Full Length Research Paper

Safety assessment of *Cymbopogon schoenanthus* (L.) Spreng. (Poaceae) essential oils: Oral toxicity, dermal and eye irritancy investigations

Sylvain Ilboudo\(^1,2\)*, Geoffroy G. Ouédraogo\(^1\), Ignace Sawadogo\(^3\), Lazare Belemnaba\(^1\), Sylvain Ouédraogo\(^1\) and Roger Ch. H. Nébié\(^3\)

\(^1\)Département Médecine, Pharmacopée Traditionnelles et Pharmacie, Institut de Recherche en Sciences de la Santé (MEPHATRA-PH/IRSS/CNRST), 03 BP 7192 Ouagadougou, Burkina Faso.

\(^2\)Unité Mixte de Recherche Internationale - Environnement, Santé et Sociétés (UMI 3189, ESS) CNRST (Burkina Faso)/ CNRS (France)/ UCAD-UGB (Sénégal)/ USTTB, Mali.

\(^3\)Département Substances Naturelles, Institut de Recherche en Sciences Appliquées et Technologies (DSN/IRSAT/CNRST), 03 BP 7047 Ouagadougou 03, Burkina Faso.

Received 13 August, 2019; Accepted 2 October, 2019

The side effects prediction is a regulatory requirement prior the effective use of a new product as drug or pesticide constituent is allowed. The handling hazards of *Cymbopogon schoenanthus* essential oils (CSEO) were determined by investigating their acute and subacute toxicities as well as irritancy potential for eye and skin in compliance with the Organization for Economic Cooperation and Development guidelines. In mice, the essential oils did not induce mortality at the limit single dose of 2000 mg/kg b.w., meaning that the test product estimated LD\(_{50}\) is 5000 mg/kg b.w. In the 28 days subacute oral toxicity study in rats, there was no mortality observed for all tested doses in both sex. Furthermore, besides body weight gain that slightly dropped, there was no significant difference observed in food and water consumption, relative organs weight, and blood biochemical profile in test animals, compared to the control. No eye irritant reaction was shown in the rabbits exposed to CSEO. To assess dermal irritation, rabbits were dermally exposed to CSEO for 4 h. The results showed that no adverse reactions such as erythema and edema were observed throughout the test. Based on these findings, CSEO do not cause either oral toxicity up to 28 days in repeated administration, nor dermal and eye irritation, and seems to be safe for animal in the study conditions. These results constitute a new scientific support for the safe use of *C. schoenanthus* essential oils as alternatives to synthetic pesticides. However, for further clinical relevance of the results and complete toxicological profile elucidation, toxicity studies must be extended to long term toxicity test as subchronic and chronic toxicities, reprotoxicity, carcinogenicity, teratogenicity, and mutagenicity investigations.

**Key words:** *Cymbopogon schoenanthus*, Wistar rat, essential oils, sub-acute toxicity, blood chemistry, naturel insecticide.

INTRODUCTION

*C. schoenanthus* (L.) Spreng.(Poaceae), also called West India citronella, is an herbaceous plant of the Poaceae
family. Beyond being native to tropical Asia, especially India (Al-Snafi, 2016), it is a common and abundant species in the West African savannas. It has a quasi-ubiquitous distribution in Burkina Faso, and even settles in environments apparently less favorable to plants. Like its ubiquitous distribution, the data from literature demonstrated that Cymbopogon species including C. schoenanthus possess various biological properties such as anti-bacterial (Hashim et al., 2017; Hellali et al., 2016), anti-diarrheal, anti-amoebic, anti-filarial, anti-fungal, anti-inflammatory, anti-mutagenicity, anti-malarial, anti-oxidants (Hellali et al., 2016), hypoglycaemic (Dutta et al., 2016), and spasmylytic (Pavlović et al., 2017). Furthermore, studies by different researchers indicate the presence of several chemical groups such as tannins, saponins, saponin glycosides, flavonoids, alkaloids, triterpens, cardiac glycosides, glycosides, steroids and volatile oils in the plant (Pavlović et al., 2017; Amina et al., 2013).

Well known for its fragrant foliage, C. schoenanthus produces essential oils (named CSEO throughout this document) that have aromatic characteristics. Essential oils are natural volatile fractions extracted from aromatic plants and formed by classes of substances such as esters of fatty acids, mono and sesquiterpenes, phenylpropanoids, aldehyde alcohols and, in some cases, aliphatic hydrocarbons, among others (Santana de Oliveira et al., 2018). Given their multiple properties, essential oils whose applications in everyday life are known since antiquity continue to attract the attention of researchers in the fields of medicine, medicine, food, cosmetics, and perfume. After a chromatographic analysis establishing that the CSEO contain 25 compounds including mainly Piperitone (42.795%), delta-2-carene (38.968%) and Limonene (5.680%), Sawadogo et al. (2018) have shown that these oils were effective against Sitophilus zeamais Motsch. and Rhysopertha dominica F., two maize seeds pests. Previous investigations in several countries confirm that some plant essential oils not only repel insects but have contact and fumigant insecticidal actions against specific pests, and fungicidal actions against some important plant pathogens (Hellali et al., 2016; Isman, 2000; Sarac and Tunc, 1995). Therefore, essential oils of C. schoenanthus have been shown to present biological effects against Anopheles gambiae and Plutella xylostella at different life stages. Furthermore, CSEO demonstrated its efficiency against the bruchid Callosobruchus maculatus (F.) insect causing major losses during the storage of seeds of Vigna unguiculata (Walp.) in West Africa (Ketoh et al., 2006). Additional investigations established that biocidal activities of CSEO could be supported by their capability to inhibit insect cholinesterase activities (Khadri et al., 2008, 2010). Essential oils potency to inhibit acetylcholinesterase is well established (Aazza et al., 2011; Kitphati et al., 2012; Owokotomo et al., 2015) and suggests that CSEO share a common mechanism of action with organophosphorus and carbamate insecticides. It could therefore be an appropriate alternative to the synthetic pesticides that induce hypothetical health and environmental effects.

Despite the abundance of data on industrial applications, traditional uses and pharmacological properties of the essential oils from C. schoenanthus, there are no scientific data available related to its toxicity. This study aims to contribute to define safe use conditions of these essential oils, specifically as grain post-harvesting insecticide. For this purpose, acute and subacute toxicity as well as eye and dermal irritation studies were undertaken.

**MATERIALS AND METHODS**

**Plant material**

Fresh leaves of C. schoenanthus were collected from the botanical garden of the "Institut de Recherche en Sciences Appliquées et Technologies" (IRSAT), Ouagadougou (Latitude Nord 12° 25’ 470”, Longitude Ouest 1° 29’ 251”). The plant sample was authenticated at "Herbier National du Burkina (HNBU)" of the “Centre National de Recherche Scientifique et Technologique (CNRST)”, Ouagadougou (Burkina Faso). Immediately after collection, the leaves were washed with tap water and dried at ambient temperature under ventilation in the shade for one week. The dried leaves were used for essential oil extraction.

**Essential oil extraction**

A portion of dried plant material (500 g) was submitted to hydro-distillation using an alembic/Clevenger-type apparatus for 3 h as previously described by Bassolé et al. (2011). Anhydrous sodium sulfate was used to remove water after extraction. The essential oil thus obtained was stored in airtight container in a refrigerator at 4°C until toxicological tests.

**Animals**

Healthy female NMRI mice weighing between 28-32 g and both male and female Wistar rats (Mean weight: 169± 22 g) were used respectively for acute and subacute toxicity studies. They were provided by the “Institut de Recherche en Science de la Santé” (IRSS), Ouagadougou, Burkina Faso, and housed in the animal cage with free access to water and standard laboratory pellet enriched with protein (29%). All animals were maintained in a
controlled temperature room of 22-25°C with a 12 h dark/light cycle. Healthy rabbits of both sex weighing between 1.5 and 2 kg were used for eyes and dermal irritation tests. They were procured from Production Department of the “Projet de Développement des Animaux Villageois (PDAV)”, Ouagadougou and acclimatized in the same conditions as mice and rats for two weeks before use. The experimental protocol was carried out in compliance with international standard protocols [Guidelines set by the European Union on the Protection of Animals (CEC Council 86/609)] and adopted by IRSS, Burkina Faso (Konaté et al., 2017).

CSEO acute oral toxicity test

The acute oral toxicity test of C. shoanathus essential oil (CSEO) was performed on female NMRI mice according to Organization for Economic Co-operation and Development (OECD) test guideline 423 (OECD, 2001). Briefly, after a fasting period (4 h of starvation only), CSEO was administered orally by gavage in single dose to the mice according to the sequential procedure. In the conducting of test, 2000 mg/kg body weight of test substance was chosen as the starting dose. After 2 h post-treatment observation of all animals, feeding was restored. They were then observed at least once daily for 14 days for mortality and signs of toxicity such as changes in skin and fur, eyes, mucus membranes, salivation, convulsion, diarhoea, lethargy, sleep and coma. A control group of female mice received a single dose of distilled water orally and have been monitored for 14 days as well as the treated group.

Subacute toxicity study of CSEO

The sub-acute oral toxicity study was carried out in accordance with OECD guideline 407 (OECD, 2008). A total number of 30 Wistar rats of both sexes were randomly selected for the subacute toxicity studies. The females were nulliparous and nonpregnant. The rats were divided into 3 groups of 10 animals each (5 males and 5 females); males and females were kept in separate polypropylene cages. Group 1, which served as the control received the vehicle (distilled water), while rats in Groups 2 and 3 respectively received daily doses of 50 and 500 mg/kg b.w. of CSEO at the same hour for 28 consecutive days. Doses were prepared by dispersion of CSEO in distilled water and dose volume was not more than 1 ml/100 g body weight. All animals were closely observed for the first 1 to 4 h post-treatment to examine any adverse toxic signs, behavioural changes and at least twice a day for morbidity and mortality.

Water intake was recorded daily, while food and body weight was recorded once in a week throughout the study period. A weekly body weight gain was calculated according to the following formula:

\[ BWG = Body\ weight_{(n)} - Body\ weight_{(n-1)} \] (1)

Where BWG represent the weekly body weight gain, Body weight\(n\) is the measure of body weight of current week to which body weight gain is calculated, and Body weight\(n-1\) is the preceding measure of body weight.

Effects on vital organs

On the 29th day of test, after an overnight fast, the rats were anaesthetized with ketamine and internal vital organs such as heart, kidneys, liver, lung, and spleen were isolated and observed macroscopically for any lesions. After that, all organs were cleaned with toilet paper and then weighed on a sensitive balance (Sartorius; precision 0.1 mg). The relative organ weight ratio (ROW) of each rat was calculated as follows:

\[ \text{ROW} (%) = 100 \times \frac{\text{Absolute organ weight (g)}}{\text{body weight of rats on sacrifice day (g)}} \] (2)

Blood analysis for effect on biochemistry

Prior to organ isolation, blood samples from animals were collected via cardiac puncture into dry vacutainers for each animal. After the blood collection, samples were then centrifuged at 3000 rpm for 10 min using a table centrifuge (ROTOFIX 32A, Mettich Zentrifugen, Germany) and the sera obtained were used for biochemical assays. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREAT), glucose, total protein (TP) and total cholesterol (TC), levels were determined using an automatic biochemistry analyzer (Mindray BS-300, China).

Eye Irritation Test of CSEO

The eye irritation study was performed according to an adaptation of the OECD guidelines 405 for determining the degree of acute eye irritation/corrosion (OECD, 2017). A total of three rabbits per group were used in the experiment test. Prior to essay, they were subjected to a rigorous observation of the ocular structures such as cornea, iris, and conjunctiva. The substance to be tested was applied in a single dose to one eye of the experimental animal whereas the untreated one served as the control.

A volume of 0.1 mL of extract was instilled to the bottom of the right conjunctival sac of one eye of each animal and, keeping eyelids together over the next 30 s. Both eyes of each animal were observed and the degree of eye irritation/corrosion is evaluated by scoring lesions of conjunctiva, cornea, and iris, at intervals of 1, 24, 48, and 72 h. Corneal damage was determined in a dark room with the use of a solution of 2% sodium fluorescein, and physiological saline was used to remove excess solution from the instilled developer substance. Finally, an ultraviolet light was used for observation. The individual irritation score was determined using the scale of weighted scores for grading the severity of ocular lesions from Draize et al. (1944). The maximum mean total score (MSTS) obtained were used for classification of eye irritability in the system of Kay and Calandra (Table 1).

The observations were allowed to continue up to 21 days to evaluate the reversibility or irreversibility of the effects.

Dermal irritation test of CSEO

The method used for acute dermal irritation was inspired by that described by Draize et al. (1944), and adapted by the OECD guideline 404 for product testing using acute dermal irritation/corrosion method (OECD, 2015).

The experiments were carried out using three adult male rabbits. Animals were shaved on the flanks 24 h before the application of the product. A single dose of CSEO (0.5 mL) is applied for 4 h to a small area (approximately 6 cm²) of one flank, and the untreated skin area of animal had served as the control. At the end of exposure period, patches were removed. Observations and rating of the skin reactions (erythema and edema) were made after 1, 24, 48 and 72 h after the removal of the patch. The observations were continued up to 14 days for possible change in behavior, general condition, posture and reflexes. The reactions, defined as erythema and edema, were evaluated according to the scoring system for skin reactions (Table 2) (OECD, 2015).
Table 1. Scale of weighted scores for grading the severity of ocular lesions.

<table>
<thead>
<tr>
<th>MMTS*</th>
<th>Irritation classification</th>
<th>Requirement for maintenance of classification¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 – 0.5</td>
<td>Non-irritating</td>
<td>Up to 0.5 at 1 h with zeros at 24 h; otherwise, increase one level</td>
</tr>
<tr>
<td>0.6 – 2.5</td>
<td>Practically non-irritating</td>
<td>With zeros at 24 h; otherwise, increase one level</td>
</tr>
<tr>
<td>2.6 – 15.0</td>
<td>Minimally irritating</td>
<td>With zeros at 48 h; otherwise, increase one level</td>
</tr>
<tr>
<td>15.1 – 25.5</td>
<td>Mildly irritating</td>
<td>With zeros at 96 h; otherwise, increase one level</td>
</tr>
<tr>
<td>25.1 – 50.0</td>
<td>Moderately irritating</td>
<td>With 7 day mean ≤ 20 and individual total scores ≤ 10 in at least 60% of the rabbits with no total score &gt;30; otherwise, increase one level</td>
</tr>
<tr>
<td>50.1 – 80.0</td>
<td>Severely irritating</td>
<td>With 7 day mean ≤ 40 and individual total scores ≤ 30 in at least 60% of the rabbits with no total score &gt;60; otherwise, increase one level</td>
</tr>
<tr>
<td>80.1 – 100.0</td>
<td>Extremely irritating</td>
<td>With 7 day mean ≤ 80 and individual total scores ≤ 60 in at least 60% of the rabbits with no total score &gt;100; otherwise, increase one level</td>
</tr>
<tr>
<td>100.1 - 110</td>
<td>Maximally irritating</td>
<td>With 7 day mean &gt; 80 and individual total scores &gt; 60 in at least 60% of the rabbits; otherwise, decrease one level</td>
</tr>
</tbody>
</table>

*MMTS: Maximum Mean Total Score.
Source: Kay and Calandra (1962).

Table 2. Classification of system for skin reaction (OECD, 2015).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema formation</td>
<td></td>
</tr>
<tr>
<td>No erythema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight erythema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Well defined erythema</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe erythema</td>
<td>3</td>
</tr>
<tr>
<td>Severe erythema (beef redness) to eschar formation preventing grading of erythema</td>
<td>4</td>
</tr>
<tr>
<td>Edema formation</td>
<td></td>
</tr>
<tr>
<td>No oedema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight oedema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Slight oedema (edges of area well defined by definite raising)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate oedema (raised approximately 1 mm)</td>
<td>3</td>
</tr>
<tr>
<td>Severe oedema (raised more than 1 mm and extending beyond area of exposure)</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3. Dermal Irritation Scores ranges for classification of substances irritating effect on the skin (Draize et al., 1944).

<table>
<thead>
<tr>
<th>Ranges of dermal irritation score</th>
<th>Classification of dermal irritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 &lt; DIS &lt; 0.4</td>
<td>Not irritant</td>
</tr>
<tr>
<td>0.4 ≤ DIS &lt; 2.0</td>
<td>Slightly irritating</td>
</tr>
<tr>
<td>2.0 ≤ DIS &lt; 5.0</td>
<td>Moderately irritating</td>
</tr>
<tr>
<td>5.0 ≤ DIS ≤ 8.0</td>
<td>Severely irritating</td>
</tr>
</tbody>
</table>

The dermal irritation score (DIS) was calculated by the following formula:

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

Data analysis

The experimental results were presented as mean ± standard deviation SD (n = 5). Data were calculated separately for males and...
females. The statistical significance of difference between treated and control groups were analyzed by two-way analysis of variance (ANOVA) using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) followed by Bonferroni post test. Differences were considered to be statistically significant at $p < 0.05$.

**RESULTS**

**Acute toxicity studies**

The results of the experimental acute oral toxicity studies indicate that the CSEO single oral dose of 2000 mg/kg b.w did not show any remarkable behavioural changes and mortality of mice in the first and second step. According to the acute toxic class method of OECD 423 guideline (OECD, 2001), the CSEO of the plant tested has a LD$_{50}$ value estimated to 5000 mg/kg b.w., and is classified to the $5^{th}$ toxicity class with.

**Sub-acute toxicity**

**General observations and mortality**

The essential oil of *C. shoehanths* was tested in male and female Wistar rats at doses of 50 and 500 mg/kg body weight. The rats received a daily oral administration of distilled water or CSEO at a dose of 50 or 500 mg/kg b.w. per day. During the study period (28 days), no behavioral symptoms of intoxication or mortality were observed in rats treated with CSEO.

**Effect of CSEO on body weight**

The means weekly body weight gains of control and daily treated rats with CSEO during 28 days are illustrated in Figures 1 and 2. Globally, both treated and control rats were shown an increase in the body weight during the 28
days of study. However, a significant reduction in weight gain was observed in both male and female rats treated with CSEO at dose of 500 mg/kg when compared to the control rats. Figures 1 and 2 represent respectively male and female rats body weight gain from the start to the end of the study, recorded weekly. Body weight gain was obtained from the difference between current and precedent weekly measures. The means (histogram) and standard deviation (error bars) of five animals (n = 5) were calculated, and comparison between values of control and test groups ones allowed to establish statistical difference for p value < 0.05.

**Effect on water and food consumption**

The results of daily water intake are presented in Table 5. The averages water consumption of treated groups was found to be similar to the control groups. However, a non-significant increase in water consumption during the 14 first days of study was observed at dose of 50 mg/kg b.w.

Table 6 summarizes the mean weekly food consumption of the different groups during study period (28 days). As shown in this table, the food consumption of treated groups was found to be unaffected by the treatment as there were no significant changes in the average food consumption when compared with the control groups.

**Macroscopic analysis and effect of CSEO on relative organ weight**

Macroscopic examination of vital organs such as liver, kidney, heart, lung, and spleen of control and treated animals with CSEO showed that extract does not affect vital organs as there was no change in color and aspect of different organs.

Table 4 presents the mean relative organ weights of liver, kidneys, heart, lungs and spleen of control and treated rats. This result shows that there were no significant changes between different values of treatment and control groups (p > 0.05).

**Effect of CSEO on biochemical parameters**

Results of the various biochemical tests on the experimentally treated animals with the CSEO and control group are summarized in Table 5. As shown in this table, the oral administration of the plant extract at doses of 50 and 500 mg/kg b.w. on rats did not cause significant changes in serum biochemical parameters such as alanine aminotransferase, aspartate aminotransferase, creatinine, fasting blood glucose, total protein and total cholesterol levels when compared to control group.

**Acute eye irritation of CSEO**

The results of primary eye irritation scores of rabbits after treatment with CSEO indicate that rabbits’ eyes treatment with extract does not cause any ocular irritation or lesion. This result indicates that CSEO is non-irritative on rabbits eye according to classification in the system of Kay and Calandra (1962).

**Acute dermal irritation of CSEO**

Rabbits showed minor signs of skin irritation after patch removal and within 72 h of the study. However, any signs of edema were not observed during the observation
Table 5. Biochemical parameters of control and treated rats with CSEO for 28 days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>Dose (mg/kg b.w.)</th>
<th>0</th>
<th>50</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>M</td>
<td></td>
<td>50.00±10.32</td>
<td>68.80±10.69</td>
<td>70.60±8.65</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>60.20±11.23</td>
<td>77.80±14.94</td>
<td>78.00±10.80</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>M</td>
<td></td>
<td>54.20±13.50</td>
<td>53.20±7.50</td>
<td>54.80±11.45</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>62.40±13.56</td>
<td>57.60±12.88</td>
<td>60.75±9.71</td>
</tr>
<tr>
<td>Creatinin (µmol/L)</td>
<td>M</td>
<td></td>
<td>57.20±8.32</td>
<td>56.00±3.00</td>
<td>56.00±5.24</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>60±9.51</td>
<td>58.80±7.40</td>
<td>58.50±3.00</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>M</td>
<td></td>
<td>5.08±1.61</td>
<td>5.60±2.19</td>
<td>5.32±0.85</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>4.64±0.84</td>
<td>4.40±0.97</td>
<td>5.45±0.45</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>M</td>
<td></td>
<td>61.20±3.70</td>
<td>64.00±4.77</td>
<td>65.80±3.19</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>66.40±0.55</td>
<td>66.60±6.56</td>
<td>65.00±3.92</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>M</td>
<td></td>
<td>1.44±0.30</td>
<td>1.50±0.20</td>
<td>1.38±0.26</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>0.86±0.17</td>
<td>1.02±0.25</td>
<td>1.08±0.36</td>
</tr>
</tbody>
</table>

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

Table 6. Score of irritation and edema after application of CSEO.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Flanks</th>
<th>24 h</th>
<th>72 h</th>
<th>Total (24+72 H)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Erythema</td>
<td>Edema</td>
<td>Erythema</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

period. The individual scores for erythema and edema are presented in Table 6.

The dermal irritation score for CSEO found was “0.16”. CSEO is then classified not irritant for skin according to scale proposed by Draize et al. (1944).

**DISCUSSION**

Essential oils of *Cymbopogon* species are renowned for their flavours, fragrances, cosmetics, perfumery, soaps, detergents and pharmaceutical properties (Ganjewala et al., 2008), giving them immense medicinal and economic potentials and various purpose uses. Specifically, several works have highlighted the insecticidal effects of *C. schoenanthus* essential oils which displays mortality against adult mosquitoes *An. gambiae* and its larvae (Nonviho et al., 2010; Musa et al., 2014), as well as the lepidoptera *P. xylostella* (Laba et al., 2012). Recently, a *C. schoenanthus* essential oils-based insecticide formulation has proved effective against *S. zeamais* Motsch and *R. dominica* F., two pests damaging corn seeds during their conservation (Sawadogo et al., 2018). These results suggest that the *C. schoenanthus* essential oils based-natural insecticides can be considered as an alternative to chemical insecticides for post-harvest preservation of grains such as corn.

In the present study, at the limit single oral dose of 2000 mg/kg b.w., the CSEO did not induce remarkable behavioural changes or mortality of mice during both the first and second steps of the test. According to the acute toxic class method of OECD guideline, the CSEO tested is classified into the 5th toxicity class with a LD50 value estimated at 5000 mg/kg b.w. CSEO are therefore...
classified as products unlikely to present acute hazard under the right conditions (WHO, 2010; ONU, 2017). We have not found study on the acute toxicity of CSEO. However, some findings report that geraniol, one of CSEO constituents, has an oral LD₅₀ in rats of 3600 mg/kg. Similarly, geraniol, another constituent, has an oral LD₅₀ in rats of 4960 mg/kg (Katiki et al., 2012). Our results are consistent with previous findings reported absence of abnormal sign and symptoms observed in test animals upon acute toxicity study of essential oil of other Cymbopogon species as C. citratus. Indeed, Costa et al. (2011) showed that essential oils of C. citratus did not present any extensive toxic effect in mice, since its oral LD₅₀ was around 3.5 g/kg b.w.

Observations during the study period revealed that none of both test and control animals showed any clinical signs of toxicity. A steady increase in the body weight was observed in both treated and control animal groups. However, there was a decrease in body weight gain in control and test animals over time. In male rats, the decrease in body weight gain was significant during the first two weeks at the dose of 500 mg/kg b.w. In females, the decrease is significant at the dose of 500 mg/mkg b.w. during the first, third and fourth weeks. Loss of body weight is an important marker of gross toxicity which drastic toxicity or interference with absorption of nutrients will be reflected in body weight reduction (Banerjee et al., 2013). Both plant extracts (Nanti et al., 2018; Ilboudo et al., 2019) and essential oils (Batubara et al., 2015) are shown to induce body weight decrease in test animals. Macroscopic examination of vital organs such as heart, lung, liver, kidney, and spleen of control and treated animals show that CSEO do not induce change in colour and aspect of the different organs. In addition, no significant differences were found in the relative organ weights of the treated animals in comparison with the control groups. The subacute oral treatment with CSEO did not appear to adversely affect the vital organ of rats. The results suggest a possible absence of extract-related organs toxicity as organ weight evaluation is an essential part of the chemicals toxicologic and risk assessment (Michael et al., 2007).

The analysis of biochemical helped to notice absence of significant change in blood chemical parameters in treated groups when compared to control with CSEO, proving that our product does not appear toxic. However, a non-significant increase was observed in serum ALT and AST of both male and female treated animals, comparatively to the control ones. These changes are not so important to presume a toxic effect of the oils on the organs studied. Also, previous studies have not reported organotoxic effects related to the plant. On the contrary, daily oral treatment with 1 ml of C. schoenanthus extract significantly corrected the incidence of nephrotoxicity, BUN, creatinine, and calcium level differences (Saeed et al., 2007). On the other hand, the 21-day treatment with C. citrus essential oils did not induce abnormalities in serum levels of aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase (GGT), urea, albumin, creatinine, total protein or triglycerides of mice. Relative to the control group, the authors noted a significant reduction in serum total cholesterol after the administration of 100 mg/kg b.w. of C. citratus essential oils (Costa et al., 2011). The results confirm absence of organ toxicity, but further investigation such as anatomopathological analysis are required to reinforce the current conclusions.

Eye irritation test was performed in compliance with international standards as OECD Test Guideline 405. Eye irritation test measures the production of changes in the eye following the application of a test substance to the anterior surface of the eye of rabbits, which are followed for reversibility for 21 days after application (OECD, 2017). As part of this study, the rabbit exposed to CSEO did not display any ocular irritation or lesion. This result indicates that CSEO is non-irritative on rabbit eye according to classification in the system of Kay and Calandra (1962). Although eye irritation is reported to be one of the most common adverse events induced by essential oils (Babar et al., 2015), CSEO are relatively safe for eye.

The results of skin irritation test indicate the absence of edema and erythema, which reflects the absence of irritation induced by CSEO. Interestingly, this absence of skin irritation is consistent with the reported action of CSEO against acute inflammation induced in animal model (Talaei et al., 2019). As inflammation is a major component of irritation reaction, the absence of skin irritation induced by the CSEO is a consistent result and translated a relative low risk for this product to induce skin damages.

**Conclusion**

The results show that the CSEO did not induce acute or subacute toxicity. Similarly, no eye or skin irritation was noted. The results suggest that CSEO would be safe in both acute and subacute exposure conditions. However, for further clinical relevance of the results and complete toxicological profile elucidation, toxicity studies must be extended to long term toxicity test as subchronic and chronic toxicities, reprotoxicity, carcinogenicity, teratogenicity, and mutagenicity investigations.

**CONFLICTS OF INTERESTS**

The authors have not declared any conflicts of interests.

**REFERENCES**

Aazza S, Lyoussi B, Miguel MG (2011). Antioxidant and


