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Antioxidant, Dermal and Acute Toxicological Effects of *Eucalyptus camaldulensis* (Dehn-Blakely) Essential Oil on Male Wistar Rats

Uju Dorathy Iliemene Ejike* and Oluchukwu Ogechukwu Anunobi

Department of Biochemistry, Faculty of Science & Technology, Bingham University, Karu. Km 26
Abuja-Keffi Expressway, P.M.B. 005, Nasarawa State, Nigeria

ABSTRACT

During the era of the COVID-19 lockdown, many Nigerians resorted to home remedies like herbal mixtures for therapy because they could not have access to orthodox medicine. *Eucalyptus camaldulensis* (*E. camaldulensis*) essential oil was one of such remedies as many believed it had the potential to treat colds, flu, sore throats, bronchitis and even prevent SARS-CoV-2 infection.

The objective of the study is to check the antioxidant, dermal and acute toxicological effects of the *E. camaldulensis* essential oil. Rats were grouped into 8 of 5 each. Normal and corn oil (2ml/kg body weight) control groups. *E. camaldulensis* essential oil from Jos, Niger, Nasarawa, Kogi, Kwara, and Benue zones were given at 2ml/kg body weight for 14 days as well as applied on the shaved skin of the rats. No mortality was recorded in the sub-acute toxicity study at low and high doses (10mg/kg and 5000mg/kg). The levels of AST, ALT, TNF- α and IL-6 did not significantly differ from normal control rats. Lungs Investigation recorded a significant increase in the TNF- α . Antioxidant enzyme assays showed a significant increase in catalase, superoxide dismutase and glutathione-s-transferase. Histological examination showed focal inflammation with moderate cytoplasmic clearing in the liver and focal mild epidermal sclerosis of the skin. Dermal application of the oil shows no significant toxic effect except some mild inflammation (skin irritation). Oral administrations were relatively safe with mild adverse effect observed in the lung inflammatory markers.

Keywords: *Eucalyptus camaldulensis*, Essential oil, COVID-19 pandemic, Infection remedies, Toxicity, Northern Nigeria

***Correspondence author: Uju Dorathy Iliemene Ejike** (uju220@gmail.com; +234 8065556861)

INTRODUCTION

Eucalyptus camaldulensis can be found in various parts of Nigeria as well as other part of the world and used widely for various home-based therapy [1]. Its leaves are known to contain 0.1–0.4% essential oil, of which 77% is 1,8 cineole. All the *E. camaldulensis* essential oil single compounds belong to chemical class of hydrocarbons terpenes, further divided according to the number of isoprene units (C_5H_8) to monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), and longer chains of isoprene units. The medicinal effects of *E. camaldulensis* can be attributed to these chemical compounds present in it. There is considerable variation in the chemical composition of essential oils extracted from *E. camaldulensis* and these differences may be due to the change in plant genes through generations and hybridizations, nutrients of different soils and their accumulation in the leaves which may result in different plant metabolism and consequently the production of different bio-products [2]. The essential oil from the leaves is used medicinally for treating gastrointestinal symptoms (including colic, diarrhea, and dysentery), respiratory disease (colds, coughs, asthma, laryngalgia, laryngitis, pharyngitis, sore throat, trachalgia) [3], arrest bleeding, open wounds, and cuts, as well as its decoctions for the relief of aches and pains in muscles, joints and even tooth [2].

In recent times as the world battled the COVID-19 pandemic amidst lockdown restrictions, most

Nigerians turned to the use of *E. camaldulensis* essential oils as it is widely believed that since it is reportedly effective against symptoms of cough and respiratory distress, it would treat COVID-19. Though studies carried out on *E. camaldulensis* in different parts of the world have been reported [4, 5, 6], however no reports of comparison of effects of *E. camaldulensis* from six different states of Nigeria bearing in mind that compositions may vary based on geographical location.

Therefore, this study was to investigate any toxicological effects of *E. camaldulensis* from six states in Nigeria by observing its effect on levels of antioxidant enzymes, the liver, lungs and skin of experimental rats.

MATERIALS AND METHODS

Plant sample collection and authentication

Fresh leaves of *E. camaldulensis* were harvested from six different states in Northern Nigeria which include Jos, Niger, Nasarawa, Kogi, Kwara, and Benue. The plant was identified at the Department of Biological Science, Bingham University, Nigeria where voucher number 27089 was assigned.

Essential oil extraction

About 50 kg of the fresh leaves of *E. camaldulensis* was loaded in an essential oil extractor with 500 ml of water. This was subjected to hydro-distillation for about three hours. The resulting volatile oils were dried over

anhydrous sodium sulphate and then stored in dark bottles in a refrigerator until used.

Experimental animals

Male Wistar rats weighing between 60 and 90 g were obtained from Animal Holding Facility at Physiology Department, Ahmadu Bello University, Zaria. The animals were pre-conditioned in plastic cages with sawdust beddings for a period of one week. They were fed with rat food (Vital Feeds Ltd, Nigeria) during the day and provided with tap water *ad libitum*. They were maintained under 12 hours' light/dark cycle.

Ethical considerations

Ethical clearance was obtained from the Ahmadu Bello University Ethical Committee in compliance with the ARRIVE guidelines following the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, and EU Directive 2010/63/EU for animal experiments.

Sub-acute toxicity determination (LD50)

Sub-acute toxicity of *E. camaldulensis* essential oil was determined according to Atawodi *et al.*, [7]. The rats were weighed and grouped into 6 groups of 3 rats each. Each group represents an individual oil sample of *E. camaldulensis* from Jos, Niger, Nasarawa, Kogi, Kwara, and Benue. The rats were administered a single dose of 1000mg/kg body weight of the oil sample orally and monitored. They were also administered a single dose of 3000mg/kg body weight as well as

5000mg/kg body weight of the oil sample and monitored.

Animal groupings and oil administration

The rats were weighed and grouped into 8 of 5 rats each. Groups 1, 2, 3, 4, 5, and 6 were administered *E. camaldulensis* essential oil from Jos, Nasarawa, Benue, Niger, Kwara, and Kogi zones (2ml/kg body weight) respectively. Group 8 was normal control, while group 7 was administered corn oil (2ml/kg body weight). The administration lasted for 14 days before sacrifice. Before the administration, the dorsal fur of the rats were shaved at a 2 cm rectangular length, then *E. camaldulensis* essential oil and corn oil were applied topically to the skin of the animals according to the groupings. The choice of the sample dose was based on the sub-acute toxicity determination.

2.7. Serum and tissue collection, homogenization and storage

Twenty-four hours after the last administration, rats were weighed and sacrificed, following mild chloroform anesthesia. The portion of the shaved skin and the liver were harvested and rinsed rapidly in ice-cold normal saline. The skin and half of the liver were stored in a sample bottle containing 10% freshly prepared formal saline for histological and immunohistochemical assays. The other half of the liver and lungs were homogenized in 10% ice-cold phosphate buffer (pH7.4) and centrifuged using a temperature-regulated centrifuge (TGL-16G. B. Bran

Scientific & Instrument England) at 3000×g for 10 min. The clear supernatants were collected. Also, at sacrifice, blood from each rat was collected from the carotid artery at the neck into previously labeled test tubes and centrifuged at 3000×g for 15 min for serum separation. Homogenates and sera samples were kept in Eppendorf tubes and stored at -20 °C for biochemical assays.

Assay for catalase activity

Catalase activity was measured using the Rat Catalase (CAT) ELISA Kit (catalog number: MBS701713). This assay employs the quantitative sandwich enzyme immunoassay technique and was conducted according to the kit manual. The color development was stopped and the intensity of the color was measured at 450nm within 5 minutes. Calculation was done using the standard curve as described by the kit manual.

Glutathione-S-Transferase (GST) Determination

Glutathione-S-Transferase was determined using Abcam (Ab65326) GST activity assay kit. The kit is based upon GST-catalyzed reaction between reduced glutathione (GSH) and the GST substrate, CDNB (1-chloro-2,4-dinitrobenzene), which has the broadest range of isozyme detectability. The GST-catalyzed formation of GS-DNB produces a dinitrophenyl thioether which was detected using spectrophotometer at OD 340 nm. One unit of GST activity is defined as the amount of enzyme producing 1µmol of GS-

DNB conjugate/min under the conditions of the assay.

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity assay was based on the SOD-mediated decrease in the rate of auto-oxidation of hematoxylin in aqueous alkaline solution, which yields a chromophore with maximum absorbance at 560 nm [8]. The SOD concentration was calculated as percentage inhibition of the rate of autoxidation of hematoxylin.

Interleukin 6 (IL-6) Determination

Quantitative determination of rat IL-6 concentrations in the liver and lungs tissue homogenates was done using Cusabio ELISA kit (Catalog Number.CSB-E04640r). This assay employs the quantitative sandwich enzyme immunoassay technique. The color development was stopped and the intensity of the color was measured at the range of 0.312 pg/ml-20pg/ml as described in the kit manual.

Determination of Tumour Necrosis Factor-Alpha (TNF-α)

Quantitative determination of rat TNF-α concentration in the liver and lung tissue homogenates was done using the Cusabio ELISA kit (Catalog Number.CSB-E11987r). This also assay employs the quantitative sandwich enzyme immunoassay technique. The color development was stopped and the intensity of the color was

measured at the range of 6.25pg/ml-400 pg/ml as described in the kit manual.

Determination of amino transferases levels

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined using the Randox laboratories Ltd. assay kit based on description of Reitman and Frankel [9]. The generated brown colour in alkaline medium was measured colorimetrically against blank at 546nm.

Histological assessment of the skin and liver

Histological investigation of the liver and skin were performed according to the method described by Drury and Wallington [10]. The selected liver and skin were excised and fixed in 10% neutral formalin fixative followed by dehydration in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax. Paraffin sections (5 µm thickness) were stained with hematoxylin-eosin (H and E) and examined under a light microscope at a magnification of ×400 (Leica Buffalo, N.Y. 14240 U.S.A. Model CME Microscope 220–240 VAC 50/60Hz).

Immunohistochemical evaluation of the skin

The method was the avidin-Biotin peroxidase complex (ABC) in immunoperoxidase techniques described by Hsu *et al.* [11] using the primary antibody pan-cytokeratin. The percentage area of expression was quantified with immunohistochemistry image analysis tool box (ImageJ computer application) which generate

automatically, the percentage of positively stained nuclear area (labelling index) and colored deconvolution algorithm separating the counter stains [12].

Data analysis

Utilizing SPSS version 23, the results obtained were analyzed using analysis of variance (ANOVA), while Duncan Multiple Range Test (DMRT) was used for post-hoc analysis ($p \leq 0.05$).

RESULTS

Sub-acute toxicity evaluation of *E. camaldulensis* essential oil from six zones

No mortality was recorded in the sub-acute toxicity study when subjected to low and high doses (10mg/kg and 5000mg/kg) of *E. camaldulensis* essential oil from six different zones (Table 1).

Effect of *E. camaldulensis* essential oil on some oxidative stress, antioxidant enzymes in the serum and liver

Liver and serum catalase was markedly elevated in animals that were administered oil from Nasarawa state. This state, out of the six states showed significant increase in liver catalase when compared with normal control but no significant increase when compared with standard oil (Table 2). Lowest level of serum SOD was observed in Benue group and highest in Kogi group. The serum SOD levels in Kogi group were markedly elevated than that of normal control and standard oil. Oil from Benue zone showed the lowest ability of increasing SOD levels in the serum,

followed closely by oil from Kwara state. However, for liver SOD levels, Niger zone showed the lowest level of SOD and a significant decrease when compared with normal control and standard oil (Table 2). Only Nasarawa state group showed significant increase in serum GST

when compared to normal control group. The remaining five states showed no significant increase or decrease when compared with normal control or standard oil. All six states showed similar liver GST levels (Table 2).

Table 1: Sub-acute toxicity evaluation of *E. camaldulensis* essential oil from six zones in rats

Samples tested	No. of rats/test	No. of death	Survival	Mortality rate
Jos oil	3	0	3	0/3
Nasarawa oil	3	0	3	0/3
Benue oil	3	0	3	0/3
Niger oil	3	0	3	0/3
Kwara oil	3	0	3	0/3
Kogi oil	3	0	3	0/3

Effect of *E. camaldulensis* essential oil on liver and lungs Interleukin 6 and TNF-Alpha of rats

Oil from Nasarawa state showed significant increase in lungs TNF- α , but a decrease in liver TNF- α . Oil from the remaining five zones did not elicit any significant increase or decrease in lungs or liver TNF- α . Comparing all with the standard control, only Jos and Kogi showed significant increase in liver TNF- α . Oil from all zones did not elicit any significant increase or decrease in IL-6 levels in the liver and lungs when compared with the normal control (Table 3).

Table 2: Effect of *E. camaldulensis* essential oil on some oxidative stress, antioxidant enzymes in the serum and liver of rats

Groups	Treatments	Serum SOD U/l	GST Serum nmole/CDNB	Serum CAT U/mg protein	GST Liver nmole/CDNB	Liver SOD U/l	Liver CAT U/mg protein
1	Jos oil	870.7±621.9 ^{bc}	9.5±0.9 ^{ab}	1509.0±46.0 ^a	9.79±0.60 ^a	4601.83±621.89 ^c	2107.9±187.6 ^a
2	Nasarawa oil	747.6±1.5 ^{abc}	19.1±3.2 ^b	4645.0±457.6 ^b	14.51±4.07 ^a	4228.86±995.03 ^c	4413.6±2117.9 ^c
3	Benue oil	248.8±0.01 ^a	8.7±1.2 ^{ab}	1852.4±110.1 ^a	9.63±1.55 ^a	4353.24±1368.16 ^c	2952.0±491.3 ^{abc}
4	Niger oil	746.3±0.01 ^{abc}	9.2±3.1 ^{ab}	2113.7±33.0 ^a	7.59±0.62 ^a	1492.53±497.51 ^a	2124.3±413.8 ^a
5	Kwara oil	372.93±24.38 ^{ab}	13.50±1.27 ^{ab}	1838.71±87.97 ^a	9.56±1.45 ^a	3731.34±4.98 ^{bc}	2231.02±19.67 ^a
6	Kogi oil	1119.40±373.13 ^c	8.29±0.79 ^{ab}	2629.06±706.97 ^a	8.14±3.98 ^a	2139.25±845.77 ^{ab}	3315.25±923.61 ^{abc}
7	Corn oil control	497.51±248.76 ^{ab}	14.59±0.23 ^{ab}	4124.30±254.39 ^b	6.92±2.13 ^a	5472.64±248.76 ^c	4021.24±35.36 ^{bc}
8	Normal control	655.22±36.99 ^{abc}	6.93±1.38 ^a	1172.50±167.21 ^a	10.13±0.96 ^a	4353.23±383.08 ^c	2536.62±551.1 ^{ab}

Values expressed as Mean ± SD; Values with different superscript down the column are significantly different (p < 0.05).

SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione S transferase

Table 3: Effect of *E. camaldulensis* essential oil on liver and lungs Interleukin 6 and TNF-Alpha of rats

Groups	Treatments	Liver TNF-Alpha		Lungs TNF-	
		Liver IL-6 pg/ml	pg/ml	Lungs IL-6 pg/ml	Alpha pg/ml
1	Jos oil	279.5±39.17 ^{ab}	714.78±5.97 ^c	177.83±7.5 ^{ab}	306.34±16.67 ^a
2	Nasarawa oil	267.0±33.3 ^{ab}	617.02±67.92 ^{ab}	178.67±16.67 ^{ab}	378.0±78.33 ^c
3	Benue oil	277.84±34.17 ^{ab}	670.75±3.74 ^{abc}	166.33±1.15 ^a	308.0±5.0 ^a
4	Niger oil	268.67±11.67 ^{ab}	699.11±81.35 ^{bc}	179.5±9.17 ^{ab}	313.0±13.33 ^a
5	Kwara oil	273.67±25.0 ^{ab}	685.67±60.45 ^{abc}	166.95±3.33 ^{ab}	299.67±30.0 ^a
6	Kogi oil	329.5±85.83 ^b	707.32±55.23 ^{bc}	187.0±13.33 ^b	311.45±18.32 ^a
7	Corn oil control	236.17±2.5 ^a	598.36±17.91 ^a	184.5±10.83 ^{ab}	339.67±20.0 ^{ab}
8	Normal control	250.34±13.34 ^a	722.24±17.91 ^c	180.34±11.67 ^{ab}	306.34±16.67 ^a

Values are expressed as Mean ± SD; Values with different superscript down the column are significantly different ($p < 0.05$).

TNF: Tumor necrosis factor, IL-Interleukin

Effect of *E. camaldulensis* essential oil on amino transferases (AST and ALT) in rats

There was no marked increase or decrease in serum AST and ALT levels when compared with normal control in all states except for Nasarawa

state group where there was statistical increase in both AST and ALT levels. None of the states showed statistical increase or decrease in AST and ALT levels when compared with standard control (Corn oil) (Figure 1)

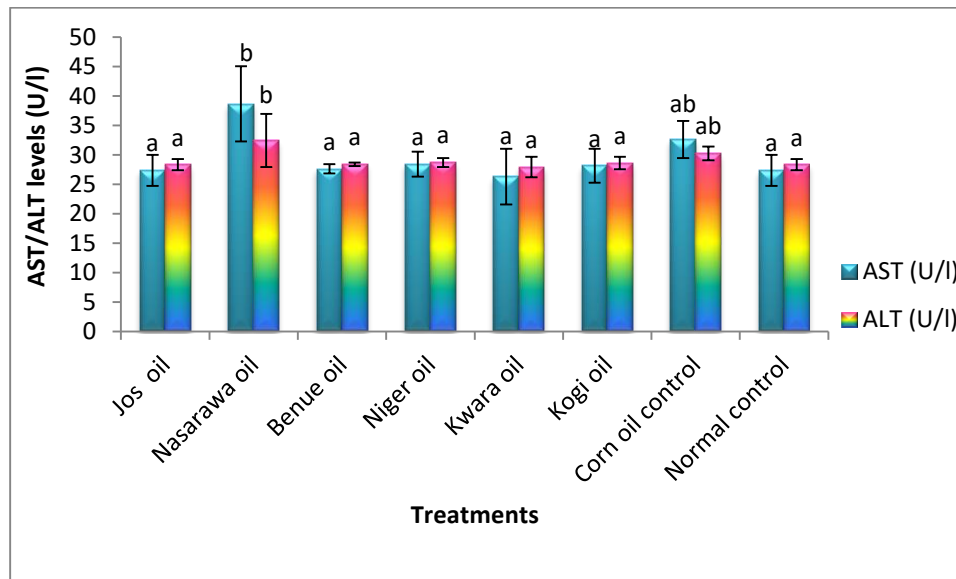


Figure 1: Effect of *E. camaldulensis* essential oil on amino transferases (AST and ALT) in rats

AST: Aspartate amino transferase, ALT: Alanine amino transferase

Histological and Immunohistochemical assessment of the skin and liver

The histology of the skin and the liver in all the groups were normal except the group of Niger zone which showed focal inflammation with mild

cytoplasmic clearing in the liver (Plate 1 group 4) and focal mild epidermal sclerosis of the skin (Plate 2 group 4). This was evident in the Immuno-Ratio where Niger zone showed the lowest expression of the antibody (pan-cytokeratin) (Plate 3 group 4).

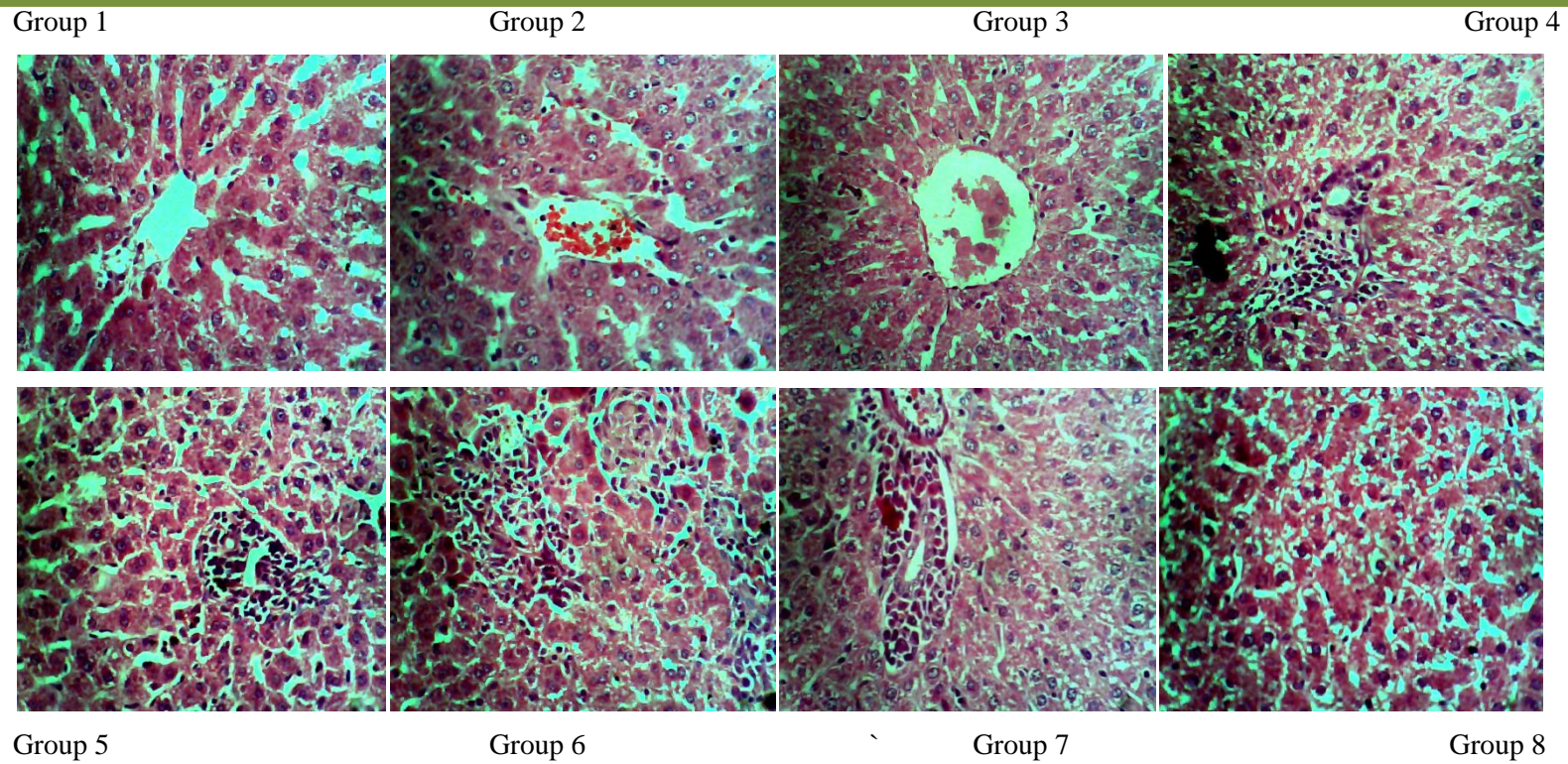
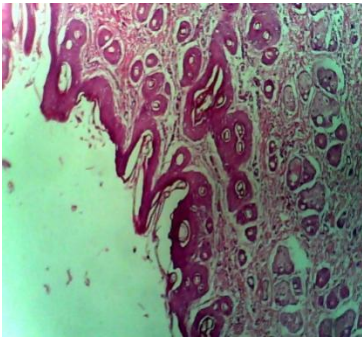
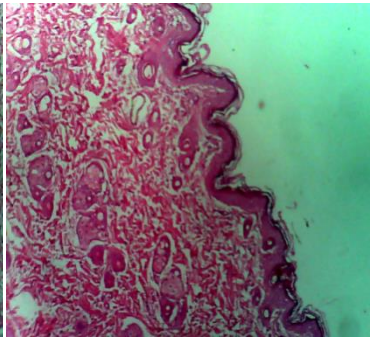


Plate 1: Histological assessment of the liver following 14 days' oral administration of *E. camaldulensis* essential oil (magnification x400)

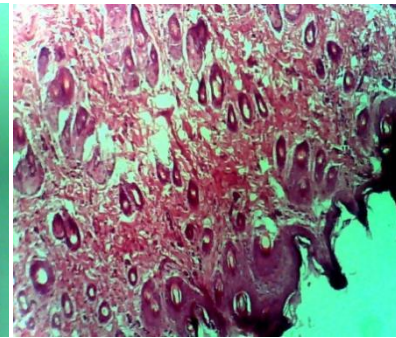
Group 1



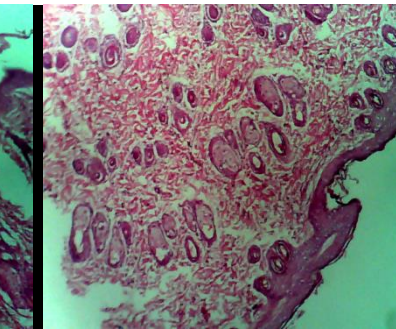
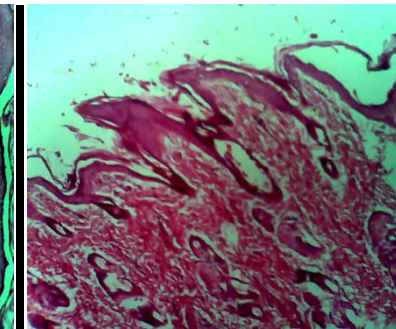
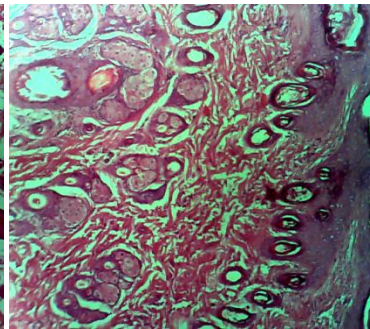
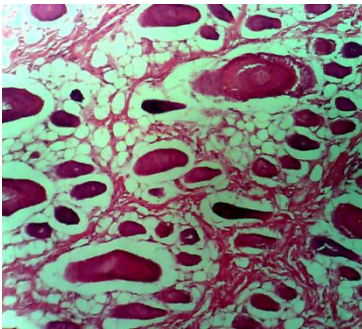
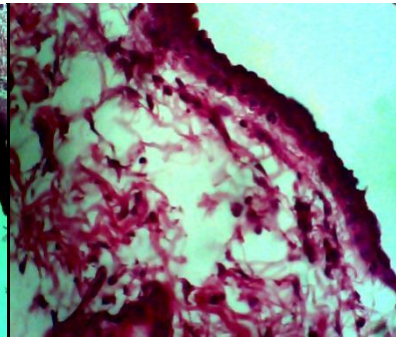
Group 2



Group 3



Group 4



Group 5

Group 6

Group 7

Group 8

Plate 2: Histological assessment of the skin following 14 days' oral administration of *E. camaldulensis* essential oil (magnification x400)

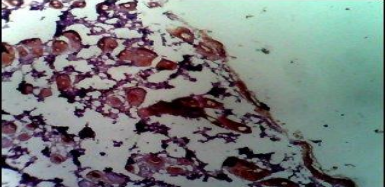
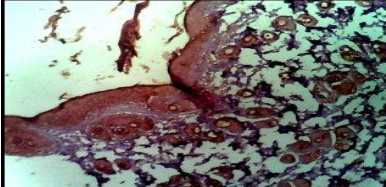
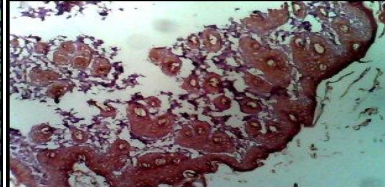
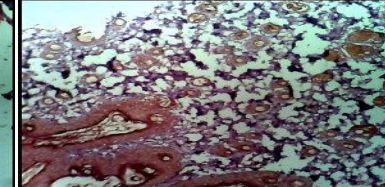
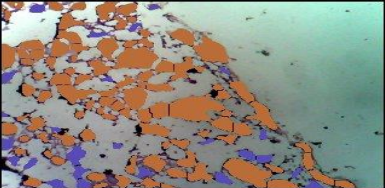
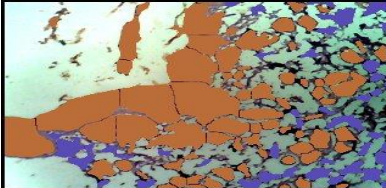
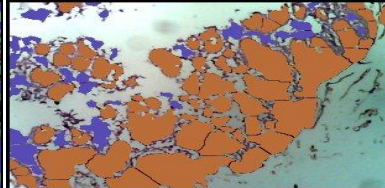
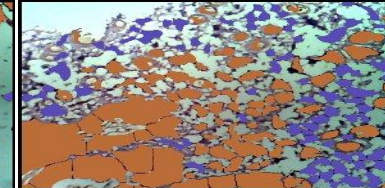
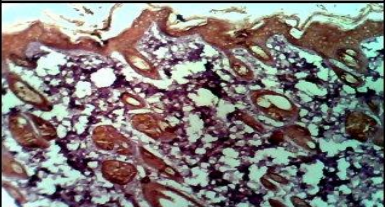

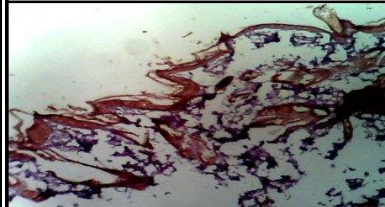
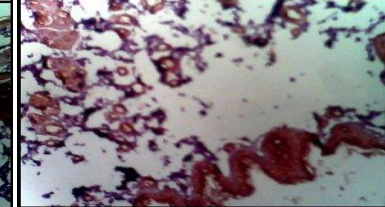
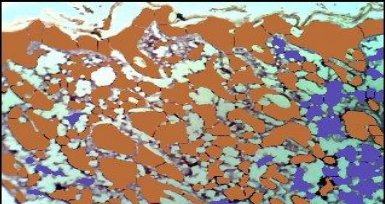

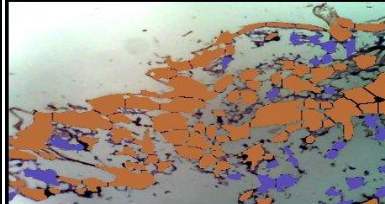
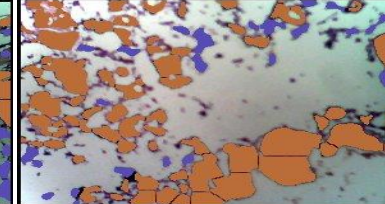
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<p>ImmunoRatio Sample ID: Kwara zone 2 Date: 10.8.2021 13:11 DAB / nuclear area: 78.7%</p>	<p>ImmunoRatio Sample ID: Kogi zone 2 Date: 10.8.2021 13:20 DAB / nuclear area: 64.6%</p>	<p>ImmunoRatio Sample ID: Corn oil control Date: 10.8.2021 13:25 DAB / nuclear area: 74.3%</p>	<p>ImmunoRatio Sample ID: Normal control 2 Date: 10.8.2021 13:29 DAB / nuclear area: 79.9%</p>
			
			

Plate 3: Immunohistochemical evaluation of the skin with percentage area of pan-cytokeratin expression following 14 days' oral administration of *E. camaldulensis* essential oil

DISCUSSION

Due to global pandemic and increase in various deadly diseases, the trend towards the use of substances from plant source as food and medicine has become a practice. Studies have shown that essential oil from plants such as *E. camaldulensis* are potent agents in combating some ailments. Increased activities of antioxidant enzymes observed in the group administered oil from Nasarawa zone may be attributed to the variation in the chemical composition of *E. camaldulensis* from different geographical location as stated by Sabo and Knezevic, [2] that variation in the chemical composition of essential oil is due to nutrients in the soil of different habitat. Decreased activity of antioxidant enzyme on the other hand, indicates low antioxidant potential of the oil and its poor ability to mop-up reactive oxygen species (ROS) [13] as observed with the group from Niger zone.

High level of ROS which may cause oxidative injury can lead to DNA modification at different levels. This can result to adduct formation and consequently mutation [14]. Mild antibody damage and inflammation observed in the Niger zone group and not in other groups indicates the presence of some phytochemicals in the other groups which are capable of protecting the skin from oxidative injury [15].

Mild irritation observed in the skin application recorded in the histology and IHC of the skin was in accordance with the findings of Hutchings *et*

al., [3], as well as Kumari *et al.*, [16] which states that increase level of ROS promotes inflammation. It also correlated with the local use by inhalation of the oil to clear air-ways, as there was no chronic effect on the lung's inflammatory markers.

Conclusion

No mortality was recorded in the sub-acute toxicity study when subjected to low and high doses (10mg/kg and 5000mg/kg) of *E. camaldulensis* essential oil from six different zones. Dermal application of the oil shows no significant toxic effect except some mild inflammation (skin irritation). Oral administrations were relatively safe with acute effect observed in the lung's inflammatory markers, liver function biomarkers, and antioxidant assays.

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Conflicts of Interest

The authors hereby declare no conflicts of interest

REFERENCES

1. Royal Botanic Garden Kew (2021). *Eucalyptus camaldulensis* In: Plants of the World Online. Published online. <http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:592777-1>
2. Sabo, V. and Knezevic, P. (2019). Antimicrobial activity of *Eucalyptus camaldulensis* Dehn. plant extracts and essential oils: A review. *Industrial Crops & Products*, 132:413–429.
3. Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A. (1996) Zulu Medicinal Plants. An Inventory. Scottsville: University of Natal Press.
4. Imam, A.A. and Wudil, A.M. (2011). Sub-acute hepatotoxicity of aqueous leaf extract of *Eucalyptus camaldulensis* in rats. *Bayero Journal of Pure and Applied Sciences*, 4(2): 118 – 120 ISSN 2006 – 6996
5. Dawoud, A.D., Shayoub, M.E., and Shayoub, S.M. (2015). Acute Toxicity Studies of Ethanolic Extract of *Eucalyptus Camaldulensis* Dehnh Leaves. *Journal of Network Communications and Emerging Technologies*, 2(2):1-5.
6. Asgar, E. and William, N. S. (2020). Analysis of the Essential Oils of *Eucalyptus camaldulensis* Dehnh. and *E. viminalis* Labill. as a Contribution to Fortify Their Insecticidal Application. *Natural Product Communications*, 15(9): 1–10. DOI: 10.1177/1934578X20946248 journals.sagepub.com/home/npx
7. Atawodi, S.E., Atawodi, J.C., Pfundstein, B., Spiegelhalder, B., Bartsch, H., and Owen, R. (2011a). Assessment of the polyphenol components and in vitro antioxidant properties of *Syzygium aromaticum* (L.) Merr. and Perry. *Electron J Environ Agric Food Chem.*, 10: 1970-78.
8. Martin, J.P., Dialely, M., and Sugarmann, E., 1987. Negative and positive assay of superoxide dismutase based on hematoxylin autoxidation. *Arch. Biochem. Biophys.*, 255 (2), 329–336
9. Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28: 56.
10. Drury, R.A.V., and Wallington, E.A., (1980). *Carltons Histological Techniques*, fifth ed. Oxford University Press, New York, Pronto 206SY.
11. Hsu, S., Raine, L., Fanger, H., (1981). Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques. *J. Histochem. Cytochem*, 29, 577–580

12. Jensen, E.C., (2013). Quantitative analysis of histological staining and fluorescence using image. *The Anatomical Record*, 296, 378–381.
13. Iliemene, U. D. and Atawodi, S. E. (2019). Preventive potential of dietary inclusion of *Brachystegia eurycoma* (Harms) seeds on N-methyl-N-nitrosourea-induced colon carcinogenesis in Wistar rats. *Journal of Ethnopharmacology*, 238 111858. www.elsevier.com/locate/jethpharm
14. Carini, F., Mazzola, M., Rappa, F., Jurjus, A., Geagea, A.G., Al-Kattar, S., Bou-Assi, T., Jurjus, R., Damiani, P., Leone, A., Tomasello, G., (2017). Colorectal carcinogenesis: role of oxidative stress and antioxidants. *Anticancer Res.* 37, 4759–4766
15. Alam, M.N., Almoyad, M., and Huq, F., (2018). Polyphenols in colorectal cancer: current state of knowledge including clinical trials and molecular mechanism of action. *BioMed Res. Int.*, 29. <https://doi.org/10.1155/2018/4154185>.
16. Kumari, S., Badana, A.K., G, M.M., G, S., and Malla, R., (2018). Reactive oxygen species: a key constituent in cancer survival. *Biomark. Insights*, 13: 1177271918755391. <https://doi.org/10.1177/1177271918755391>