

Repeated-Dose Dermal Toxicity of *Tagetes erecta* Essential Oil in Wistar Rats

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Abstract

Marigold essential oil has gained widespread acceptance in medicinal and cosmetic applications due to its reported therapeutic properties. However, emerging evidence suggests potential toxicity effects may occur following topical application and exposure of some marigold species. This study aimed to investigate the effect of single and multiple dermal exposure of the African marigold (*Tagetes erecta*) essential oil and evaluate its impact on hematological (blood) parameters in experimental animals (wistar rats). Twenty wistar rats were used for this experiment, the rats were grouped into four with each group containing five rats with no vehicle control, the essential oil was applied at 0.1ml/BSA (10% Body Surface Area) on the cleared surface of the skin as the test was a dermal test and the period of exposure was varied between 2 weeks to 6 weeks. At the end of the experiment, parameters such as packed cell volume (PCV), hemoglobin (HB), red blood cell count (RBC), and white blood cell count (WBC) were measured. The mean values observed were PCV: 40.53% $\pm$ 0.86, HB: 12.76 g/dL $\pm$ 0.49, RBC: $7.07 \times 10^{12}/L \pm 0.32$, and WBC: $4665.14 \times 10^9/L \pm 984.65$. Statistical analysis revealed no significant differences between groups ($p > 0.05$), indicating that the treatment had no significant effect on the blood parameters of the treated animals. Additionally, no physiological signs of toxicity were observed in the animals. This study shows that at the applied quantity of the essential oil; it does not have any significant toxic effect on the test animals.

Keywords: *Tagetes erecta*, Essential Oil, Dermal Toxicity, Experimental Animals, African Marigold.

Introduction

The genus *Tagetes* belongs to the Asteraceae family and comprises 56 species, 27 annuals and 29 perennials (Swapnanjali *et al.*, 2025; Vasudevan *et al.*, 1997). *Tagetes* species are native to America but have been naturalized in other parts of the world, including Africa,

Asia, and Europe (Salehi *et al.*, 2018). The most common *Tagetes* species are *T. minuta*, *T. erecta*, *T. patula*, and *T. tenuifolia* (Joshi, 2022). African marigold (*Tagetes erecta*) is an annual houseplant known for its large flower heads. It is an ornamental plant used as cut flowers for aesthetic purposes and its essential oil is used in the perfume and pharmaceutical industries. Marigold is among the most commonly grown flowers for garden decoration and is used largely as loose flowers for making garlands for religious and social functions. Most marigold flowers have a strong, pungent smell and are valuable in cosmetic treatment (Jadhav *et al.*, 2014).

Tagetes species have found widespread applications in the field of agriculture due to their fungicidal, bactericidal, and insecticidal activities, as well as their anticancer properties (Salehi *et al.*, 2018). These plants are widely used in folk medicine to treat various health problems, including dental, stomach, intestinal, emotional, and nervous disorders, as well as muscular pain (Joshi, 2022; Bohatu *et al.*, 2024). These plants contain diverse bioactive compounds including triterpenoids, alkaloids, carotenoids, thiophenes, fatty acids, tannins, flavonoids, and essential oils (Bohatu *et al.*, 2024). Multiple *Tagetes* species such as *T. minuta*, *T. erecta*, *T. patula*, and *T. tenuifolia* demonstrate significant pharmacological activities including antibacterial, antifungal, insecticidal, antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties (Joshi, 2022; Bohatu *et al.*, 2024). Specifically, *T. minuta* has been scientifically validated for its anti-inflammatory activity through inhibition of NF- κ B signaling pathway, with pheophytins identified as the active compounds responsible for treating stomach and intestinal discomfort (Ticona *et al.*, 2021). The primary pigment present in the flower is xanthophyll particularly lutein that accounts for 80 to 90% in the form of esters of plamitic and myristic acid (Jadhav *et al.*, 2018). *Tagetes minuta* L. essential oils demonstrate significant antimicrobial properties and diverse bioactivities, supporting their traditional uses. The oils exhibit broad-spectrum antimicrobial activity against various pathogens, with particular effectiveness against Gram-positive bacteria like *Staphylococcus aureus* (Walia *et al.*, 2020). Beyond antimicrobial properties, *T. minuta* essential oils possess multiple bioactivities including anthelmintic, insecticidal, fungicidal, antiviral, and nematocidal properties against plant, human, and animal pathogens (Wanzala *et al.*, 2016). Additionally, *T. minuta* has exhibited cytotoxic (Sahranavard *et al.*, 2014) and anti-inflammatory activities (Devika and Koilpillai, 2015).

Problem Statement

While marigold oils are extensively used in dermal applications, comprehensive *in vivo* safety data regarding repeated exposure to *Tagetes erecta* essential oil remain limited. The growing commercialization of African marigold products necessitates systematic evaluation of their toxicological profile. This study addresses the critical knowledge gap by investigating hematological responses in Wistar rats following single and repeated dermal exposure to *T. erecta* essential oil, providing essential safety data for clinical and commercial applications.

Objectives

- To evaluate the effect of marigold essential oil on skin of wistar rats.
- To investigate the effect of marigold oil on the blood of Wistar rats.
- To quantify clinical, hematological, and dermal histopathology effects under repeated dermal application of African marigold (*Tagetes erecta*) essential oil.
- To explore exposure duration trends by examining the effects over different time periods (2, 4, and 6 weeks).

Literature Review

Tagetes erecta, commonly known as marigold, has garnered attention for its essential oils, which are rich in bioactive compounds. This literature review synthesizes recent findings on the chemical composition of *T. erecta* oils, their potential for dermal irritation and sensitization, effects observed in repeated dermal dosing paradigms in rodents, and their relevance to hematology under dermal exposure.

Chemical Composition of *T. erecta* Oils

The essential oils of *Tagetes erecta* (commonly known as marigold) are characterized by a complex mixture of volatile compounds, primarily composed of various monoterpenes and sesquiterpenes. Key components often identified include compounds such as limonene, α -terpinene, and β -caryophyllene. These constituents contribute not only to the aromatic properties of the oil but also to its potential therapeutic effects. The specific profile of these compounds can vary based on factors like the extraction method and the plant's geographical origin, influencing both their efficacy and safety (Moghaddam *et al.*, 2021). Recent studies employing gas chromatography-mass spectrometry (GC-MS) have revealed significant variability in the chemical profiles depending on factors such as geographic origin and extraction methods. For instance, a study conducted on *T. erecta* fruits from northwest Iran identified 2-isopropyl-5-methyl-3-cyclohexen-1-one (19.99%), d-limonene (12.75%), and terpinolene (11.64%) as major constituents (Ahmadpour *et al.*, 2024). Another investigation of *T. erecta* cultivated in Northeastern Iran highlighted neophytadiene (17.22%), piperitone (12.52%), and β -caryophyllene (8.7%) (Moghaddam *et al.*, 2021). These findings underscore the necessity of considering the plant's origin and extraction method when discussing the oil's chemical properties.

Dermal Irritation, Sensitization, and Phototoxicity Data

The dermal application of *Tagetes erecta* oils may result in irritation, sensitization, and phototoxicity. The European Commission Scientific Committee on Consumer Products has classified *Tagetes* extracts and oils as phototoxic, indicating a risk for adverse skin reactions upon exposure to sunlight (Coenraads, 2016). Additionally, the Environmental Working Group (EWG) has reported that *T. erecta* oil can cause allergic skin reactions and irritation, citing references from the European Chemicals Agency (ECHA). However, a study on a 95%

ethanolic extract of *T. erecta* flower extract found no significant skin irritation in healthy volunteers, suggesting its potential for cosmeceutical applications (Mekjaruskul *et al.*, 2021). These results highlight the dual nature of *T. erecta* oils, necessitating careful consideration in their application.

Repeated Dermal Dosing Paradigms in Rodents

In rodent models, repeated dermal dosing of *T. erecta* oils has been utilized to evaluate chronic exposure effects. Studies often focus on the histopathological changes in the skin, as well as systemic effects. Results show that prolonged exposure can lead to skin irritation. A study examining *Tagetes erecta* Linn. leaf extract cream on rat dermal wound healing revealed that both 3% and 4% cream formulations significantly increased wound contraction and accelerated epithelialization (Parwanto *et al.*, 2021). Furthermore, Sultana *et al.* (2021) noted modulated inflammation and enhanced wound healing in a caprine model. These findings suggest potential therapeutic benefits.

Hematology Relevance under Dermal Exposure

The impact of dermal exposure to *T. erecta* oils on hematological parameters remains an area requiring further exploration. While limited recent data directly addresses this topic, studies on related plant oils, such as *Calendula officinalis*, have monitored hematological changes (Lagarto *et al.*, 2011). These studies indicate that repeated dermal exposure may influence blood parameters, such as white blood cell, red blood cell, hemoglobin, and lymphocyte counts.

Materials and Methods

The experiment was carried out in the National Horticultural Research Institute, Ibadan as well as the Veterinary and Physiology Department of the University of Ibadan, Oyo State, Nigeria.

Plant Extraction and GC-MS Analysis

The extraction procedure, reference number and GC-MS analysis of the marigold essential oil are reported in a separate manuscript.

Dose Preparation and Administration

The evaluation was carried out according to the method described by Parasuraman *et al.* (2010). Twenty wistar rats were used for the experiment containing both males and females with average weight of 100g, animals were grouped and placed in plastic cages. Acclimatization was done for 10 days after which treatment commenced. The animals were grouped randomly into 4 containing 5 rats each of both gender (Male & female) and treatments were based on the period/time of exposure to the essential oil which was 2weeks, 4weeks and 6 weeks. The 4th group served as the control and received no treatment at all. Then, 10mg/kg of ketamine was injected to the animal intraperitoneally (IP), after

10min the animal fore/hair were scrapped off on the right side with soap and water with the use of a blade and exposing the skin in preparation for treatment application. After hair removal, the animal skin was observed with hand lens for possible surface damage and after 48 hours treatment commenced. Two drops of marigold essential oil 0.1ml/BSA (10% Body Surface Area) was applied and evenly spread on the exposed skin every other day, it was allowed to dry so as to prevent oral contamination, and then animals were returned to their cages. Food and water were given to the animals *ad-libitum*.

Clinical Observations

At the end of the experiment, the blood of animals was collected through ocular veins, animals were sacrificed through cervical dislocation and the organ of interest (skin) was carefully harvested. Data collected include weight gain or loss, physical changes, behavioral changes, skin and blood.

Statistical Analysis

Data was analyzed using one-way ANOVA with Tukey post-hoc analysis

Results and Discussion

On the third day of treatment, one animal in the two-week exposure group exhibited mild discoloration at the application site, which resolved completely by day five. Upon study completion, both dermatological assessment (demonstrated by normal hair growth) and hematological evaluation confirmed the absence of any treatment-related damage to skin or blood parameters. This agrees with the findings of Devika and Koilpillai (2015) who concluded that *T. erecta* showed that its compounds; flavonoids and salicylic acid have anti-inflammatory potential with no sign of toxicity after inhibiting α -glucosidase enzyme with RAW 264.7 cells activated with lipopolysaccharide. It also agrees with the findings of Mahmoud (2013) who stated that the treatment of wistar rat with marigold extract had no significant effect on the blood parameters. No behavioural changes were observed in any of the treated animals; none were deformed or aggressive.

Table 1: Body Weight Changes

Group	Day 0 (g)	Day 14 (g)	Day 28 (g)	Day 42 (g)
Control	74.75 $\pm$ 3.63	76.75 $\pm$ 7.08	82.75 $\pm$ 47.93	69.5 $\pm$ 69.80
T1	88 $\pm$ 12.19	89 $\pm$ 12.9	78 $\pm$ 45.21	71.25 $\pm$ 72.71
T2	95.75 $\pm$ 9.81	86.25 $\pm$ 8.73	95 $\pm$ 56.39	70.25 $\pm$ 71.21
T3	86 $\pm$ 17	92.25 $\pm$ 12.81	80.25 $\pm$ 46.50	76 $\pm$ 76.11
T4	84.25 $\pm$ 7.36	83.75 $\pm$ 6.94	84.75 $\pm$ 49.33	78.5 $\pm$ 78.96

T1 - Treatment 1, T2 - Treatment 2, T3 - Treatment 3, T4 - Treatment 4

Dose of 1000 mg/kg daily oral administration of *T. erecta* EO for 42 days did not cause any treatment-related toxicity signs, behavioural changes, and death in both sexes of wistar rat.

The body weight measurements showed several fluctuations in treated animals compared to the control (Table 1). Nevertheless, the difference was not significant statistically.

Table 2: Blood parameters measured throughout the treatment period.

Parameters	Control	2wks	4wks	6wks
PCV (%)	39.42 ± 0.50	41.25 ± 0.60	39.00 ± 0.45	40.50 ± 0.55
HB (g/dL)	13.00 ± 0.25	13.125 ± 0.30	12.100 ± 0.35	12.800 ± 0.40
RBC (×10 ¹² /L)	7.1231 ± 0.10	6.8150 ± 0.12	7.4175 ± 0.11	7.1800 ± 0.13
WBC (×10 ⁹ /L)	4986.83 ± 20	5073.00 ± 25	3183.13 ± 30	5435.50 ± 35
PLT (10 ⁹)	120006.00±500	118000.00±400	105750.00±600	122000.00±450
LYM (10 ⁹ /L)	68.12 ± 0.5	70.25 ± 0.4	62.00 ± 0.3	67.50 ± 0.5
NEUT (10 ⁹ /L)	25.40 ± 0.3	26.00 ± 0.4	24.50 ± 0.3	27.25 ± 0.4
MON (10 ⁹ /L)	1.35 ± 0.1	1.75 ± 0.2	1.25 ± 0.1	1.75 ± 0.2
EOS (10 ⁹ /L)	2.43 ± 0.1	2.50 ± 0.1	2.75 ± 0.1	2.25 ± 0.1

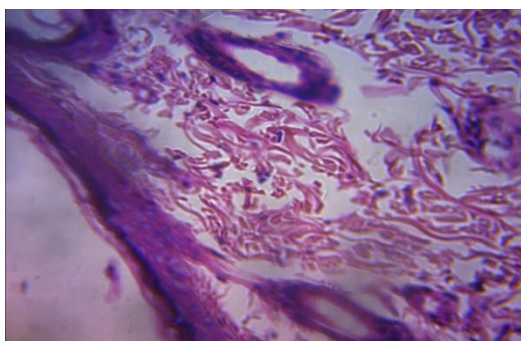
PCV; pack cell volume, HB; hemoglobin, RBC, red blood cell, WBC, white blood cell, PLT; Platelet, LYM; Lymphocytes, NEUT; Neutrophil, MON; Monocytes, EOS; Eosinophil

a = means on the same row with same letter are not significantly different (p>0.05).

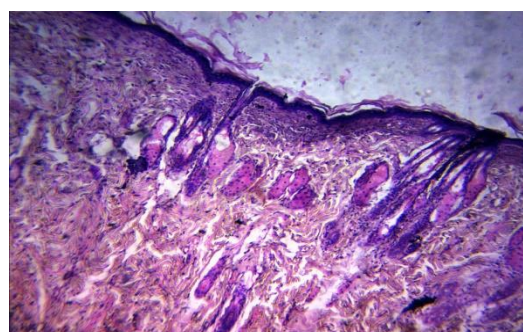
Table 2 illustrates no significant differences among blood parameters throughout the treatment period. While the PCV showed no significant differences across the weeks, the highest value was observed at 2 weeks (41.25%) and the lowest at 4 weeks (39.0%). The confidence intervals around these means (e.g., PCV: 41.25 ± 0.60) indicate that there is no clinically relevant harm associated with these values. These results are consistent with those of Oguwike et al. (2013), who observed that while marigold extract slightly increased packed cell volume in rats after 28 days of feeding, the increase was not significant. Same trend follows for HB, where the highest value was at 2 weeks and the lowest at 4 weeks; this is in agreement with the work of Emelike and Dapper (2013), who reported that the marigold extract slightly increased the haemoglobin concentration.

The same trend occurred for white blood cell counts, with increases at 2 weeks and 6 weeks, respectively, and this is in agreement with the findings of Oguwike et al. (2013), who observed that white blood cell counts were increased due to their glycoside content. Platelets (PLT) and Lymphocytes (LYM) had highest values at 2weeks and lowest at 4weeks respectively. But for RBC, NEUT, MON and EOS, there were variations in the trend where highest value in NEUT was at 6weeks and lowest at 4weeks respectively. For RBC, the highest was observed at 4weeks while the lowest was at 2weeks, EOS had the highest at 4weeks and lowest at 6weeks, and MON had highest at both 2 and 6 weeks and lowest at 4 weeks. These align with the findings of (Shetty, 2008; Shetty *et al.*, 2009; Manisha *et al.*, 2013) who reported that both aqueous and ethanolic fractions of flowers of *T. erecta* were found to be safe during an acute toxicity study. This study was in accordance to the Organisation for Economic Co-operation and Development (OECD) Guidelines for repeated-dose dermal toxicity testing, specifically following protocols for assessing dermal

irritation and sensitization (OECD Test Guideline 404) and for repeated-dose toxicity (OECD Test Guideline 411).

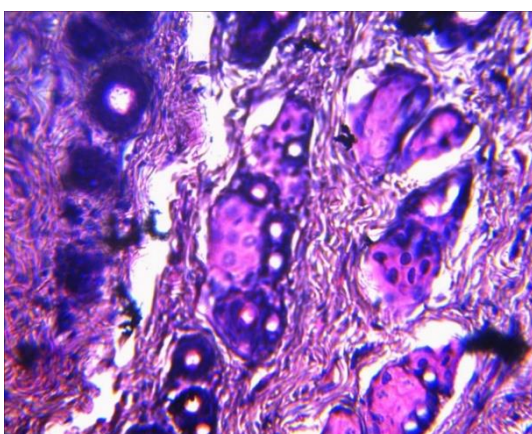


Skin 1: No visible lesions seen.



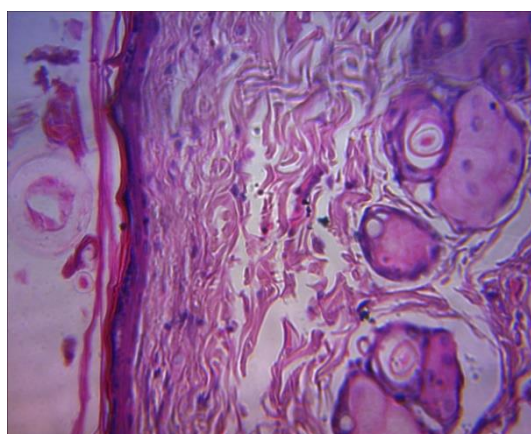
Skin 2: No visible lesions seen.

Hair follicles and sebaceous glands seen (arrows)



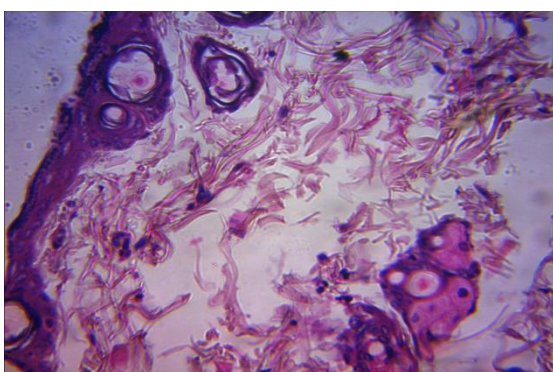
Skin 3: No visible lesions seen.

High population of sebaceous glands (arrowheads) seen.



Skin 4: No visible lesions seen. No hair follicle

Sebaceous glands present (arrows)



Skin 5: No visible lesions seen. Few sebaceous glands (arrowheads) and follicles (arrows) seen.

(A supplementary material showing the arrowheads is attached).

Plates showing histopathology and photomicrographs of skin

Conclusion

Despite the methodological limitation of not including a vehicle control group and organ histology, the present findings indicate that dermal application of *Tagetes erecta* essential oil at 0.1 mL/BSA produced no significant alterations in hematological parameters, suggesting an acceptable safety profile at the tested dose level.

Recommendation

Future investigations should address the current study's limitations by including vehicle control groups and employing dose-response designs with multiple application rates. Such studies are essential to establish comprehensive safety profiles and identify optimal therapeutic dosing parameters for *T. erecta* essential oil in dermal applications.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' contributions

EOA, FBA, DOA: conceptualization, methodology

EOA, AAA, DOA: data curation, writing—original draft preparation

EOA, POI: visualization, investigation

ROA, POI: supervision

DOA: resources

POI, EOA, AAA: writing—reviewing and editing.

POI: validation

All authors have read and agreed to the published version of the manuscript.

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