Toxicological Studies of *Eucalyptus camaldulensis* Dehnh. (*Myrtaceae*) Essential Oil for the Safety of Its Insecticidal Application

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Abstract

Introduction: *Eucalyptus camaldulensis* Dehnh. (*Myrtaceae*) (E. camaldulensis) or red gum is a plant whose essential oil (EO) is recognized for its insecticidal, antibacterial, and antifungal properties. Imported from Australia and introduced to other continents whose Africa, its EO is used in Burkina Faso to formulate biopesticides.

Method: The current study evaluated the toxicological profile of this oil by evaluating the acute and sub-acute oral toxicity in Wistar rats and dermal and eye irritancy in rabbits, according to the guidelines of the Organization for Economic Cooperation and Development (OECD).

Result: The administration of a single dose of the EO to 2000 mg/kg b.w. did not result in mortality. The LD₅₀ of this EO is estimated to 5000 mg/kg b.w. The sub-acute toxicity study conducted for 28 days at doses of 100, 500, and 1000 mg/kg b.w. showed no toxicity signs or mortality for all tested doses in both sexes. However, no significant difference was observed in average food consumption level, body weight gain, and relative organ weights in test groups compared to control ones. The differences were significant for mean water consumption level in the females treated to 500 and 1000 mg/kg b.w. in the fourth week compared to the control. There was a significant decrease in biochemical parameter levels such as AST at 1000 mg/kg in both sex compared to control ones. The Dermal Irritation Score (DIS) was 0.16 and the Maximum Mean Total Score (MMTS) was 4.

Conclusion: *E. camaldulensis* EO was a low acute and sub-acute oral toxicity in rats. It also had very little irritant power on the rabbit’s skin and eye. This EO can constitute an alternative to the use of chemical pesticides in pest control.

Keywords

*Eucalyptus camaldulensis*, Essential oils, Acute and subacute toxicity, Biochemical parameters, Dermal and eye irritation, Burkina Faso


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Introduction

Agricultural and public health pest control has been a human concern for years. Indeed, losses in pre-harvest production of world crops, mostly due to insects, microorganisms, and weeds, are estimated at 35%, and these losses would be 70% without adequate protection [1]. Ticks and other microorganisms are vectors of many diseases. However, synthetic pesticides have proven to be very effective in combating these pests, thereby improving agricultural yields and considerably limiting the proliferation of disease-carrying insects. Nowadays, while synthetic pesticides have many advantages on the one hand, their use is the source of severe health and environmental problems on the other. Indeed, several synthetic pesticides are sources of acute and chronic intoxications, often with residuals in the environment [2-4]. This is why research efforts are being made to find less harmful pesticides to both health and the environment. As a result, biopesticides are presented as a veritable alternative to synthetic pesticides. These biopesticides are classified into three main categories: microbial, plant, and animal [5]. This study focused on natural pesticides of plant origin, specifically on essential oils (EO) from plants used as active substances in the formulation of biopesticides.

Eucalyptus camaldulensis Dehnh. (E. camaldulensis) is a fast-growing plant species of Australian origin, introduced to the rest of the continents and then to Africa, including Burkina Faso [6]. It is a woody plant of the Myrtaceae family, which is commonly cultivated. It is a tree up to 20 meters tall with branches with drooping ends and a trunk diameter of one to two meters [6]. Eucalyptus has become one of the world’s most widely planted genera (Akin et al., 2010). Eucalyptus camaldulensis (formerly Eucalyptus rostrata Sch.), also known as long-beak eucalyptus, Murray red gum, red gum, river gum, and red river gum, is one of the most widely distributed Eucalyptus species. It is also considered one of the most widely planted trees in the world (ca. 5,000,000 ha planted) (N.A.S., 1980; Boland et al., 1984).

In Burkina Faso, the EO of this plant is used to formulate biopesticides. Indeed, a study carried out in Morocco has shown that the majority of molecules of E. camaldulensis EO are p-cymene (6.50%), α-pinene (28.30%), and 1,8-cineole (42.30%). This oil also has insecticidal properties [7]. Other work has shown that E. Camaldulensis EO has antibacterial and antifungal properties [8,9]. In Burkina Faso, a study has shown that the leaves of E. camaldulensis are traditionally used to control disease-carrying insects in the post-harvest period [10-12]. Several studies have shown the pesticide properties of E. camaldulensis EO, but few authors have been interested in studying its toxicological profile. This study aimed to study the toxicological profile of E. camaldulensis EO in order to provide further data on its safety and use. This consisted of determining the oral LD₅₀, physical and biochemical parameters, dermal and eye irritancy of this EO.

Material and Methods

Plant material and essential oil extraction

The fresh leaves of Eucalyptus camaldulensis were collected from the botanical garden of the Research Institute of Applied Sciences and Technologies located at Bama, Burkina Faso (Latitude Nord 11° 22’ 00”, Longitude Ouest 4° 25’ 00”). Sampling took place in June 2019. After being transferred to the laboratory, leaves were washed with tap water and extracted by hydrodistillation using an alembic/Clevenger-type apparatus for 3 hours. For EO extraction, 40 kg of fresh leaves were poured into a 150 L round-bottom flask of the Clevenger apparatus. Thus, the EO obtained was stored in an airtight container in a refrigerator at 4 °C until various toxicological tests.

Animals

Healthy female and male Wistar rats weighing respectively between 127-140 g and 110-140 g were used for acute and sub-acute toxicity studies. These animals were supplied by the Research Institute for Health Sciences pet shop in Ouagadougou, Burkina Faso. They were housed in the animal cage with free access to water and a standard laboratory pellet enriched with protein (29%). All rats were housed under laboratory standard conditions of temperature (22 ± 3 °C), relative humidity (60 ± 5%), and circadian cycle (12 hours dark/light).

Male and female New Zealand albino rabbits weighing between 1.67 and 2.09 kg were used for dermal and eye irritation tests. They were provided by the breeders from the city of Ouagadougou and housed in an enclosure in standard laboratory conditions of temperature (25 °C) and a dark/light cycle of 12 hours for two weeks prior to the tests. They also had free access to water and a standard laboratory pellet.

All experimental protocols followed international standards for animal testing, including the UE “Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes” (Louhimies, 2002).

Acute orale toxicity test

For the acute toxicity test, animals were examined to check for the absence of pathology, then fasted four (04) hours before the test. The Acute oral toxicity test was carried out in accordance with the OECD guideline 423 [13]. A starting single dose of 2000 mg/kg b.w. of the E. camaldulensis EO was orally administered to three female rats. A control group of rats received distilled water instead of EO. Animals of both test and control groups were observed every 30 min for two
hours after administration, then daily until the 14th day after administration. Observations focused on mortality and signs of toxicity (changes in skin and fur, eyes, salivation, convulsion, diarrhea, sleep, and coma). If the mortality is more than 2, the dose is reduced to 300 mg/kg b.w., but for less than 2 mortality, the same dose of 2000 mg/kg b.w. is repeated, and the OECD sequencing scheme (OEC, 2001) is followed for the rest.

**Sub-acute oral toxicity test**

The test was performed on Wistar rats in accordance with OECD guideline 407 [14]. Forty Wistar rats were used to form four groups of ten animals (five per sex): Group I, group II, group III, and group IV. *E. camaldulensis* EO was administered to groups II, III, and IV animals at doses of 100, 500, and 1000 mg/kg b.w. respectively. Administration was made at the same hour for 28 consecutive days. Group I was considered as the control and received only distilled water. After treatment, animals were observed for the first to fourth hour post-treatment to examine any adverse toxic signs and behavioral changes and at least twice a day for morbidity and mortality. Water intake was recorded daily, while food and body weight were recorded once a week during the 28-day study period.

**Blood analysis**

After the four-week study period, animals were fasted (deprived of food but no water) the night before the blood sample. On day 29th, all animals were anesthetized with ketamine (150 mg/kg) and sacrificed. Blood samples were collected from each animal via cardiac puncture into dry vacutainers and centrifuged at 3000 rpm for 10 min using a table centrifuge (ROTOFIX 32A, Mettich Zenfrifugen, Germany). The serum from centrifugation was used to determine biochemical parameters levels such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREAT), glycemia (GLY), total protein (TP) and total cholesterol (TC). These parameters were determined using an automatic biochemistry analyzer (MINDRAY BA-88A, China).

**Effects on vital organs**

After blood samples collection, the animal organs such as the heart, liver, lungs, kidneys, and spleen were removed, observed macroscopically and weighed using by the mean of balance brand METTLER TOLEDO of type PB3002-S (3.1 kg maxima and minimum 0.0005 kg minima). The Relative Organ Weight ratio (ROW) of each organ was calculated according to the following formula:

\[
ROW(\%) = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100
\]

**Dermal irritation test**

The test was performed according to the protocol of OECD guideline 404 for product testing using acute dermal irritation/corrosion method, which is an adaptation of the method of Draize, et al. (1944) [15,16]. Three (03) healthy male rabbits were chosen from the group of rabbits acclimated to the breeding conditions of the IRSS pet store. The day before, a small area (≈ 6 cm²) was shaved on the two flanks of each rabbit. A single dose of the EO (0.5 ml) was applied for four hours to a small area of one flank, and the untreated skin area of the animal was used as the control. After the exposure period, clinical parameters such as erythema and edema levels were observed after patch removal. Observations and ratings of edema and erythema levels were done after 1, 24, 48, and 72 hours after the patch’s removal. Observations continued for fourteen (14) days to detect possible reversibility of the observed effects. Dermal Irritation Score (DIS) was calculated using the following formula:

\[
DIS = \frac{\text{Value (erythema + edema)}}{\text{Number of animals} \times \text{Number of observations}}
\]

*E. camaldulensis* EO DIS was classified according to the scale proposed by Draize, et al., adapted by Dutok, et al. and adopted by OECD for determining the level of acute dermal irritation/corrosion [16,17].

**Eye irritation test**

The test was performed according to OECD guideline 405 to determine the level of acute eye irritation/corrosion [18]. Three (03) healthy rabbits were chosen from the group of rabbits acclimated to the breeding conditions of the IRSS pet store for at least two weeks. They were examined to check for any infection or pathology, and marked for easy identification. For each animal, one eye is taken as a test and the second one as a control. A volume of 0.1 mL of *E. camaldulensis* EO was instilled to the bottom of the right conjunctival sac of test eye of each animal and keeping eyelids together over the next 30s. Eyes of each animal were observed and the level of eye irritation/corrosion is evaluated by scoring lesions of conjunctiva, cornea, and iris after 1; 24; 48 and 72 hours after the instillation, using an ultraviolet light for observations. The irritation score was determined using the weighted scale to grade the severity of eye lesions from Draize, et al. [16]. The Maximum Mean Total Score (MMTS) is determined according to the following formula:

\[
\text{MMTS} = \frac{\text{Sum of total imum score rabbit rabbit rabbit}}{3}
\]

The values obtained were used for classification of eye irritability in the system of Kay and Calandra [19]. Observations were continued up to 21 days in order to verify the reversibility or not of the possible toxic effects.

**Data analysis**

Results were presented as mean ± standard deviation SD (n = 5). The statistical significance of the difference
Table 1: Mortality of female rats in acute oral toxicity study

<table>
<thead>
<tr>
<th>Substance and dose</th>
<th>Rats mortality</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First test</td>
<td>Second test</td>
</tr>
<tr>
<td>E. camaldulensis EO (2000 mg/kg b.w.)</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Table 2: Mean daily water consumption (mL/day/rat) during 28 days of treatment with E. camaldulensis EO.

<table>
<thead>
<tr>
<th>Week</th>
<th>Sex</th>
<th>Doses</th>
<th>Control</th>
<th>100 mg/kg</th>
<th>500 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>33.57 ± 3.41</td>
<td>32.43 ± 2.23</td>
<td>35.86 ± 6.39</td>
<td>35.00 ± 5.83</td>
</tr>
<tr>
<td>Week 1</td>
<td>F</td>
<td>35.14 ± 2.12</td>
<td>33.29 ± 6.24</td>
<td>35.57 ± 3.82</td>
<td>32.57 ± 3.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>35.75 ± 5.91</td>
<td>35.29 ± 3.20</td>
<td>38.71 ± 6.34</td>
<td>42.14 ± 7.78</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>F</td>
<td>39.71 ± 2.98</td>
<td>38.14 ± 3.53</td>
<td>40.29 ± 4.46</td>
<td>40.00 ± 6.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>39.86 ± 3.85</td>
<td>40.43 ± 3.16</td>
<td>40.86 ± 4.34</td>
<td>42.57 ± 5.50</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>F</td>
<td>41.14 ± 4.91</td>
<td>42.57 ± 7.09</td>
<td>41.86 ± 4.06</td>
<td>41.86 ± 6.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>47.14 ± 5.30</td>
<td>47.14 ± 5.21</td>
<td>46.57 ± 6.40</td>
<td>48.86 ± 5.93</td>
<td></td>
</tr>
</tbody>
</table>

(n = 10; 5/sex) M = Male; F = Female
*p < 0.01 => highly significant; p < 0.05 => significant difference; p > 0.05 => no significant difference

Animals and treated showed that the EO does not affect vital organs as there was no change in color and aspect of different organs. The relative organ weights of the control rats and those treated with doses of 100, 500, and 1000 mg/kg b.w. with E. camaldulensis EO are presented in Table 4. No significant change was noticed in these relative organ weights of control and treated animals at 100, 500, and 1000 mg/kg b.w. (p > 0.05).

Effect on biochemical parameters

Analysis of biochemical parameters of control and treated rats are shown in Table 5. These results showed that the daily oral administration of E. camaldulensis EO to 100, 500 and 1000 mg/kg b.w. on animals during the four weeks did not cause significant changes in ALT, total protein, creatinine, glycemia and total cholesterol levels (p > 0.05). However, statistical analysis showed a significant increase in AST levels in males and females treated to 1000 mg/kg b.w. (p < 0.05).

Acute dermal irritation study

Result of E. camaldulensis EO dermal irritation scores is shown in Table 6. This result showed that the plant’s EO is very mildly irritating to the skin. Indeed, the DIS for E. camaldulensis EO found was “0.16”. This product is classified not irritant for skin according to scale proposed by Draize, et al. [16].
EO is classified as a substance with slight irritation on the rabbit’s eye.

**Discussion**

Acute oral toxicity study of *E. camaldulensis* EO gave an LD<sub>50</sub> estimated at 5000 mg/kg b.w. According to the OECD guideline 423 [13], the EO tested is classified as a substance with slight irritation on the rabbit’s eye.
in the fifth class of toxicity, that means in the class of substances with low acute oral toxicity according to the categorization of acute oral toxicity classes of the General Harmonized System (GHS) [20]. To our knowledge, no data on the oral toxicity of crude *E. camaldulensis* EO was available. However, oral toxicity studies of molecular components of EO and extracts belonging to different parts of *E. camaldulensis* exist.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sex</th>
<th>Control</th>
<th>100 mg/kg</th>
<th>500 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (UI/L)</td>
<td>F</td>
<td>32.40 ± 6.19</td>
<td>30.20 ± 4.44</td>
<td>35.60 ± 4.67</td>
<td>39.40 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>39.80 ± 2.59</td>
<td>34.20 ± 6.67</td>
<td>32.20 ± 5.26</td>
<td>33.00 ± 3.74</td>
</tr>
<tr>
<td>AST (UI/L)</td>
<td>F</td>
<td>126.20 ± 15.51</td>
<td>114.0 ± 18.21</td>
<td>122.6 ± 15.14</td>
<td>83.40 ± 5.81***</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>105.20 ± 6.26</td>
<td>98.40 ± 4.67</td>
<td>95.60 ± 6.47</td>
<td>94.00 ± 6.56</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>F</td>
<td>61.18 ± 2.23</td>
<td>60.16 ± 2.88</td>
<td>62.44 ± 5.14</td>
<td>65.60 ± 2.74</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>63.32 ± 2.92</td>
<td>61.90 ± 2.56</td>
<td>60.64 ± 4.73</td>
<td>64.32 ± 2.26</td>
</tr>
<tr>
<td>CREAT (µmol/L)</td>
<td>F</td>
<td>64.38 ± 2.97</td>
<td>61.48 ± 1.45</td>
<td>68.16 ± 5.96</td>
<td>69.12 ± 7.91</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>58.24 ± 5.23</td>
<td>65.48 ± 5.74</td>
<td>63.30 ± 3.08</td>
<td>60.96 ± 3.66</td>
</tr>
<tr>
<td>GLY (mmol/L)</td>
<td>F</td>
<td>4.38 ± 0.32</td>
<td>4.96 ± 0.63</td>
<td>5.18 ± 0.61</td>
<td>4.52 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>4.04 ± 0.57</td>
<td>4.70 ± 1.07</td>
<td>4.62 ± 0.78</td>
<td>5.11 ± 0.31</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>F</td>
<td>2.06 ± 0.67</td>
<td>2.07 ± 0.29</td>
<td>2.06 ± 0.43</td>
<td>2.75 ± 0.84</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.78 ± 0.20</td>
<td>2.13 ± 0.22</td>
<td>2.40 ± 0.45</td>
<td>2.13 ± 0.66</td>
</tr>
</tbody>
</table>

Mean and standard deviation are presented (n = 10; 5/sex) M = Male; F = Female

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; CREAT: Creatinine; GLY: Glycemia; TP: Total Protein; TC: Total Cholesterol

**p < 0.001; *p < 0.05; p > 0.05 vs. controls**

<table>
<thead>
<tr>
<th>Test conditions</th>
<th>24 hours</th>
<th>72 hours</th>
<th>Scores</th>
<th>Total (24 + 72 H) Erythema + Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit N° Flanks</td>
<td>Erythema</td>
<td>Edema</td>
<td>Erythema</td>
<td>Edema</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>Treated</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>Treated</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>Treated</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (T)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DIS = T/12 = 2/12 = 0.16 Conclusion: 0 < DIS = 0.16 < 0.4; => Not irritant

<table>
<thead>
<tr>
<th>Rabbit N° 1</th>
<th>Rabbit N° 2</th>
<th>Rabbit N° 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours</td>
<td>Hours</td>
<td>Hours</td>
</tr>
<tr>
<td>I. Cornea</td>
<td>A × B × 5 (≤ 80)</td>
<td>0</td>
</tr>
<tr>
<td>II. Iris</td>
<td>A × 5 (≤ 10)</td>
<td>0</td>
</tr>
<tr>
<td>III. Conjunctiva (A + B + C) × 2 (≤ 20)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

MMTS 4

Conclusion: 2.6 < MMTS = 4 < 15 => Slightly irritant
An acute oral toxicity study carried out on rats has given an oral LD₅₀ value estimated at 3700 mg/kg b.w. for α-Pinene, a component of *E. camaldulensis* EO [21]. Acute toxicity studies carried out on mice have given an oral LD₅₀ estimated at 3849 mg/kg b.w. for 1,8-Cineole, which is the main component of *E. camaldulensis* EO [22]. An acute toxicity study conducted in Sudan in 2015 in Wistars rats with the ethanolic extract of leaves of *E. camaldulensis* gave an LD₅₀ ≥ 5000 mg/kg b.w. [23]. The difference between the results of these studies and the current ones could be explained by the nature of the extracts used and the methods, but also by the animal model used for the various tests.

During the 28-day study period, there was no mortality or signs of toxicity. Daily oral administration of *E. camaldulensis* EO to 500 and 1000 mg/kg b.w. induced an increase in the average water consumption in the female treated rats compared to control ones in the fourth week (p < 0.05). However, the average food consumption of treated groups was similar to the control ones. The increase in mean water consumption can be explained by the progressive weight gain of animals [23]. The non-significant increase in the weight of animals treated with *E. camaldulensis* EO compared to the controls shows that it had no toxic effect on the weight of rats treated up to 1000 mg/kg for 28 days. Macroscopic observation showed no lesions, change in color, or shape in the organs of test groups compared to control ones. There was a non-significant increase in organ weights of treated animals compared to control ones. This shows that the plant’s EO did not have any harmful effect on the organs of treated rats compared to control ones during the sub-acute toxicity study up to 1000 mg/kg b.w. Another study carried out on mice reported that the majority of chemical compounds of this EO (1,8-cineole or eucalyptol) have low toxicity on organs such as the liver, spleen, and kidneys at low doses [22]. The difference between the results of these studies and this one could be explained by the edaphic factors, which influence the synthesis of secondary metabolites, as well as by the model of animal species used.

Some authors have reported that certain plant-based EOs can cause hepatotoxicity, nephrotoxicity, and cardiotoxicity [24]. The four-week sub-acute toxicity study showed that ALT, total protein, creatinine, glycemia, and total cholesterol levels were similar in treated groups compared to control one in males and females. These results show that EO had no toxic effect on these biochemical parameters up to 1000 mg/kg b.w. The significant decrease of AST level (p < 0.05) to 1000 mg/kg b.w. in males and females could mean that *E. camaldulensis* EO has a toxic effect on this biochemical parameter at high doses. However, another study in Nigeria in 2012 with the aqueous extract of leaves of *E. camaldulensis* showed that this extract had no toxic effect on the liver up to 1000 mg/kg [25].

Dermal irritancy of *E. camaldulensis* EO has been evaluated. The extract’s DIS is between 0 and 0.4. According to OECD guideline 404, the plant’s EO is therefore classified in the first class [15]. The EO is classified as not irritating to the skin. A study showed that α-pinene had a low irritant power on the rabbit’s skin up to a dose of 500 mg/kg for 24h [21]. A European Chemicals Agency (ECHA) study showed that 1,8-cineole had a very low irritant power on the skin [24]. A study examining the anti-inflammatory properties of EOs from other species of *Eucalyptus*, showed that the EOs of *Eucalyptus citriodora*, *Eucalyptus tereticornis*, and *Eucalyptus globulus*, possess anti-inflammatory properties with a significant reduction of edema [26]. The ocular irritancy gave a MMTS equal to four (4) for treated group. This value is between 2.6 and 15, and *E. camaldulensis* EO is classified in the third class according to OECD guideline 405 [18]. This means that it is classified as a mild irritant. A study showed that the mixture of α-terpinene, p-cymene with d-limonene was not irritating to the eyes [27].

**Conclusion**

The study of acute oral toxicity showed a relatively low toxicity of *E. camaldulensis* EO with an LD₅₀ estimated to 5000 mg/kg b.w. The sub-acute toxicity study showed low toxicity with minimal impact on exposed animals’ physical and biochemical parameters. The irritancy potential assessment shows that the EO is non-irritant to the skin and slightly irritates the eyes. The results suggest that *E. camaldulensis* EO would be safe in both acute and subacute exposure conditions and is an excellent alternative to synthetic chemical pesticides. However, for the further clinical relevance of the results and complete toxicological profile elucidation, toxicity studies must be extended to other toxicity tests such as subchronic and chronic toxicities, reprotoxicity, carcinogenicity, teratogenicity, and mutagenicity investigations.

**Conflicts of Interests**

The authors have not declared any conflicts of interest.

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