Review of: "Toxicity of Olea africana in Artemia Salina and Mice"

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Potential competing interests: No potential competing interests to declare.

General

The study assessed the cytotoxicity of ethanol extract of*Olea Africana* on *Artemia salina*. It also investigated its acute and subacute toxicity on mice with a view to establishing its safety and use in the treatment of various infections and diseases. The study established that *O. africana* exhibited acute and sub-acute toxic effects including significant haematological, histological, and biochemical derangements. Overall, the study was well defined with appropriate evidence. The study provided valuable information on the toxicity of *O. africana* and its safety for both alternative and modern medicine. However, there are major concerns to be addressed for a better logical flow and coherence of the manuscript for its overall quality improvement:

1. Lack of novelty: **"Sawsan A. Omer, M.A. Elobeid, M.H. Elamin, Z.K. Hassan, P. Virk, M.H. Daghestani, E.M. Al-Olayan, Nadia A. Al-Eisa and Z.M. Almarhoon, 2012.** Toxicity of Olive Leaves (Olea europaea L.) In Wistar Albino Rats. Asian Journal of Animal and Veterinary Advances, 7: 1175-1182" provided information on haematology, biochemistry, and histopathology of O. africana. **Gaube Guex, Camille; Reginato, Fernanda Ziegler; Figueredo, Kássia Caroline; da Silva, Andreia Regina Haas; Pires, Fernanda Brum; da Silva Jesus, Roberta; Lhamas, Cibele Lima; Hübscher Lopes, Gilberti Helena; de Freitas Bauermann, Liliane (2018).** Safety assessment of ethanolic extract of Olea europaea L. leaves after acute and subacute administration to Wistar rats. Regulatory Toxicology and Pharmacology, S0273230018301132–. doi:10.1016/j.yrtph.2018.04.013. **Ibrahim Hinad, 2021.** Acute and subacute toxicity study of the methanolic extract of olea europea.L leaves in Wistar rat. Recently, its in vitro toxicity and genotoxic activity was reported for aqueous extracts by **Verschaeve et al. (2023)** in Asian Journal of Animal and Veterinary Advance, 7(11): 1175 - 1182
2. The general English structure needs to be tightened with specific with consistency of technical terms and remove typographical errors. E.g., the word ~~hematological~~ should be haematological, and histopathology should read "histomorphology"
3. **Topic**

i. I suggest the topic be modified to "Toxicity of*Olea africana* **Leaf on** *Artemia salina* and Mice

1. **Abstract:** The statement "The ~~current~~ study investigated the toxicity of~~the~~ ethanol…" should read "The study investigated the toxicity of ethanol…". Also, Artemia salina is redundant in this abstract and should be expunged.

2. “Subacute toxicity of the extract was studied in mice for 28 days using animal weight, organ-to-body weight ratio, and haematological, biochemical and histological parameters” should read “Subacute toxicity of the extract was carried out on haematological, biochemical, and histomorphological parameters”
3. **Keywords:** Mice should not be a keyword for this study, in its place, herbal medicine could be used. Histomorphology instead of histological parameters, haematology instead of hematological parameter
4. **Methods:** Collection of plant should read “collection and identification of plant". The authors need to justify the use of ethanol for extract in place of water (aqueous) or a milder solvent like methanol. 20 Kgs of air-dried Olea africana leaves… should read “Twenty kilograms (20 kg) of air-dried Olea africana leaves was….” The statement, “…and pulverized into a fine powder.” Should read “…and pulverized into fine powder.” For the analysis of brine shrimp cytotoxicity, Finney, D.J., 1971. Probit Analysis, third ed. Cambridge University Press, Cambridge England, pp. 269–282 is a more recent reference. For acute toxicity study, the Lorke (1983) method would have been more appropriate for this acute study with assessment of effect at 10, 100, 1000 mg/kgBW in Phase I and 1600, 2900 and 5000 mg/KgBW in Phase II instead of starting off at 2000 mg/kgBW with your extract. What informed the choice of this initial concentration of 2000 mg/kgBW administration? For a substance whose perceived toxicity is being investigated, this is alarmingly high. For Sub-acute toxicity study, how did investigators distribute 28 mice into four groups with each group containing 4 animals each? This will give a total of 16 animals and not 28. The concentration of ketamine administered to anaesthetize test animals should be stated. Blood for assessment of haematological parameters is best collected in EDTA K_3 bottles as heparin is known to interfere with blood components. Assay methods employed for each biochemical parameter should be stated and referenced.
5. **Result:** I am curious as to why author estimated percentage yild of the extract and didn’t present the result. The result of LD_{50} is outrightly missing in this manuscript. Also, for all the groups of animals tested in the sub-acute study, why are their results of observation not tabulated to show the degree of toxicity reported in this study. It will be appropriate to show the correlation between the cytotoxic effect of the extract and control (e.g., thymol) on *A. salina.* Authors should put scale bar on the photomicrographs of the liver and kidney in Figures 3 and 4. It will be appropriate to at least determine the phytoconstituents of the ethanol extract which may be implicated for its toxic activity in comparison to those of aqueous and methanol that are in literatures For all the figures under the result section, all abbreviations such as “ns”, “mg/kg”, etc., should be defined. According to Meyer et al. (1982), substances with LC50 value <1000 ppm are toxic while ones with values >1000 ppm are non-toxic. Also, OECD (2001) and Lorke (1983) categorized substances having LD_{50} greater than 5000 mg/kgBw as safe. The assertion that this extract is toxic cannot be validated based on the cytotoxicity, acute, and subacute studies. In Table 3, the normal range of the blood parameter for a healthy animals should be provided in the first row
6. **References:** A majority of the references cited is older than 5 years, especially in the review section of the background study where 1984, 1985, 2003, 2005, etc., articles were referenced. Authors are encouraged to review recent positions in recent articles.

Suggested articles are:

a. Odongo et al. (2022): A Systematic Review of Medicinal Plants of Kenya used in the Management of Bacterial

Infections

b. Msomi and Simelane (2017). Olea europaea subsp. africana (Oleaceae). In: Active Ingredients from Aromatic and Medicinal Plants.
c. Adem et al. (2020). Phytochemical screening and antimicrobial activity of Olea
d. europaea subsp. africana against pathogenic microorganisms.