajasekharan SN. Sub-chronic oral toxicity assessment (90 days) of ethanolic fraction of leaves of *Neurocalyx calycinus* (R. Br. ex Benn.) Rob. in rodents: A lesser known ethnomedicinal plant from the Cholanaickan tribal community, India. *Interdiscip. Toxicol.* 2018; 11. 221–235. https://doi.org/10.2478/intox-2018-0021. PMCID: PMC6853008; PMID: 31736637.
12. Wang L, Li Z, Li L, Li Y, Yu M, Zhou Y. et al. Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Cortex Dictamni* in mice and rats, J Ethnopharmacol. 2014; 158; *Pt A*, 207–215. https://doi.org/10.1016/j.jep.2014.10.027.
13. Ntchapda F, Abakar D, Kom B, Hamadjida A, Dimo D. Acute and sub-chronic oral toxicity assessment of the aqueous extract leaves of *Ficus glumosa* Del. (Moraceae) in rodents, J Intercult Ethnopharmacol. 2014; 3: 206–213. doi: 10.5455/jice.20140913021547. PMCID: PMC4576810.
14. Quaye O, Cramer P, Ofosuhene M, Okine L, Nyarko AK. Acute and Subchronic Toxicity Studies of Aqueous Extract of *Desmodium adscendens* (Sw) DC, *J Evid Based Complementary Altern Med.* 2017; 22, 753–759. https://doi.org/10.1177/2156587217736587. PMCID: PMC5871315; PMID: 29228815.
15. Balogun FO, Ashafa AO. Acute and Subchronic Oral Toxicity Evaluation of Aqueous Root Extract of *Dicoma anomala* Sond, in Wistar Rats, Evid Based Complement Alternat Med. 2016;

3509323. https://doi.org/10.1155/2016/3509323. PMCID: PMC4846747; PMID: 27200099.

16. Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, et al. Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats, Heliyon, 2019; 5, e01179. https://doi.org/10.1016/j.heliyon.2019.e01179. PMCID: PMC6356088; PMID: 30775575.
17. Nsonwu-Anyanwu AC, Nsonwu MC, Bebia DP, Fabian UA, Offor SJ, Egete PD, Usoro CAO. Chronic Exposure to Toluene and Heavy Metals and Changes in Indices of Liver Function, Inflammation and Oxidative DNA Damage among Automobile Workers. Asia Pac J Med Toxicol 2021; 10 (2):53-60. https://apjmt.mums.ac.ir/article_18229_7e6de9e61fb3cc2b33412924d075d613.pdf.
18. Wattanathorn J, Wannanon P, Muchimapura S, Thukham-Mee W, Tong-Un T, Polyiam, P. Toxicity Evaluation of *Anacardium occidentale*, the Potential Aphrodisiac Herb, *BioMed Research International.* 2019; 1459141. https://doi.org/10.1155/2019/1459141. PMCID: PMC6409010; PMID: 30915346.
19. Ashafa AO. Kazeem MI. Toxicopathological Evaluation of Hydroethanol Extract of Dianthus basuticus in Wistar Rats, Evid Based Complementary Altern Med. 2015; 348519. https://doi.org/10.1155/2015/348519. PMCID: PMC4609415; PMID: 26504473.
20. Adusei-Mensah GF, Tikkanen-Kaukanen C, Kauhanen J, Henneh IT, Agyei PEO, Akakpo PK, et al. Sub-chronic toxicity evaluation of top three commercial herbal antimalarial preparations in the Kumasi metropolis, Biosci. Rep. 2020; 40; 1-16. https://doi.org/10.1042/BSR20192536. PMCID: PMC7276653; PMID: 32420605.
21. Kpemissi M, Metowogo K, Melila M, Veerapur VP, Negru M, Taulescu T, et al. Acute and subchronic oral toxicity assessments of *Combretum micranthum* (Combretaceae) in Wistar rats, *Toxicol Rep*. 2020; 7; 162–168. doi: 10.1016/j.toxrep.2020.01.007. PMCID: PMC6976914.
22. Nigatu TA, Afework M, Urga K, Ergete W, Makonnen E. Toxicological investigation of acute and chronic treatment with *Gnidia stenophylla* Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice. BMC Res Notes, 2017; 10. 625. https://doi.org/10.1186/s13104-017-2964-3. PMCID: PMC5704563; PMID: 29183389.
23. Shafaei A, Esmailli K, Farsi E, Aisha AF, Abul AM, Ismail Z. Genotoxicity, acute and subchronic toxicity studies of nano liposomes of *Orthosiphon stamineus* ethanolic extract in Sprague Dawley rats, BMC Complement Altern Med. 2015; 15; 360. https://doi.org/10.1186/s12906-015-0885-z. PMCID: PMC4604773; PMID: 26467526.
24. Tang R, Tian RH, Cai JZ, Wu JH, Shen XL, Hu YJ. Acute and sub-chronic toxicity of *Cajanus cajan* leaf extracts, *Pharm Biol.* 2017; 55. 1740–1746. https://doi.org/10.1080/13880209.2017.1309556. PMCID: PMC6130582; PMID: 28494681.
25. Olorunnisola OS, Afolayan AJ, Adetutu A. Sub-chronic Administration of Methanolic Whole Fruit Extract of *Lagenaria breviflora* (Benth.) Roberty Induces Mild Toxicity in Rats, Pharmacogn Mag. 2015; 11; S516–S521. https://doi.org/10.4103/0973-1296.172955. PMCID: PMC4787082; PMID: 27013788.
26. Lyoussi B, Cherkaoui K, Tangi N, Morel M, Haddad J. Quetin-Leclercq, Evaluation of cytotoxic effects and acute and chronic toxicity of aqueous extract of the seeds of *Calycotome villosa* (Poiret) Link (subsp. *intermedia*) in rodents, *Avicenna J Phytomed*. 2018; 8. 122–135. PMCID: PMC5885326; PMID: 29632843.
27. de Fátima M, Formiga Melo Diniz H, de Luna Freire Pessôa CB, de Sá, AB, Lira L, da Silva Nunes Ramalho KM,et al. Non-clinical acute and chronic toxicity evaluations of *Cissus sicyoides* L. (*Vitaceae*) hydroalcoholic leaf extract, Toxicol Rep. 2018; 5; 890–896. https://doi.org/10.1016/j.toxrep.2018.07.001. PMCID: PMC6120429; PMID: 30181957.
28. Akindele AJ, Oladimeji-Salami JA, Oyetola RA, Osiagwu DD. Sub-Chronic Toxicity of the Hydroethanolic Leaf Extract of *Telfairia occidentalis* Hook. f. (Cucurbitaceae) in Male Rats, Medicines *(Basel, Switzerland)*. 2018; 5; 4. https://doi.org/10.3390/medicines5010004. PMCID: PMC5874569; PMID: 29316640.
29. Nguenang GS, Ntyam A, Kuete V. Acute and Subacute Toxicity Profiles of the Methanol Extract of *Lycopersicon esculentum* L. Leaves (Tomato), a Botanical with Promising *In Vitro* Anticancer Potential, Evid Based Complement Alternat Med. 2020; 8935897. https://doi.org/10.1155/2020/8935897. PMCID: PMC7077039; PMID: 32215048.
30. Deyno S, Abebe A, Tola MA, Hymete A, Bazira J, Makonnen E, et al. Acute and sub-acute toxicity of *Echinops kebericho* decoction in rats, BMC Complement Med Ther. 2020; 20. https://doi.org/10.1186/s12906-019-2794-z. PMCID: PMC7076833; PMID: 32020865.
31. Lan Z, Wang L, Chong Z, Yang G, Yu X, Chen L, et al. Evaluation of the acute and chronic toxicity of the jiangu capsules, Exp Ther Med, 2017; 14;6229–6237. https://doi.org/10.3892/etm.2017.5341. PMCID: PMC5729389; PMID: 29250145.