Evaluation toxicologique de l'huile essentielle de *Cymbopogon citratus* Stapf en vue de son utilisation dans une formulation d'insecticide

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Résumé

Cymbopogon citratus Stapf. est une plante traditionnellement utilisée pour traiter la toux, la fièvre, la douleur, l'inflammation et comme répulsif contre les moustiques. Des études toxicologiques ont été menées sur les risques pour la santé humaine liés à l'utilisation des huiles essentielles (HE) de cette plante pour la formulation de biopesticides. La toxicité orale aiguë et la toxicité subaiguë de ces huiles ont été évaluées chez des rats Wistar, selon les lignes directrices de l'Organisation de coopération et de développement économique (OCDE). Le pouvoir irritant de ces huiles pour la peau et les yeux a été déterminé chez le lapin conformément aux lignes directrices de l'OCDE. Lors du test de toxicité orale aiguë, aucun changement lié au traitement n'a été observé. La dose létale 50 % (DL_{50}) a été estimée à 5 000 mg/kg, ce qui montre que l'HE de *Cymbopogon citratus* Stapf. sont peu susceptible de présenter un danger aigu. Dans le test de toxicité subaiguë, l'analyse biochimique a montré une augmentation de l'ALAT, la créatinine, les protéines totales et la glycémie à 500 et 1000 mg/kg chez les deux sexes. Les tests d'irritation ont montré que les huiles essentielles de la plante sont légèrement irritantes pour les yeux et la peau. En conclusion, les huiles essentielles de *C. citratus* S. sony peu susceptible de présenter un danger aigu par voie orale en administration aiguë mais une faible toxicité orale en administration subaiguë à des doses plus élevées. En outre, elles sont légèrement irritantes pour les yeux et la peau.

Mots clés : *Cymbopogon citratus,* rats Wistar, lapins, huile essentielle, toxicité, irritation.

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Toxicological assessment of the essential oil from *Cymbopogon citratus* Stapf for its use in insecticidal formulation

Abstract

Cymbopogon citratus Stapf. is an aromatic perennial plant traditionally used to treat coughs, fever, pain and inflammation, as well as a mosquito repellent. To determine the risks to human health associated with the use of essential oils (EO) from this plant for the formulation of biopesticides, toxicological studies were carried out. Acute and subacute oral toxicities of these oils were evaluated in Wistar rats, respectively, according to the guidelines 423 and 407 of the Organization for Economic Co-operation and Development (OECD). These oils' skin and eye irritancy were determined in rabbits following OECD guidelines 405 and 404. During acute oral toxicity testing, no treatment-related changes were observed during the study period. The 50% lethal dose (LD_{50}) was estimated to be 5,000 mg/kg, showing that EO was practically non-toxic. In the subacute toxicity test, biochemical analyses performed at the end of the study showed increased ALT, Creatinine, total protein and blood sugar at 500 and 1000 mg/kg in both sexes. The determination of the irritant powers showed that the plant's essential oils are slightly irritant to the eyes and the skin.

In conclusion, the plant's essential oils do not exhibit oral toxicity in acute administration but low oral toxicity in subacute administration at higher doses. In addition, they are slightly irritant to the eyes and the skin. Skin-to-mucous contact should be avoided when used in a biopesticide formulation.

Keywords: *Cymbopogon citratus,* Wistar rats, rabbits, essential oil, toxicity, irritation.

Introduction

Over time different substances, natural or synthetic origin, have been used in agriculture for crop improvement and in the health field (1). In effect, losses caused by pests can reach 46% in developing countries (2). Synthetic pesticides can be effective, but they can cause resistance in pests and negatively impact the environment and human health (3). This is what gave rise to the removal of some pesticides, such as DDT, chlordane, cyanazine and methyl bromide (4). Alternatives to synthetic pesticides are being explored. One of the approaches involves research into plant-derived substances.

Currently, the research is directed towards the essential oil plants (EO) that are used as biopesticides. Biopesticides cause a low impact on human health and the environment (5). EO showed its property pesticide in many studies against weeds (6, 7), insects (8-9, 9-10), and mushrooms (11,12).

In addition to their many traditional uses, their pharmacological and insecticidal properties, are capable of causing side effects. Several

studies have shown the toxicity of EOs on various species of rodents and humans (13-14)

This study focuses on assessing the toxicity of *Cymbopogon citratus* Stapf EO (Poaceae) (*C. citratus*) for use in the biopesticide formulation in Burkina Faso. It is an aromatic plant used in traditional medicine in many countries as an antitussive, antipyretic, analgesic, anti-inflammatory, antispasmodic, antimalarial and repellent against mosquitoes (15,16). Several studies have demonstrated insecticidal activity of EOs of *C. citratus* on *Aedes aegypti* L., *Culex quinquenfasciatus* Say and *Anopheles dirus* (17,18). However, the different effects and properties of EO may vary their compositions, which depend on various factors such as the environment, genotype, geographical origin, the place and time of harvest, part of the plant studied, the age of the plant, instead of drying temperature and drying time (19,20). To consider a common use of this EO as pesticides must ensure that their toxicological profiles are acceptable. The purpose of this study was to investigate the toxicity profile of EO oil from this plant, estimating its LD_{50}, the parameters of the subacute toxicity, skin and eye irritancy.

I. Material and methods

Test material

The fresh leaves of *C. citratus* were harvested in Bama (rural district of Burkina Faso). The leaves were dried in the shade for seven days. The dried leaves have been used for the extraction of essential oils. The extraction of EO was performed at “Institut de Recherche en Sciences Alimentaire et Technologique” (IRSAT) of “Centre National de la Recherche Scientifique et Technologique” (CNRST) in Ouagadougou. The EO was extracted by steam distillation of dried leaves by the method of steam distillation of water produced with a still of stainless steel (150 L) for 3H. It was a classic distillation process using a boiling still in which the plant material (40 kg) did not come into contact with the water. The steam carried the volatile aromatic compounds, which condensed in the condenser, and the distillate collected allowed the essential oil to be separated from the hydrolate by difference in density. The collected essential oils were left to rest in a funnel for one hour away from light in order to eliminate any traces of water. They were then stored in colored glass bottles and kept at 4 ° C in a refrigerator away from light until use (21).

Experimental animals

It consisted of rats of both genders and male rabbits.
Both male and female rats weighing 119 ± 14 g and 227 ± 30 g respectively from the animal facility of the "Institut de Recherche en Sciences de la Santé" (IRSS), Ouagadougou were used. The rats were maintained under standard conditions: 22 ± 3 °C, 60 ± 5% humidity and 12 hours light/12 hours dark with free access to water and food (flour wheat or enriched corn fish) throughout entire experimental period.
Albino rabbits New Zealanders weighing between 1800 - 1950 g were purchased from breeders in Ouagadougou. They were placed in the cages at room temperature. The rabbits were fed with corn bran and lettuce leaves and water at will. Before their use for the different tests, the rabbits were acclimatized to laboratory conditions for two weeks.
The experiments were conducted in accordance with the international standards established by the European Union for animal protection (CEC Council 86/609), validated by the Institute for Health Sciences Research (IRSS, Burkina Faso).

Acute toxicity test

Toxicity studies were conducted in the toxicology laboratory of MEPHATRA-PH, at IRSS.
The test of acute toxicity was carried out according to the method of guideline 423 of the OECD (22). To carry out the test of acute toxicity, the animals were divided into batches of 3 in plexiglass cages while taking care of a homogeneity of the body weights. The rats were examined to check the absence of pathology, then put at fast during 4H (deprivation of food but not of water). The test was carried out in two stages: A first stage where a batch of 3 rats received, EO with the amount of 2000 mg/kg of body weight and a pilot batch which received distilled water (1%). Water and EO were administered orally using intubation probe . The pilot rats and tests were kept with fast during two overtimes after the administration, then the food was restored. The test was taken again with the same amount, according to the same procedure with the second phase.
All the rats were observed during the first two hours following the administration, then with twice by days until the 14th day of the test. The observation related to the number of died to 24 H, 48 H and 72 H and the signs of toxicity in particular the modification of peeling, the locomotion, the tremors, breathing, the aspect of the saddles, mobility as well as mortality.

Subacute toxicity study

The study was led according to guideline 407 of OECD (23). The EO amounts were selected on the basis of result of the tests of acute toxicity.

The test was carried out on 4 batches of 10 rats (5 females and 5 males) of which a pilot batch. Batch 1, the rats daily received distilled water. Batches 2 - 4, they received amounts of respectively 100, 500 and 1000 mg/kg. Distilled water and the various amounts of EO were managed with the rats by cramming, once by day during 28 days. The rats were observed twice a day, at the beginning and the end of the day in order to detect the symptoms of toxicity. In addition to mortality, the raised symptoms were inter alia: changes affecting the skin, the fur, the eyes, the frequency of secretions and the excretions, the secretion of tears, abnormal breathing. The water consumption was measured each day and that of food each week. The rats were weighed every seven days. The bodies (kidneys, liver, heart, lungs, spleen) were taken and weighed at the end of the test after a macroscopic examination. The relative weight of each organ (ROW) was calculated according to the following formula:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)} X 100}{\text{Body weight of the rat on sacrifice day (g)}}$$

At the end of the treatment, the rats were put on an empty stomach during 16 H, after administration of the ketamine, at a rate of 150 mg/kg of bw, the blood were collected by cardiac puncture. The blood of each rat was taken in a tube without anticoagulant, in order to proportion the biochemical parameters. The samples of blood contained in the tubes without anticoagulant were centrifuged with 3000 turns/min during 10 min. The serums collected were preserved at -20°C, and were used to quantify the biochemical parameters following: Cholesterol, the glycemia and the total proteins were proportioned by the colorimetric method; alanine aminotransferase by kinetic method UV IFCC optimized, by using kit GPT-ALT SPINREACT and the aspartate aminotransferase by kinetic method UV IFCC optimized, by using kit GOT-AST SPINREACT, creatinine by the colorimetric method of Jaffé, using the kit creatinine SPINREACT. A semi-automatic spectrophotometer (Mindray BA-88A) was used for the proportioning of the biochemical parameters.

Ocular test of irritation

The study was led according to guiding line 405 of OECD (24). The test was carried out on three rabbits. EO with the amount of 0.1 ml were introduced into the conjunctival cul-de-sac of one of the two eyes of rabbit, after having delicately drawn aside the lower eyelid of the ocular sphere. The two eyelids were brought back then delicately one against

the other and were maintained in this position during approximately 30 second in order to avoid any loss of substance. The eye untreated was used as witness. The score was evaluated by dimensioning the gravity of the lesions affecting the conjunctivitis, the cornea and the iris, with given time intervals (see appendix 3). In addition to the ocular lesions, the clinical signs such as the excessive cillement one and the excessive whimpering were evaluated. The eyes were the subject of complete examination to locate possible ocular lesions one hour after the application of EO, then this procedure was repeated at least once per day in D1, D2, D3, D4, D7 and D21.The scores of the ocular lesions were given after an evaluation to the natural light and under a lamp UV in the presence of fluorescein to 1H, 24H, 48H and 72H. The classification of EO was made, according to the modified interpretation of irritations of the eyes of Draize (34-36). It is based on the Maximum Average Total Score (MATS).

Test of cutaneous irritation

The study of cutaneous irritation was undertaken according to Guideline 404 of OECD (25). The test was carried out on three rabbits. Approximately 24 hours before the test, the dorsal area of the side of the animals was mowed with short-nap cloth. A volume of 0.5 ml of the EO of the plant was then applied to the skin of one of the sides of the rabbit (approximately 6 cm^2 of the skin) and covered by a compress with gauze, maintained in place by means of an adhesive plaster not irritating. The other side of the skin of rabbit was used as witness. Precautions were taken to prevent the rabbit from having access to the compress during the period of exposure of 4H the observation of the signs of erythema and of edema was made at the end of 4H, 24H, 48H, and 72H after the removal of the stamp, in all rabbits. The observations continued up to 14 days for the reversibility of the effects. The erythema and the edema were used to make a quotation of the scores of cutaneous irritations. In addition to the observation of the symptoms of irritation, the observations also related to all the local toxic effects. To classify EO we have calculated the score of dermic irritation (DIS), by the modified method of Draize. It is done according to the relation:

$$\mathrm{DIS} = \frac{Value\ (erythema + oedema)}{Number\ of\ animals\ X\ number\ of\ \text{à}bservtion}$$

Statistical analysis

The data were analyzed using the Software Graph Pad PRISM. 5. The results were expressed as means ± standard deviations (SD) separately

for the females and the males, and represented in the form of graphs and tables. The statistical study of the difference between the treated batches and the pilot batches was made using a variance analysis (ANOVA, One-way analysis off variance). The differences were considered significant with the threshold in probability of 5% ($p < 0.05$).

II. Results

Acute Oral Toxicity of essential oils of *C. citratus*

The administration of a single amount of 2000 mg/kg of body weight of the EO of *C. cictratus* did not involve remarkable behavioral change nor mortality of the rats. LD_{50} of this oil was estimated to be 5000 mg/kg, according to the OECD test guideline.

Subacute toxicity of essential oils of *C. citratus*

Toxidrome

The amounts of 100; 500 and 1000 mg/kg bw of the EO of *C. citratus* did not cause toxic demonstrations in the rats.

Mortality

There was no mortality in the various groups of rats treated with the EO of *C. citratus.*

Weight change

Figure 1 illustrates the weekly ponderal evolution in the groups of male and female rats having daily received water distilled (pilot) or treated with the EO of *C. citratus*. The *C. citratus* EO resulted in a significant decrease in average body weights in the group of male rats.

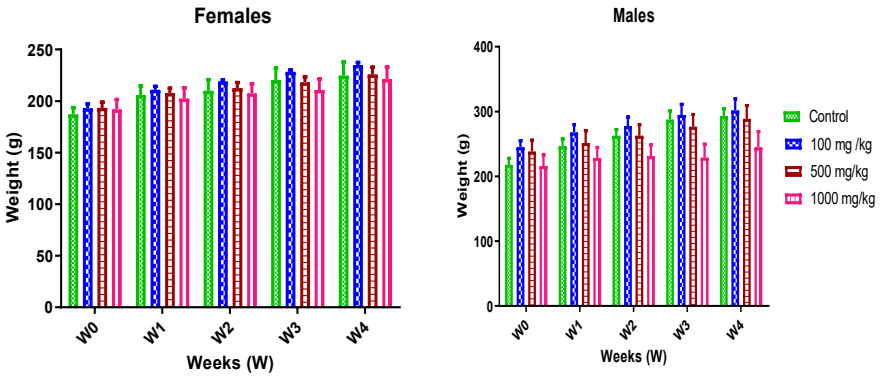

Figure 1: Ponderal evolution of the rats pilot and treated with various EO amounts of *C. citratus*. The results are expressed as means ± standard deviations (n= 5).

Consumption of water and food

The results of daily food and water consumption are presented in Table I. These results show that daily administration of *C. citratus* essential oil did not significantly alter water or food consumption in treated rats compared with controls.

Table I: Average daily consumption of water (in mL/day/rat) and food (g/day/rat) of the pilot rats and those treated with EO of *C. citratus* during 28 days.

Treatment	Gender	Water /food	Weeks			
			Week 1	Week 2	Week 3	Week 4
Control	M	Water	52.00 ± 6.19	45.00 ± 6,22	54.00 ± 4.00	57.00 ± 4.20
		Food	24.94	26.97	26.20	24.80
	F	Water	43.57 ± 4.47	37.47 ± 5.29	37.71 ±6.18	41.71 ± 5.94
		Food	19.14	21.14	17.37	17.71
100	M	Water	55.00 ± 5.80	44.14 ± 6.84	54.71 ± 4.42	54.29 ± 4.07
		Food	23.14	23.91	22.60	21.51
	F	Water	40.00 ± 5.51	37.00 ± 4.12	39.29 ± 3.20	39.14 ± 5.24
		Food	19.83	21.63	18.51	20.31
500	M	Water	46.86 ± 2.48	39.43 ± 4.35	49.00 ± 3.32	50.57 ± 4.96
		Food	21.60	24.49	21.23	21.09
	F	Water	39.71 ± 3.40	35.43 ± 2.37	41.00 ± 4.24	41.00 ± 3.83
		Food	19.80	20.60	18.11	18.43
1000	M	Water	49.00 ± 3.10	42.57 ± 3.36	48.86 ± 4.56	50.71 ± 5.91
		Food	19.51	19.97	18.23	19.11
	F	Water	38.14 ± 2.34	35.71 ± 2.63	39.14 ± 7.56	40.29 ± 6.13
		Food	16.91	18.97	17.31	18.11

The data are expressed in the form of means ± standard deviation; n=5; M: males; F: females

Effect of the EO of *C. citratus* on the relative weight of the organs of the rats

The macroscopic examination of vital organs such as the heart, the lungs, the liver, the kidneys and spleen of the rats treated with the EO of *C. citratus* did not reveal of change of color or aspect of the various organs.

The relative average weights of the organs of the rats treated with the EO of *C. citratus* are indicated in table II. The EO did not induce a significant change of the relative weight of the bodies of the rats treated compared to the witnesses ($p > 0.05$).

Table II: Average relative weight (in g) of the organs of the pilot rats and those treated with EO of *C. citratus* daily during 28 days.

Organs	Gender	Control	Amounts		
			100 mg/kg	500 mg/kg	1000mg/kg
Heart	F	0.43 ± 0.09	0.39 ± 0.03	0.46 ± 0.02	0.39 ± 0.05
	M	0.33 ± 0.04	0.35 ± 0.04	0.37 ± 0.05	0.37 ± 0.09
Lungs	F	0.64 ± 0.10	0.56 ± 0.03	0,62 ± 0.08	0.61 ± 0.07
	M	0.52 ± 0.05	0.52 ± 0.03	0.48 ± 0.02	0.53 ± 0.08
Liver	F	3.18 ± 0.40	3.25 ± 0.36	3.51 ± 0.26	3.69 ± 0.21
	M	2.69 ± 0.51	2.96 ± 0.33	2.87 ± 0.65	3.49 ± 0.69
Kidneys	F	0.69 ± 0.06	0.68 ± 0.07	0.71 ± 0.06	0.71 ± 0.06
	M	0.67 ± 0.09	0.65 ± 0.06	0.68 ± 0.05	0.83 ± 0.29
Misses	F	0.28 ± 0.06	0.24 ± 0.02	0.30 ± 0.03	0.30 ± 0.06
	M	0.23 ± 0.03	0.25 ± 0.05	0.28 ± 0.06	0.26 ± 0.09

The data are presented in the form of means ± standard deviations. F: female; M: male.

Effects of essential oils on the biochemical parameters in the rats

The results of the analysis of the biochemical parameters of the pilot rats and those treated with the EO of *C. citratus* are presented in table III.

Oral administration of *C. citratus* EO at various doses to rats for 28 consecutive days resulted in significant increases in alanine aminotransferase (at 1000 mg/kg bw in females and males), creatinine (at 500 and 1000 mg/kg bw in females and males) and blood glucose (at 500 mg/kg in females), compared with control batches ($p < 0.05$). The other parameters (total protein, total cholesterol and aspartate aminotransferase) were not significantly altered.

Table III: Biochemical parameters of the pilot rats and those treated during 28 days with the EO of *C. Citratus*.

Parameters	Gender	Control	Treatment		
			100 mg/kg	500 mg/kg	1000 mg/kg
ALT (UI/L)	F	38.60 ± 1.82	37.20 ± 2.28	41.20 ± 3.63	45.80 ± 1.64***
	M	37.60 ± 3.72	34.20 ± 4.32	42.80 ± 4.97	47.20 ± 2.59**
AST (UI/L)	F	122.80 ± 8.47	116.60 ± 12.82	111.20 ± 17.06	108.09 ± 9.54
	M	113.60 ± 18.77	111.40 ± 16.83	105.40 ± 13.90	99.40 ± 11.28
TP (g/L)	F	69.24 ± 4.22	62.26 ± 4.30	64.58 ± 6.28	63.86 ± 2.72
	M	54.70 ± 4.64	59.52 ± 2.96	57.88 ± 4.14	55.68 ± 2.88
CREAT (µmol/L)	F	59.60 ± 5.41	55.28 ± 0.99	68.48 ± 1.88**	65.04 ± 2.43*
	M	53.40 ± 5.86	55.92 ± 6.15	64.84 ± 4.05*	64.42 ± 5.96*
GlY (mmol/L)	F	6.32 ± 1.49	6.19 ± 0.68	8.83 ± 1.83*	9.10 ± 1.05*
	M	6.77 ± 2.22	6.97 ± 0.40	8.83 ± 2.92	8.02 ± 1.53
TC (mmol/L)	F	1.58± 0.72	2.32 ± 0.31	1.75 ± 0.64	1.60 ± 0.43
	M	1.10 ± 0.32	1.69 ± 0.27	1.67 ± 0.42	1.47 ± 0.59

The data are presented in the form of means ± standard deviations; ALT = Alanine aminotransferase; AST= Aspartate aminotransferase; TP= total Proteins; CREAT= Creatinin; GlY = Glycemia; TC = total cholesterol; F = female; M = male; * = significant difference with $p < 0.05$; ** = significant difference with $p < 0.01$; *** = significant difference with $p < 0.001$.

Ocular irritant power of the essential oil of *C. citratus* in rabbits

Application of *C. citratus* EO to the eyes of rabbits caused corneal opacity, iris reactivity to light, with blood vessels difficult to distinguish from one another, and discharges during the first 72 hours. Scoring these observations gave a Maximum Mean Total Score (MMTS) of 18.33 for the EO of *C. citratus*. This score indicates that *C.* citrtus EO is mildly irritant, according to Draize's modified classification rule for

eye irritants. Other eye reactions were not observed. The results of the eye irritation scores are shown in Table IV.

Table IV: Scores of ocular irritations of the EO of *C. citratus*

	Rabbit N° 1				Rabbit N° 2				Rabbit N° 3			
	Duration (Hours)											
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A. Degree of opacity	0	0	1	1	0	1	1	1	0	1	1	1
B. Opacity surface	0	0	1	1	0	1	1	1	0	1	1	1
A × B× 5 (≤ 80)	0	0	5	5	0	5	5	5	0	5	5	5
II. Iris												
A. Values	0	0	1	0	0	0	0	0	0	0	1	0
A × 5 (≤ 10)	0	0	5	0	0	0	0	0	0	0	5	0
III. conjunctive												
A. Reddening	1	1	2	1	1	2	2	1	1	1	2	1
B. Chemosis	1	1	1	2	1	1	1	1	1	1	1	2
C. Flow	1	1	2	0	1	2	2	1	1	1	2	0
(A + B + C) × 2 (≤ 20)	6	6	10	6	6	10	10	6	6	6	10	6
Total	6	6	20	11	6	15	15	11	6	11	20	11
MMTS	**18.33**											
	15.1 < 1.33 < 25.0											
Conclusion	**Slightly irritant**											

Dermal irritancy testing of *C. citratus* essential oils in rabbits

Application of *C. citratus* essential oils to rabbit skin caused skin lesions.

At 24 h, *C. citratus* EO caused well-defined erythema and very slight edema. At 72 h, very slight erythema was observed. The DIS for this EO was 0.92, classifying *C. citratus* EO as a mild skin irritant. Table V shows the results of skin irritation scores 24 and 72 hours after application of *C. citratus* EO.

Table V: Scores of dermic irritation after application of EO of *C. citratus in rabbits*

		Duration				
		24 hours		72 hours		
Rabbit Nº	Side	Erythema	Edema	Erythema	Edema	Total (24 + 72 H) Erythema + Edema
1	Control	0	0	0	0	0
	Treated	2	2	1	0	5
2	Control	0	0	0	0	0
	Treated	2	1	0	0	3
3	Control	0	0	0	0	0
	Treated	2	1	0	0	3
Total (T)						**11**
DIS = T/12 = 11/12 = 0.92				**0.4 < 0.92 < 2.0**		
Conclusion :				**Slightly irritant**		

DIS = Dermic Irritation Score; H = hour

III. Discussion

For the safety of use of *C. citratus* EO entering the formulation of biopesticides in Burkina Faso, our experimental study evaluated acute, subacute toxicity and the ocular and cutaneous irritant power of the EO of the sheets of this plant *in vivo* in rats and rabbits.

The results of this study showed that the *C. citratus* EO is tolerated by oral administration with a dose of 2000 mg/kg in rats. LD_{50} of this oil was estimated at 5000 mg/kg. According to the system of classification overall harmonized and the United Nations (21,22), *C. citratus* EO tested could be classified in the 5th category of substances without acute danger. Other studies, have reported the LD_{50} of the EO of *C. citratus* to be 3250 and 3500 mg/kg bw in the rat and the Swiss male mouse, respectively (18). The LD_{50} of citral and β- myrcene, the mean components of the EO of *C. citratus* was reported to be higher than 125 mg/kg and 1200 mg/kg. These variations of the DL_{50} can be related to the chemical composition of EO depending on the place and the season of harvest (19,20).

During our study, the daily administration of the EO of *C. citratus* did not involve any statistically significant difference in the ponderal growth of the rats treated compared to the pilot rats. For the 28 days period, the EO of *C. citratus*, involved a reduction not statistically significant in the water consumption in the batches treated compared to the pilot batches. Concerning the relative weight of the organs, statistically significant differences were not found between the pilot batch and those treated with the various amounts of EO of *C. citratus*.

In addition to the relative organ weights, biochemical parameters of rats indicate the physiological state. The rise and fall of biochemical parameters can indicate the toxicity of specific organs (26).

In this study, biochemical analyzes showed significant increases respectively ($p < 0.05$) in ALT, Creatinine, total protein and blood glucose at a dose of 500 and 1000 mg / kg in both gender.

Transaminase or amino-transferases are enzymes catalyzing the tissue transport of radicals alpha-amino alanine and aspartic acid at the alpha-ketoglutaric acid. Transaminases are present in the liver, but also in muscle and AST in the kidney, pancreas, and other tissues. They are synthesized in the cytoplasm of the cells of these organs and are unloaded into traffic, when these cells are damaged. These enzymes increase in myopathy, rhabdomyolysis or myocardial infarction and AST, particularly in cases of hemolysis. ALT is more specific to liver damage, but the AST are slightly more sensitive (27). The *C. citratus* EO at a dose of 1000 mg / kg p.c probably caused liver damage. Usually for plants extracts would cause hepatocellular injury, cholestatic or

necrotic cirrhosique (28). In addition, studies have shown that d-limonene, citrala (geranial) and citralb (neral) which are components of the EO of *C. citratus* may be responsible for hepatotoxicity (29,30). Furthermore, transaminases and total protein (TP) provide information on the physiological condition of the liver. TPs are globular proteins; there are biosynthesis is the liver. They consist mainly of globulin and albumin. The latter is the most important protein in the blood plasma and a useful indicator of liver function. A low rate of synthesis by the liver, of distribution or biotransformation volume may decrease the rate of albumin (31). This decrease was not observed between the treated and control groups.

Another parameter, Creatinine is a breakdown product of creatine. It is located at 98% in muscle. The increase in serum creatinine reflects a decrease in the glomerular filtration rate therefore renal impairment (32). In the study, the EO of *C. citratus* at a dose of 500 and 1000 mg/kg bw in both sexes, resulted in a significant increase of Creatinine ($p < 0.05$). This dose-dependent increase would cause harmful effects on the kidneys. Given that studies on the β-myrcene and d-limonene, acyclic monoterpene present in the EO of *C. citratus* caused hypertrophy of the kidney, associated with histological lesion of renal glomeruli (33).

In the study C. *citratus* EO involved a statistically significant increase in the glycemia ($p < 0.05$). The animal cells reserve the glycose in the form of glycogen. Its degradation and its synthesis utilize many proteins. The role of these proteins, is the inhibition of glycogénolyse and the activation of the glycogénogenèse. This high blood glucose level could be due toa stimulation of the receivers of glucagon or certain receivers adrenergic or a fall of insulin (34).

The measure of eye irritation test the effect of changes on consecutive eyes to the application of a test substance to the anterior surface of the eye of the rabbit, followed by their reversibility during 21 days after application (24). In this study, rabbits exposed to *C. citratus* EO showed mild irritation. This eye irritation EO is one of their most common side effects (35), but in a similar study, the EO of *Cymbopogon shoenanthus* L. did not cause eye damage.

The result of skin irritation test showed the presence of signs of edema and erythema with *C. citratus* EO. A similar study showed severe irritation of the skin by the EO of *C. citratus*. In addition, the EO of *C. citratus* used in the formulation of a cream caused a low skin irritation (33). These results show that the EO of *C. citratus* is slightly irritating to eyes and skin.

Conclusion

The work focused on the evaluation of acute, subacute, and in addition to the determination of eye irritancy and skin of the EO of *C. citratus*. The EO administered orally at a dose of 2000 mg/kg, showed that it is unlikely to present an acute hazard via no toxicity. In subacute toxicity assessment, *C. citratus* EO did not result in a change in the weight growth, the consumption of water and food. Also, it did not induce a change in the relative weight of organs. However, in daily administration for 28 days in rats orally we observed an increase in ALT and Creatinine. In the local application in the eye and skin irritation was mild. Given these results, *C. citratus* EO could be used as an active material for the biopesticide formulation in the field of agriculture against insects, weeds and during grain storage against fungi. Occupational safety measures should be established for the applicant. This study is a contribution to the formulation of biopesticides after other toxicity tests (chronic, mutagenicity, ecotoxicity).

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