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Acute and Repeated-Dose Dermal Toxicity Assessment of *Coriandrum Sativum* Essential Oil-Loaded Microemulsion in Wistar Albino Rats

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ABSTRACT

Background: *Coriandrum sativum* essential oil is attracting a lot of interest because of its different pharmacological properties, as well as its potential to be used in cosmetic and topical pharmaceutical formulations. But there is limited information on the dermal safety of its microemulsion delivery system. So, the present study was designed to investigate the acute and repeated dose dermal toxicity of coriander essential oil containing microemulsion in Wistar albino rats as per OECD guidelines. **Methods:** The acute dermal toxicity was determined according to the OECD guideline 402 after a single application of the microemulsion (2000 mg/kg) and the observation of the animals during 14 days. The repeated dose dermal toxicity was assessed by daily topical application of the formulation on to the skin of rats for 28 days as per OECD guideline 410 at 1000 mg/kg/day. Clinical signs, mortality, behavioral changes, body weight, food and water consumption, hematological and biochemical parameters, and histopathological changes of the liver and kidney were studied. **Results:** No acute or repeated dose toxicity was seen. No variations were observed in the behavior, body weight, food and water intake, hematological and biochemical markers (AST, ALT and BUN) parameters between the treated animals and the control group ($p>0.05$). Liver and kidney were free from any treatment-related abnormality, as clinically confirmed on histopathological examination. The acute dermal LD_{50} was determined to be more than 2000 mg/kg of body weight and the repeated dermal administration no observed adverse effect level (NOAEL) was greater than 1000 mg/kg/day. **Conclusion:** The results of the present study showed that the coriander essential oil microemulsion was not found to be toxic to skin, when applied as an acute or repeated dose under the conditions of the experiment. These findings indicate that it may serve as a topical delivery system for drugs and cosmetics in the future without causing any adverse effects.

INTRODUCTION:

Medicinal plants have been the focus of interest for their essential oils because of their multiple biological activities and the growing use in pharmaceutical and cosmetic products. Of these, the use of *Coriandrum sativum* L. [1]. The essential oil of (Apiaceae) is known for its antimicrobial, antioxidant, anti-inflammatory and protective action on the skin, mainly due to the presence of the main constituent linalool [2]. These properties render it a potential material for topical formulations for

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therapeutic purposes.

Direct topical use of essential oils is sometimes hindered by their volatility, low aqueous solubility, and potential to be irritating to the skin or sensitizing. The ability to increase lipophilicity, improve formulation stability, and achieve controlled release of lipophilic compounds has led to the design of microemulsion based delivery systems [3] as an effective solution to these drawbacks. But, the dermal penetration through the microemulsion systems could change the local and systemic exposure levels, so safety evaluation of these systems is crucial before their long-term use in topical applications [4], [5], [6]

The pharmaceutical potential of coriander essential oil loaded microemulsion has been previously reported but there is limited data on the acute and repeated dose dermal safety of the microemulsion. For this reason, the present study has been designed to assess the acute and subacute dermal toxicity of *Coriandrum sativum* essential oil loaded microemulsion in Wistar albino rats, according to Organization for Economic Co-operation and Development (OECD) Guidelines 402 and 410. The developed formulation was clinically evaluated by clinical observation, body weight, food intake, water intake, hematological and biochemical parameters and histopathological changes to establish its dermal safety profile.

MATERIAL AND METHODS

Authentication of plant material

CS plants and seeds were procured from the Amroha local market, Uttar Pradesh, India. A herbarium specimen was prepared for plant identification. Dr. Sunita Garg, a botanist at the Council of Scientific and Industrial Research (CSIR, NIScPR)-National Institute of Science Communication and Policy Research in New Delhi, India, identified and authenticated the plant under authentication number NIScPR/Raw Materials Herbarium and Museum (RHMD)/consult/2022/4288-89.

Extraction of essential oil

the collected CEO samples were stored in tubes and shielded with aluminium foil at 18 °C until subsequent examination. The percentage yield following the effective extraction of the CEO sample was 0.71%, and the sample exhibited a pale yellow colouration.

Previous Findings

Preparation and Characterization of CEO-Loaded Microemulsion

We previously reported the successful development of a coriander essential oil (CEO)-loaded microemulsion for topical delivery. The goal was to develop a formulation suitable for topical application, assess its stability, and evaluate its effectiveness in delaying in vivo skin photoaging. The microemulsion was prepared using CEO as the oil phase, combined with an appropriate surfactant/co-surfactant mixture and purified water. Pseudo-ternary phase diagram studies helped identify the microemulsion region and optimise the formulation's composition. The final formulation demonstrated favourable physicochemical properties for topical use. Characterisation revealed that the CEO-loaded microemulsion had a mean droplet size indicative of nanosized droplets, which can enhance skin penetration. The droplet size distribution was narrow, with a polydispersity index (PDI) indicating uniformity. Additionally, the zeta potential indicated good colloidal stability. Physical stability tests, including centrifugation, heating/cooling cycling, and storage stability studies, showed that the formulation remained clear, homogeneous, and free from phase separation, creaming, or precipitation throughout the testing period [7], [8].

Evaluation for dermal toxicity

Experimental animals

The study used healthy young adult Wistar albino rats (Female). The rats weighed between 190 and 210 g at the start of the trial. The research was conducted in accordance with ethical standards. The study was approved by the Institutional Animal Ethics Committee of the Teerthanker Mahaveer Medical College and Research Centre, Moradabad, India (Registration No. CCSEA/1205/2024/14). The animals were housed in an air-conditioned, controlled laboratory environment. Room temperature was kept between 21.0 and 24.0°C, and Rh ranged from 57% to 65%. Temperature and Rh readings were taken every day. Each animal was housed in a conventional poly-sulphonate cage. The cage had a stainless-steel mesh top, facilitating the provision of pelleted food and drinking water through a water bottle equipped with a stainless-steel sipper tube. The rats had full access to food throughout both the acclimatization and research phases [9], [8].

Acute dermal toxicity evaluation

The acute dermal toxicity assessment was conducted in accordance with the OECD guidelines. Before the experiment began, Wistar albino rats were carefully selected and given at least 5 days to adjust to the controlled laboratory environment. In the current study, the dorsal fur of all subjects was properly excised by clipping or shaving. Particular attention was directed towards preventing skin damage, and only animals with unblemished skin were included in the study [10]. At least 10% of each animal's total body surface area was treated, with both the control

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and experimental medicines uniformly applied to this specified region. Two groups of Wistar albino rats were used for the range-finding investigation and the acute dermal toxicity assessment (Table 1).

A limit test was performed in accordance with OECD Guideline 402 because the test formulation was anticipated to have low dermal toxicity based on the natural origin of coriander essential oil and the absence of any reported severe toxic effects from previous studies. Therefore, a single dose of 2000 mg/kg body weight was selected as the limit dose and applied topically to the shaved dorsal skin of rats for 24 hours under a semi-occlusive non-irritating dressing.

In the experiment, the ME formulation and the control were topically applied to a specified area of the skin at a dose of 2000 mg/kg body weight. Group I served as the control, whereas Group II received the formulation treatment (ME) with semi-occlusive dressing. Animals were housed individually, and behavioural changes, toxicity, and mortality indicators were systematically observed for the first 4 hours, followed by continuous monitoring for 24 hours. Subsequent to the exposure period, the remaining test material was removed, and observations were conducted daily for an additional 14 days (OECD, 2004) (11). The duration of this application was 24 hours. The animals were assessed for clinical symptoms of toxicity and mortality at intervals of 30-40 minutes, 1 hour, 2 hours, 3 hours, and 4 hours after treatment during the 14-day observation period. After that, observations were taken every day (OECD, 1987). The examination was conducted using the "Acute Dermal Toxicity - Fixed Dose Technique" in accordance with the OECD Guidelines for Testing of Chemicals (No. 402, Section 4: Health Effects). The significant findings focused on alterations in the test subjects. The animals' body weight was recorded before the experiment and two weeks after the analysis. The food and water consumption of Wistar albino rats was monitored frequently throughout a 14-day duration from the beginning of the experiment. The research utilized a completely randomized design (CRD) and followed the protocols specified in OECD 402 (Acute Dermal Toxicity) [11].

Repeated-dose dermal toxicity study

The Organization for Economic Co-operation and Development (OECD) Guideline 410 was followed in the subacute dermal toxicity investigation. The control and test groups underwent a limit test at 1000 mg/kg body weight. The animal grouping for the subacute dermal toxicity study was the same. The ME formulation was applied topically for up to 28 days. Animals were fasted for 2 hours before treatment [12] . On the first day, subjects were observed every 30 minutes for eight hours. After administration and at the end of each day for the duration of the 28-day therapy. Throughout this period, mortality, signs of toxicity, and symptoms of illness were recorded. Throughout the trial, daily food intake was recorded, and body weight was measured once a week to assess any changes that might occur during use of the formulation. After the experiments, surviving animals were euthanized and examined by necropsy to assess gross pathological changes [13].

Cage-side observation

Daily investigation and monitoring for symptomatic abnormalities and toxicity indicators. After the medicine was administered once daily throughout the trial, rats were monitored for clinical symptoms and mortality at 5–10 minutes, 30–45 minutes, 1, 2, 4, 6, and 24 hours. Each animal was checked twice daily for illness and mortality.

Analysis of body weight

Every animal's body weight was recorded on the first day of the experiment, and then weekly thereafter.

Analysis of food consumption

Rats' weekly feed intake was determined by calculating the leftover feed from the quantity given in the subsequent weeks [13].

Blood collection

Animal handling, dosing, and sample collection were conducted in strict accordance with humane care principles, minimizing stress and pain at all times. At the end of the observation period, blood samples were collected from representative surviving animals before euthanasia to assess biochemical parameters. Rats' blood was taken on day 29 in order to examine changes in their hematology and biochemistry. The rats were anaesthetized with diethyl ether after fasting throughout the whole night, and blood samples were taken through the retro-orbital plexus. Heparinized microcentrifuge tubes were used for biochemical tests, while K3EDTA vacutainers were used for haematology. Following heparinization, the blood was centrifuged for 15 minutes at 4000 rpm to extract plasma, which was then used to measure biochemical parameters (15).

Biochemical and haematological parameters

The plasma was examined for biochemical parameters, including aspartate transaminase (AST), alanine transaminase (ALT), and blood urea nitrogen (BUN), along with hematological profiles comprising hematocrit (Hct), hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, neutrophil (Neut %), lymphocyte (Lym %), monocyte (Mon %), eosinophil (Eos %), basophil

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percentage (Bas %), and platelet count (Plt) [14], [15].

Necropsy and histopathology

On the 29th day of the subacute study, after blood collection, the animals were euthanised with an overdose of diethyl ether, followed by a comprehensive post-mortem examination. The application site (skin), kidney and liver, in particular, were removed and fixed in 10% neutral buffered formalin. The fixed tissues were stained with haematoxylin and eosin, embedded in paraffin, sectioned at 5 µm, and examined under a microscope. After a necropsy to identify gross and histological findings, each rat was properly disposed of. Data were analyzed using one-way analysis of variance (ANOVA) (18) [16].

Statistical analysis

Data are shown as mean ± standard deviation (mean ± SD). It was analyzed using a one-way analysis of variance (ANOVA). To determine statistical significance, a one-way ANOVA was performed at a $p<0.05$ level.

RESULT:

Acute dermal toxicity study

The Wistar albino rats given a single dose of CEO-loaded ME did not exhibit any behavioural changes during the first 4 hours. No mortality or treatment-related clinical signs were observed throughout the 14-day observation period following acute dermal exposure to CEO-loaded microemulsion. There was no discernible weight loss or mortality. A concentration of 2,000 mg/kg did not cause rashes or a burning sensation on any of the animals' skin during the dermal toxicity test. Furthermore, neither the treatment groups nor the control group experienced any deaths during the entire observation period. The CEO-loaded microemulsion did not produce any treatment-related signs of acute dermal toxicity at the tested dose of 2000 mg/kg. No evidence of clinical toxicity was observed. According to OECD Guideline 402, the absence of mortality at the limit dose of 2000 mg/kg indicates that the dermal LD_{50} of the CEO-loaded microemulsion is greater than 2000 mg/kg body weight. Table 1 shows that there were no treatment-related differences in body weight, food consumption, or water intake.

Table 1 Effect of microemulsion formulation treatment on body weight change, food consumption, and water consumption in rats during acute dermal toxicity evaluation

Group	Body weight gain (g)			Food consumption			Water consumption (ml)		
	Day (0)	Day (7)	Day (14)	Day (0)	Day (7)	Day (14)	Day (0)	Day (7)	Day (14)
Control	194.25±1.50	196.10±1.60	197.25±2.10	11.1±0.41	10.21±0.45	11.68±2.57	14±0.31	14.5±1.50	14±2.50
ME F1 (2000mg/kg)	194.50±1.60	196.75±1.80	197.95±2.20	11.15 ±0.25	11.10±0.73	12±0.87	15.32±0.3	15.98±1.10	15±2.20

n = 5; The data are presented as mean ± SD. To analyse the data, one-way ANOVA was used. *P < 0.05 versus control.

Repeated-dose dermal toxicity study

Clinical signs, animal behaviour, and mortality

Mild transient hair straightening (HS) was noticed in the test group on the 26th day of the trial, whereas the control and test groups remained normal and showed no evidence of intoxication. When comparing the rats' topical treatment with the ME formulation to the control group, cage-side observations revealed no changes in any parameters. Over the course of the examination, no mortality was observed.

Impact of the treatment on rats' body weight and feed intake

Compared with control rats in subacute dermal toxicity examinations, the CEO-loaded formulation did not significantly alter body weight or feed consumption ($P > 0.05$) (Table 2). The body weight of ME-treated rats increased with age and did not differ substantially from that of control rats.

Table 2 Evaluation of the impact of repeated 28-day dose application of CEO loaded microemulsion on weekly changes in body weight and feed consumption of rats

Week	Body weight (g)		Feed consumption (g)	
	Control	ME formulation 1000 mg/kg	Control	ME Formulation 1000 mg/kg
1	195.16 ± 4.13	195.16 ± 4.35	167.6± 9.96	165.2±17.61
2	198.83 ± 5.11	199.16 ± 5. 12	157.0± 4.64	156.0± 5.51
3	200.83 ± 6.08	203.16 ± 6. 12	168.2± 8.62	164.0± 9.62

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4	206.36 ± 7.14	205.16 ± 7.20	122.8± 10.20	124.8± 10.60

Haematological and Biochemical Parameters

Haematocrit, haemoglobin, erythrocyte count, differential leucocyte count and percentage, platelet count, and other associated corpuscular parameters were found to be non-significant ($P > 0.05$). The topical application of ME did not cause any significant changes ($P > 0.05$) in biochemical parameters, including AST, ALT, and BUN, in control rats (Table 3). According to the findings of the haematological study, treatment with 1000 mg/kg for 4 weeks did not significantly alter the rats' haematological parameters compared with the control group (Table 4).

Table 3 Effect of repeated 28-day topical application of CEO loaded microemulsion formulation on biochemical parameters

Parameters	Reference values (Magpale 2026)	control (Group 1)	1000 mg/kg (Group 2)
AST (IU/L)	37-205	115.30± 6.71	118.72± 4.42
ALT (IU/L)	6-114	50.40± 2.57	48.50±1.70
BUN (mg/dl)	13-29	21.21± 3.21	23.88 ± 1.85

Table 4 Study of Haematological Changes in Control and treated rats

Parameters	Reference values (Magpale 2026)	Control (Group 1)	1000 mg/kg (Group 2)
RBC (10^6µL)	6.39-8.01	8.10± 0.10	8.95± 0.20
WBC (10^3µL)	3.00-9.22	8.92±4.81	9.10± 2.05
Hb (g/dL)	12.9-15.4	14.40±0.51	15.10±0.20
Hct (%)	42-49	43.20±1.40	44.58±0.03
Neut (%)	6.14-22.95	15.64±1.50	14.21±1.50
Lym (%)	69.88-86.89	80.08±2.24	81.44±1.50
Mono (%)	3.77-10.82	3.05±0.01	2.98±0.10
Eos (%)	0.54-3.39	3.05±0.35	3.50±0.10
Baso (%)	0.04-0.44	0.20±0.03	0.21±0.03
Plt (10^9/L)	923-1580	1270.3±80.55	1301.5±58.26

Note: Haematological Parameters Including Red Blood Cells, White Blood Cells, Hemoglobin, Hematocrit, Differential Leukocyte Count, and Platelet Count in Control and Treated Rats.

Macroscopic observation and histological analysis of rat organs

According to histopathological analysis. No treatment-related macroscopic findings were observed in treated animals at necropsy. For the histological investigation, no pathological changes were observed in the skin of animals in any group (Figure:1). The kidneys and liver of the treated rats were examined under a microscope, the appearance of the organs did not differ from that of the control group. Rats in the control group had normal, unaltered livers and kidneys. (Figure:2 liver A control and B test formulation, Kidney C control and D test formulation).

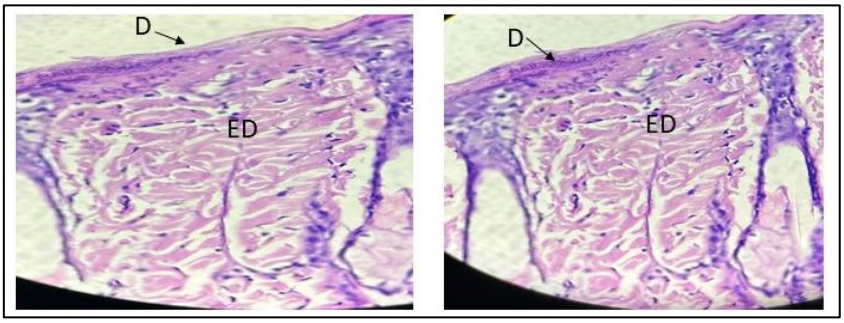

Figure 1 Histomorphology of skin samples (a) skin from the control group, and (b) skin treated with microemuslion, prepared at 4-5 µm thickness and stained with haematoxylin and eosin, are shown at 10X magnification. Abbreviations: D for Dermis; ED for Epidermis.

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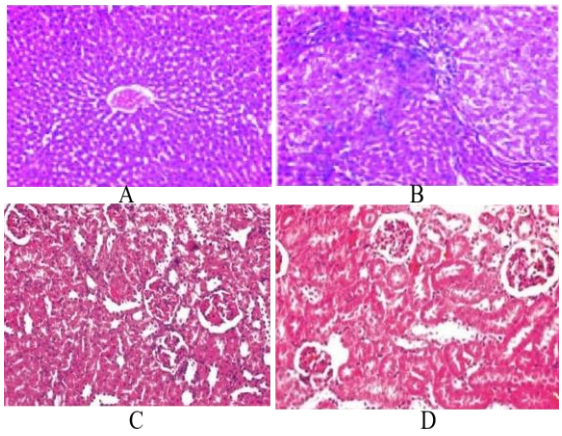

Figure 2 (A) Histological part of the liver from control rats administered distilled water and maintained on a standard diet for 28 days, displaying normal hepatic architecture. (B) Liver part from rats treated with 1000 mg/kg coriander essential oil (CEO) loaded microemulsion for 28 days, showing no signs of toxicity or pathological changes. (C) Kidney section from control rats receiving distilled water and a standard diet for 28 days, exhibiting normal renal histology. (D) Kidney parts from rats given a 1000 mg/kg CEO-loaded microemulsion for 28 days, demonstrating normal kidney structure without notable abnormalities.

During a 28-day repeated-dose dermal toxicity study (Figure. 1), histopathological assessment of the liver and kidney in the control group and the CEO-loaded microemulsion group revealed no treatment-related effects. Both the CEO-loaded microemulsion and control groups exhibited mild to reasonable hyperkeratosis, possibly due to repeated rubbing during topical application. However, no increase in hyperkeratosis or other related skin abrasions was observed with the test medication. Therefore, the organs showed no histological alterations linked to the treatment.

DISCUSSION:

Accumulating uses of essential oil-based formulations in pharmaceutical and cosmetic products have made it more important than ever to assess their safety systematically after topical exposure. While the essential oil of Coriandrum sativum shows several biological activities, the addition of the essential oil into a microemulsion system could affect the penetration into the skin and systemic exposure. In light of this, a safety assessment of its dermal application is necessary prior to considering its use for an extended period of time in topical application [17],[16].

The present investigation showed that coriander essential oil loaded microemulsion at the limit dose of 2000 mg/kg when applied acutely during observation period for 2 weeks, did not cause any mortality, abnormal behavioural changes or visible signs of toxicity. In accordance with OECD Guideline 402, there is no mortality at the limit dose, hence the dermal LD_{50} of the formulation is also considered to be low, >2000mg/kg bwt. The same is observed in dermal toxicity studies of essential oils and herbal products where the topical exposure did not show significant systemic toxicity or pathological changes in experimental animals [17],[10]

Significant effects were not observed on clinical signs, body weight, food and water intake following repeated dermal exposure to the formulation at 1000 mg/kg/day for 28 days, suggesting that the formulation did not affect normal physiological functions. The repeated dose dermal toxicity experiments conducted with plant extracts and topical formulations of these extracts also did not show any significant alterations in body weight or in general behavior after prolonged dermal exposure [16].

AST, ALT, and BUN are routinely used to assess possible liver and kidney damage when conducting toxicity studies. No significant changes in these parameters were observed after dermal delivery of the CEO-loaded microemulsion suggesting that there was no detectable liver or kidney toxicity. Similar observations have been made during repeated dermal exposure studies with herbal products which exhibit normal biochemical parameters [16].

The hematological parameters are important parameters for the physiological and immunological status of experimental animals. As there were no significant changes in red blood cell count, white blood cell count, haemoglobin concentration, hematocrit and differential leukocyte count, it is suggested that the formulation had no negative impact on the hematological functions or any systemic inflammatory response. These findings are comparable to those reported in the assessment of the safety of topical herbal preparations [10]

Histopathological examination is an important tool to confirm biochemical results and to detect microscopic tissue damage as a result of toxic exposure. In the present study, histopathology analysis of skin did not reveal any toxic lesions or alteration in the natural architecture of the skin [18]. The liver and kidney sections in treated animals revealed no evidence of cellular degeneration, necrosis, or inflammatory changes and normal architecture. This result corroborates the earlier reports of no treatment related histopathological changes following dermal application of plant based formulation [16],[10]

The favourable safety profile noted may also be related to the properties of the microemulsion system, that provides a uniform dispersion of the essential oil, and may minimise direct contact with

concentrated constituents to the skin. Such systems have been extensively studied to increase the tolerability and topical delivery.

The good dermal safety seen in the present study could also relate to the properties of the microemulsion delivery system. Microemulsions are thermodynamically stable colloidal systems that can help to disperse and solubilize lipophilic substances, including essential oils, and create a more even distribution on the skin surface. Furthermore, the constituents of essential oils can be encapsulated in the microemulsion droplets which may decrease direct contact with highly concentrated phytochemicals on the skin, thus decreasing local irritation, but still allowing for controlled and effective topical delivery. Appropriately designed microemulsions have been previously reported to be useful in topical delivery of drugs, in formulation stability and in providing acceptable tolerability to the skin, because of the small droplet size and optimized composition of the microemulsions. Hence, the absence of apparent toxic effects observed in the present study could be due partly to the protective and controlled delivery properties of the microemulsion system.

But there are some restrictions to be taken into account. The present study was limited to 3 weeks of exposure and biochemical panel was limited. More in-depth studies with extended treatment times, skin histopathological evaluations, dermal sensitization tests, and a larger selection of biochemical and oxidative stress markers are needed to generate a full long-term safety profile of the formulation.

Overall, the results showed that neither the dermal acute nor repeated dose toxicity of the essential oil of Coriandrum sativum loaded microemulsion was found to be significant under the experimental conditions used. The fact that there were no changes in clinical observations, biochemical and hematological parameters, and histopathological architecture, indicates its potential use as a safe delivery system for future pharmaceutical and cosmetic applications for topical delivery.

CONCLUSION:

In the present study, it is observed that topical application of Coriandrum sativum essential oil microemulsion at the tested experimental conditions does not show any significant acute or repeated dose toxicity on the dermal administration in Wistar albino rats. There was no mortality or apparent treatment-related toxic effects following dermal exposure to the formulation at 2000 mg/kg b.w., which is consistent with OECD Guideline 402 for dermal LD_{50} > 2000 mg/kg b.w. Additionally, a NOAEL > 1000 mg/kg/day was postulated based on the results of repeated topical exposures for 28 days with no significant changes in clinical observations, body weight, food and water consumption, biochemical and hematological parameters, or liver and kidney histopathological architecture.

In conclusion, the results indicated that the developed microemulsion has a safe dermal safety profile and can be used as a potential carrier system for the topical application of coriander essential oil. More long-term studies, such as dermal sensitization testing, skin histopathology, and other tests to assess for toxicity would support a broader safety assessment prior to clinical or commercial use.

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CONFLICTS OF INTEREST

The authors disclosed no financial or other potential conflicts of interest in this study.

ABBREVIATION

CS - *Coriandrum sativum*ME- Microemulsion
CEO- Coriander essential oil
WHO -World Health Organization
OECD -Organization for Economic Cooperation and Development
Rh- Relative humidity
ADT- Acute dermal toxicity
EDTA - Ethylenediaminetetraacetic Acid
AST- Aspartate transaminase
ALT- Alanine transaminase
BUN- Blood Urea Nitrogen
RBC -Red Blood Cell Count
WBC - White Blood Cell Count
HB -Haemoglobin Level
Hct- Hematocrit
Neut -Neutrophil
Eos - Eosinophil
Baso - Basophil
Lym-Lymphocyte
Mono- Monocyte
NOAEL- No-observed-adverse-effect level

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