ORIGINAL ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2025.038

The Nigerian Journal of PHARMACY
AS MEN OF HONOUR WE JOIN HANDS
THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Evaluation of Acute Dermal Toxicity and Acute Oral Toxicity of Neurovoral® Cream and Tea Extract in Rats

Osiyoye, Kehinde Oluwaseyi [1,4], Mudathir, Aisha Doyinsola [1], Mafo, Rapheal Omoniyi.[2,6], Olayemi, Jacob Olubunmi[2], Femi-Akinlosotu, Omowumi [5], Bakre, Adewale Ganiyu[1,3*]

[1]Department of Pharmacology and Therapeutic, University of Ibadan, Ibadan, Nigeria
[2]Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria
[3]Department of Pharmacology and Toxicology, University of Ibadan, Ibadan, Nigeria
[4]Department of Pharmaceutical Technology, Gateway ICT Polytechnic, Saapade, Nigeria
[5]Department of Anatomy, University of Ibadan, Ibadan, Nigeria
[6]Colleagnant International Limited, Ibadan, Nigeria

ARTICLE INFO

Article history:

Received 9th September 2025
Revised 13th October 2025
Accepted 15th October 2025
Online Published

Keywords:

dermal toxicity,
erythema,
hyperemia,
tea extract,
Traditional African Medicine

**Corresponding Author:*
Bakre, Adewale Ganiyu
Phone: +2348081062365
ORCID I.D.: 0000-0001-9769-8510
Email: ag.bakre@mail.ui.edu.ng

ABSTRACT

Herbal products have become a common remedy used in treatment and management of many diseases. The safety ascribed to natural products because of their origin is now doubtful as side effects and toxicities have been recorded.
The aim of the study is to determine acute dermal toxicity and acute oral toxicity of Neurovoral® cream and tea extract.
Thirty albino rats were used to investigate the acute dermal toxicity (12) and acute oral toxicity (18) of Neurovoral® cream and tea respectively. Neurovoral® cream (0.5 g) was applied to the trimmed dorsal area of the trunk of rats, signs of toxicity (erythema, oedema, irritation) were observed periodically at 24, 28 and 72 h, and again after 14 days. The extract from the tea was administered at 2000 and 5000 mg/kg, and the rats were observed for the first 6 hours, and there after daily for 14 days. All the animals were euthanised at the 14th day and vital organs (skin, kidney, liver, brain) were collected for subsequent histological analysis.
Dermal toxicological report showed negligible erythema during the period of application of cream, and acute oral administration of 5000 mg/kg did not result in death or any significant toxicity signs. There was no significant alteration in the histology of organs observed.
The study results indicated that Neurovoral® cream and tea are relatively safe and non-toxic to rats in acute exposure.

INTRODUCTION

Neurovoral® cream and tea are 100 percent herbal products derived from same combination of parts plants and are used for treatment of different forms of paralysis (plegias) and movement disorders in Traditional African Medicine (TAM) and ethnomedicine. Several herbal products including turmeric and *Ginkgo biloba* have been claimed to be of benefit in treatment and recovery from stroke, paralysis and several other movement disorders[1]. Most of these herbal products, though derived from mainly from one plant, are presented and used in largely unquantified forms that could cause toxicity as a result of the dose. Research works have shown that all herbal medicines could be toxic[2]. Studies on some traditional herbs have reported side effects such as diarrhea and skin necrosis[3].

Herbal products derived from more than one beneficial plant are theoretically expected to be more potent and thus more toxic. Only few of these herbal products are prepared in more than one pharmaceutical dosage form that help to increase the overall bioavailability of the active compounds and reduce its toxicity. Neurovoral® herbal products are similar compounds derived from same combination parts of plants presented in different pharmaceutical dosage forms.

About two thirds of the world populations consume tea as a beverage, presenting an opportunity for its use as a delivery source of herbal extract[4]. Tea has the ability to deliver a wide range of heterogeneous macromolecules including benzene rings, hydroxyl functional groups, carboxyl groups, methyl groups and amino groups, which have many health benefits, such as antitumor activity and blood lipid-lowering effects[5]. The main ingredients of tea determine its benefit and toxicity, and how it impacts human health[6]. They have drawn an increasing attention for their bioactive phytochemicals and beneficial properties. However, the fact that they are products from natural sources do not ensure their safety, since some natural plants are reported to produce side effects[7]. Vital organs (brain, liver, and kidney) can be observed when rats are given very high amounts of tea extract[8], as a number of studies have indicated that large dose and excessive consumption of tea in humans could be deletorious[9], thus, more focus should be placed on the safety of tea and processed tea products. When rats are exposed to a high dose of tea extract, vital organ (brain, liver and kidney) were observed[8]. Research has shown that excessive consumption of tea is harmful to human beings[9].Thus, the safety of tea and processed tea products should be given more attention.

Generally, it is believed that herbal products are safe and free of side effects because of their origin, but there is paucity of scientific data in support of their safety and toxicological profiles. Hence proper scientific knowledge on toxicity and safety is crucial. Toxicological studies on medicinal plants are limited, raising a legitimate concern regarding the potential toxic effects associated with their use[10]. Combined herbal remedies are rarely available because of the expected side effects. This study seek to determine the toxic effect of the Neurovoral® cream on the skin of rats using the OECD test guidelines which measure irritation, erythema and oedema and also determine the safety profile of the tea extract (lethal effect of tea extract on the brain, kidney and liver) after single exposure and its reversibility if at all response occurs.

Materials and Method

Experimental Animals

A total of thirty albino rats weighing between 300-350 g were obtained from the Laboratory Animal House of the University of Ibadan, Ibadan, Nigeria. All animals were housed in stainless steel cages and allowed to acclimatize to the laboratory conditions for 14 days before the experimental procedures were carried out. The animals were maintained on a 12 h dark/light cycle at about 22 ± 3°C and allowed free access to standard laboratory diet (Ace Feeds Plc., Ibadan, Nigeria) and water *ad libitum*.

Ethical Approval

Rats were handled in accordance with guidelines for care and use of laboratory animals laid down by University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/007-0123/27) and the National Institute of Health (NIH, Department of Health and Human Services Publication No. 5, Revised 1985).

Acute Dermal Toxicity Studies

Twelve animals were used for the acute dermal toxicity test that was performed in compliance with the OECD test guidelines 402 OECD[11]. The fur (2-3 cm) of the test animals were removed from the dorsal area of the trunk of the animals. The test cream (0.5 g) was applied to each of the animals in the test treatment group, while distilled water was applied to each animal in the control group (n=6). The application was done at 3 min, 1 h and 4 h to test the animals and the animals were observed for signs of oedema, erythema, irritation and inflammation at 24 h, 48 h and 72 h after the application of the creams as described by Awodele et al., (2022)[12] . The observation of the test animals for dermal toxicity and possible reversibility of toxicity continued for 14 days post-cream administration.

The following scores were adopted in this study: Erythema (0 = no erythema; 1 = very slight erythema; 2 = well defined erythema; 3 = moderate to severe erythema; 4 = severe erythema with injuries); Oedema (0 = no oedema; 1 = very slight oedema; 2 = well defined oedema; 3 = moderate oedema; 4 = severe oedema); Irritation (0 = non-irritant; 0-0.5 = negligible irritant; 0.5-2.5 = mild irritant; 2.5-5.0 = moderate irritant; 5.0-8.0 = severe irritant).

Histological Examination of the Skin

Tissues taken from the skin of the animals upon sacrifice were fixed in 10% formo1-sa1ine (buffered formalin) for 24 h. Thereafter, they were processed for 17 h using the Leica"

TP 1 0 semi-enclosed automatic tissue processor which performed actions including dehydration in graded alcohol, clearing in xylene and impregnation with molten wax. Tissues were embedded on edge in paraffin wax and then sectioned at 3 microns using a rotary microtome, Leica" RM2125. Tissues were stained using the conventional haematoxylin and eosin for histopathological analysis. The tissue slides were viewed under a light microscope.

Acute Oral Toxicity Study

The acute-toxicity test was conducted according to OECD Guideline 423 (OECD 2002) with slight modifications. In this study, the up and down procedure was used to determine the median lethal dose LD_{50} [13]. The animals were divided into three groups (n=6) and orally administered distilled water (10 ml/kg), 2000 mg/kg and 5000 mg/kg Neurovoral® tea extract (NTE) according to the animal body weight. All the animals were observed closely for mortality, diarrhea, tremors, salivation and lethargy, for the first 6 hours, and there after daily for 14 days. The animals were sacrificed at the 14th day and vital organs (brain, liver and kidney) were collected for subsequent histological analysis.

Results

Acute Dermal Toxicity Study

Neurovoral® cream does not cause any acute toxicity on the skin

The sum of scores for erythema (E), oedema (O), and irritation (I) based on the scoring scale described in the methodology is shown in the Table 1 below. At 24 h there was negligible erythema and irritation that were reversed almost completely as at 72 h.

Table 1: **Effect of Neurovoral® cream on dermal toxicity of the skin**

Group	Skin reactions								
	24 hours			48 hours			72 hours		
	E	O	I	E	O	I	E	O	I
Control	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Treated	7/6	0/6	5/6	2/6	0/6	3/6	0/6	0/6	0.5/6

The following scores were used in this study: Erythema (0 = no erythema; 1 = very slight erythema; 2 = well defined erythema; 3 = moderate to severe erythema; 4 = severe erythema with injuries); Oedema (0 = no oedema; 1 = very slight oedema; 2 = well defined oedema; 3 = moderate oedema; 4 = severe oedema); Irritation (0 = non-irritant; 0-0.5 = negligible irritant; 0.5-2.5 = mild irritant; 2.5-5.0 = moderate irritant; 5.0-8.0 = severe irritant).

Complete recovery of removed dorsal fur on skin after using Neurovoral® cream

The sum of scores for erythema (E), oedema (O), and growth (G) based is shown in the Table 2 below. At 14 days, the scrapped hair has completely grown

Table 2: Sum of scores for dermal changes for reversal after 14 days

Group	Skin reactions		
	E	O	G
Control	0	0	6
Treated	0	0	6

The score for hair growth is 1

3.1.3 Histological Study

Histological section of skin section showed more cellular organelles and infiltration of more vascular cells in the treated group in comparison with the control (Plate 1). Also, there was more proliferative and adipose layer in treated group in comparison with the control. There were no evidences of tissue necrosis, heamorrhage and dermal atrophy. There were no changes in the thickness of dermal and sub curtis layer, but the epidermal layer in the treated group were more proliferative.

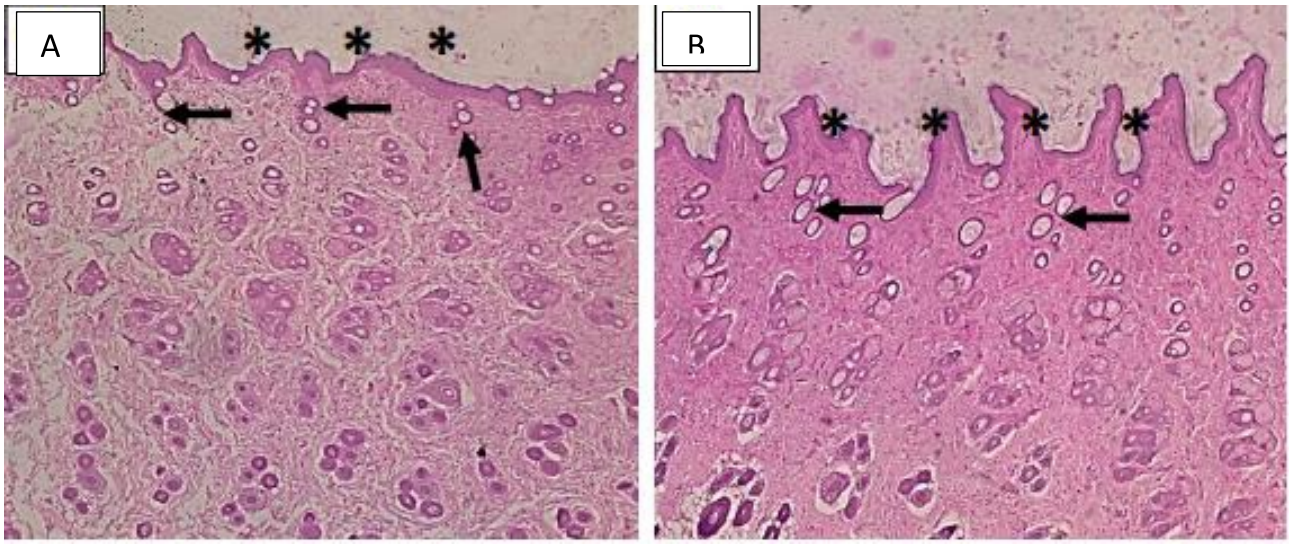

Plate 1: Histology of the skin (A) Skin of control rats. (B) Skin of treated rats Heamatoxylin and Eosin (H&E) stained representative photomicrograph of skin of albino rats of control. X100 (A) showing normal cellular organelles and a few scattered hair follicles (arrows) while in the treated section (B), there are more and robust hair follicles. Note the appearance of a more proliferative epidermal layer (asterisks) in the (B).

Acute Oral Toxicity of Neurovoral® Tea Extract (NTE)

Sign of oral acute toxicity of Neurovoral® tea extract

The administration of 2000 and 5000 mg/kg did not cause mortality in 24 h (Table 3). Convulsion, tremor, salivation, lethargy and other possible acute symptoms of toxicity were not observed.

Table 3: **NTE did not cause mortality at 5000 mg**/kg

Dose (mg/kg)	N	Mortality	% Mortality
10 ml/kg	6	0	0
2000	6	0	0
5000	6	0	0

3.2.2 Effect of NTE on brain tissues

Administration of 5000 mg/kg NTE did not cause any visible lesions in the histological section of brain of rats given 10 ml/kg dH_2O and 5000 mg/kg NTE (Plate 2)

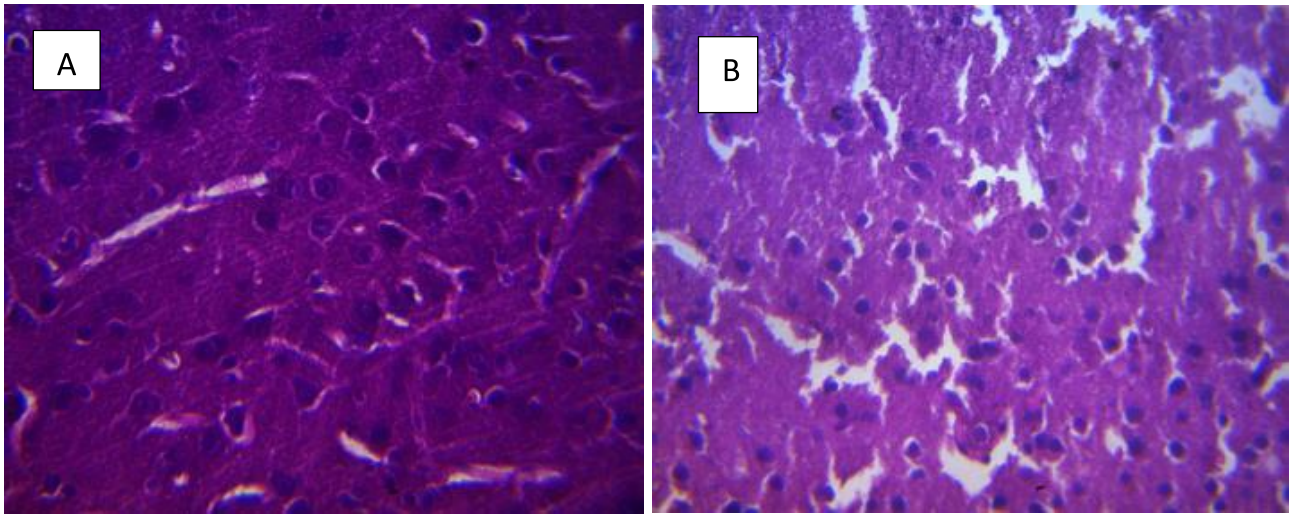

Plate 2: H & E stained representative photomicrograph of cerebellum (brain tissue) of rats

(A) Brain tissues of control (10 ml/kg dH_2O) (B) Brain tissue of treated albino rats (5000 mg/kg) showing no visible lesion 14 days after acute administration. X100

3.2.3 Effect of NTE on Kidney tissues

Administration of 5000 mg/kg NTE did not cause any visible lesions in the histological section of kidney of rats given 10 ml/kg dH_2O and 5000 mg/kg NTE (Plate 3)

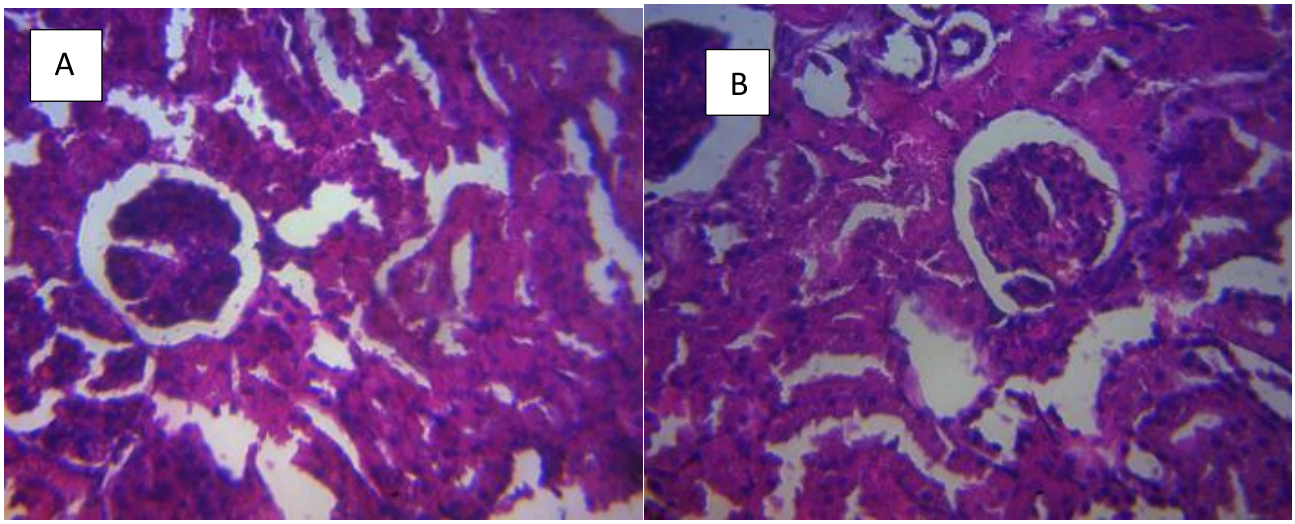

Plate 3: H and E stained representative photomicrograph of kidney tissue

(A) Kidney tissues of control (10 ml/kg dH_2O) (B) Kidney tissue of treated albino rats (5000 mg/kg) showing no visible lesion 14 days after acute administration. X400

3.2.4 Effect of NTE on Liver tissues

Administration of 5000 mg/kg NTE did not cause any visible lesions in the histological section of liver of rats given 10 ml/kg dH_2O and 5000 mg/kg NTE (Plate 4)

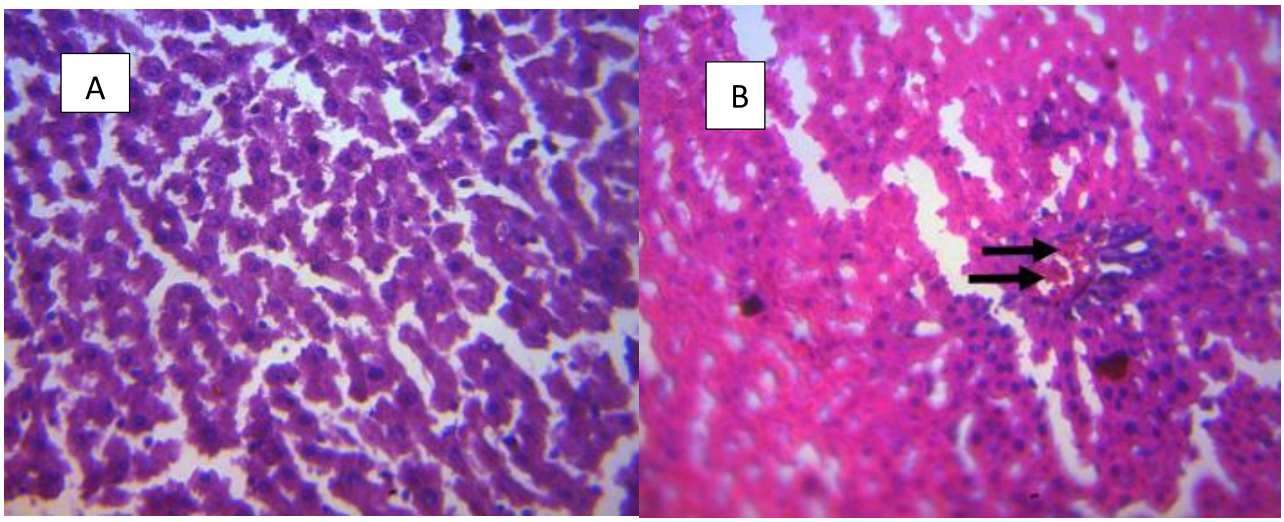

Plate 4: H & E stained representative photomicrograph of liver tissue
(A) Liver tissues of control (10 ml/kg dH_2O) (B) Liver tissue of treated albino rats (5000 mg/kg) showing no visible lesion 14 days after acute administration. X100.

4.0 DISCUSSION

Establishment of the safety profile of herbal product is now more than ever becoming very vital because of its emerging acceptability and popularity in prevention and cure of diseases. In this study, topical application of Neurovoral® cream and oral administration of 5000 mg/kg Neurovoral® (NTE) did not cause any sign of toxicity or mortality acutely and after the reversibility. The cream did not cause erythema, oedema, irritation but allowed hair regrowth, while the tea extract did not cause histopathological lesions in brain, kidney and liver.

Irritation of the skin involves local inflammation, a direct skin injury presenting physiologically as erythema and oedema[14]. The skin irritation test has become very important in evaluation of safety of consumer products[15]. The rat model for dermal toxicity studies, a well-established model was used in this study[16-17]. The study showed that components of the Neurovoral® cream are negligible skin irritants as very slight erythema were observed at 24 h to 48 h, and no oedema were observed in the test group at all times employed in the study. The erythema scores were (1) for all the animals except one with a score of (2) suggesting a physiological response to the cream. At all the other time points, there were no sign of well-defined erythema and irritation. Erythema is redness of the skin or mucous membranes, caused by hyperemia of superficial capillaries while edema is a buildup of excess serous fluid between tissue cells[18]. This physiological process does not occur in isolation, thus no oedema (0) score for same animals confers the negligibility on the erythema score. Hair re-growth was observed in all the test animals by the 14th day. The test was performed according to the OECD guidelines, providing a basis for subsequent long term toxicity tests and establishment of a suitable dose regimen.

Histopathological assessment of the skin, which is the target organ under study in the dermal toxicity test further validates the result of the study[19]. The more cellular organelles and infiltration of vascular cells in the treated skin might be a signal for immunologic response to the cream which might be beneficial in removal of any inflammatory agent present. Also, the epidermal layers remain intact, though there was more proliferative and adipose layer in treated group in comparison with the control. There is no evidence of tissue necrosis, heamorrhage and dermal atrophy in the treated group. Also no changes in the thickness of dermal and sub curtis layer, but the epidermal layer in the treated group were more proliferative.

Histopathological examination of organs is most suitable for evaluating pathological changes related to treatment[9]. The evaluation of pathological changes in the vital organs of animals exposed to xenobiotic compounds is the basis of toxicological evaluation[20]. In the present study, no toxicity signs or lesions were found in the histology of the brain,

kidney and liver of rats in all the experiment groups from a macroscopic perspective. In addition, the results of histopathological examination showed no pathological signs in the rats of the control and test groups.

The results from this acute oral toxicity study suggested that NTE is relatively nontoxic and the no-observed-adverse-effect level (NOAEL) was determined as 5000 mg/Kg body weight/day. However, further toxicity assessment such as subacute, chronic, or genotoxic studies using repeated dose NTE should be conducted to confirm its safety on prolonged use.

CONCLUSION

The present study showed the absence of acute toxicity attributable to application of Neurovoral® cream and drinking of NTE at the dose of 5000 mg/kg. Moreover, the data obtained from the present study are relevant as they contribute to the safety and toxicological profiles of Neurovoral® products. However, studies on chronic toxicity of the oil should be conducted in the future to evaluate the safety of this oil for human use because of its proposed duration of use.

Acknowledgements The authors thank Mr. O. Akintoye and Mr. M. Adegoke for their assistance during the course of the experiment.

Funding: The authors appreciate Mr. R. O. Mafo and Dr. J. O. Olayemi for providing the cream and tea used in the experiment

Conflict of Interest: There are no conflicts of interest.

Author Contributions: Bakre AG: Experiment design, manuscript review and writing; Olayemi JO: Writing manuscript discussion, review; Mafo RO: Writing introduction aspect of manuscript
Osiyoye KO: Benchwork, writing methodology aspect of manuscript; Mudathir AD: Benchwork, writing the result aspect of manuscript; Femi-Akinlosotu: Methodology, result and discussion on histological slides.

References

1. Kurn, SJ, Shook S. Herb and Nutrients for Neurologic Disorder: Treatment strategies for Alzheimer`s Parkinson, Stroke, Multiple Sclerosis, Migraine and Seizures. Simon and Schuster; 2016 June 16
2. Wannissorn B, Jarikasem S, Siriwangchai T, Thubthimthed S. Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia. 2005 Mar 1;76(2):233-6. doi.org/10.1016/j.fitote.2004.12.009
3. Yayesh Limenih YL, Shemsu Umer SU, Messay Wolde-Mariam MW. Ethnobotanical study on traditional medicinal plants in Dega Damot Woreda, Amhara Region, North Ethiopia.
4. Ding X, Han C, Hu W, Fu C, Zhou Y, Wang Z, Xu Q, Lv R, He C, Zuo Z, Huang J. Acute and subacute safety evaluation of black tea extract (herbt tea essences) in mice. Toxics. 2022 May 27;10(6):286.
5. Xu J, Yan B, Zhang L, Zhou L, Zhang J, Yu W, Dong X, Yao L, Shan L. Theabrownin induces apoptosis and tumor inhibition of hepatocellular carcinoma Huh7 cells through ASK1-JNK-c-Jun pathway. OncoTargets and therapy. 2020 Sep 9:8977-87.
6. Tang GY, Meng X, Gan RY, Zhao CN, Liu Q, Feng YB, Li S, Wei XL, Atanasov AG, Corke H, Li HB. Health functions and related molecular mechanisms of tea components: an update review. International journal of molecular sciences. 2019 Dec 8;20(24):6196.
7. Bello I, Bakkouri AS, Tabana YM, Al-Hindi B, Al-Mansoub MA, Mahmud R, Asmawi MZ. Acute and sub-acute toxicity evaluation of the methanolic extract of Alstonia scholaris stem bark. Medical sciences. 2016 Mar 8;4(1):4.
8. Wang D, Xiao R, Hu X, Xu K, Hou Y, Zhong Y, Meng J, Fan B, Liu L. Comparative safety evaluation of Chinese Pu-erh green tea extract and Pu-erh black tea extract in Wistar rats. Journal of Agricultural and Food Chemistry. 2010 Jan 27;58(2):1350-8.
9. Gardner EJ, Ruxton CH, Leeds AR. Black tea–helpful or harmful? A review of the evidence. European journal of clinical nutrition. 2007 Jan;61(1):3-18.
10. Lulekal E, Tesfaye S, Gebrechristos S, Dires K, Zenebe T, Zegeye N, Feleke G, Kassahun A, Shiferaw Y, Mekonnen A. Phytochemical analysis and evaluation of skin irritation, acute and sub-acute toxicity of Cymbopogon citratus essential oil in mice and rabbits. Toxicology reports. 2019 Jan 1;6:1289-94.
11. Guideline O. Acute Oral Toxicity-Acute Toxic

Class Method. OECD Guidelines for the Testing of Chemicals.
12. Awodele O, Awolola A, Oladimeji-Salami JA, Ayinde OO. Investigation of Acute Dermal Toxicity of Some Hydroquinone Containing Cosmetics in Rats. University of Lagos Journal of Basic Medical Sciences. 2022 Sep 2;4(8).
13. Rispin A, Farrar D, Margosches E, Gupta K, Stitzel K, Carr G, Greene M, Meyer W, McCall D. Alternative methods for the median lethal dose (LD50) test: the up-and-down procedure for acute oral toxicity. ILAR journal. 2002 Jan 1;43(4):233-43.
14. Alavi A, Sibbald RG, Ladizinski B, Saraiya A, Lee KC, Skotnicki-Grant S, Maibach H. Wound-related allergic/irritant contact dermatitis. Advances in skin & wound care. 2016 Jun 1;29(6):278-86.
15. Mathur AK. Safety of industrial chemicals and finished product. Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology. 2005;6(1).
16. Han SM, Lee GG, Park KK. Acute dermal toxicity study of bee venom (Apis mellifera L.) in rats. Toxicological research. 2012 Jun;28(2):99-102.
17. Guaouguaou FE, Taghzouti K, Oukabli M, Masrar A, Chabraoui L, Bouabdellah M, Es-Safi NE. Acute and subchronic oral and dermal toxicological studies of salvia verbenaca extracts in mice and rats. Journal of Herbs, Spices & Medicinal Plants. 2019 Jan 2;25(1):33-42.
18. Gatne M, Tambe K, Adarsh A, Ravikanth K. Acute dermal irritation study of polyherbal gel mastilep in rabbits.
19. Hothorn LA, Hajian G. Biostatistics in toxicology. Toxicology. 1999 Jan 1:25-41.
20. Ramaiah SK. Preclinical safety assessment: current gaps, challenges, and approaches in identifying translatable biomarkers of drug-induced liver injury. Clinics in Laboratory Medicine. 2011 Mar 1;31(1):161-72.