

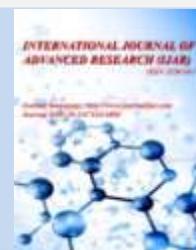


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RESEARCH ARTICLE

ASSESSMENT OF ORAL TOXICITY, SKIN AND EYE IRRITATION OF CYMBOPOGON NARDUS (L.) RENDLE (POACEAE) ESSENTIAL OIL FROM BURKINA FASO

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Abstract

Cymbopogon nardus (L.) Rendle (Poaceae) (*C. nardus*) still known as Ceylon citronella is an aromatic plant imported from India, Sri Lanka and introduced to the rest of the world including Africa. In Burkina Faso, the essential oil (EO) of this plant is used to formulate biopesticides. The objectives of this study consisted of evaluating the toxicological profile of the EO of *C. nardus* for its safe use. Acute and sub-acute oral toxicity was assessed on Wistar rats and skin, and eye irritant power was determined on rabbits according to the Organization for Economic Cooperation and Development (OECD) guidelines. In Acute oral toxicity test, a single administration of EO at a dose of 2000 mg/kg.b.w. to rats did not result in mortality or sign of toxicity. The LD50 of this EO is estimated to 5000 mg/kg.b.w. The 28 days sub-acute toxicity study with doses of 100, 500 and 1000 mg/kg.b.w of EO did not show mortality in both sexes. Furthermore, there was no significant difference observed in body weight gain, relative organ weights, food and water consumption in test groups compared to control ones. However, there was a significant increase in AST, creatinine levels at a dose of 1000 mg/kg.b.w. and total protein at tested doses in both sexes. The skin and eye irritation studies on rabbit show that the EO had a very weak irritant power on the skin and the eye. These results suggested that *C. nardus* EO can constitute an alternative to synthetic pesticides and used in biopesticides formulation.

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Introduction:-

Synthetic pesticides are a heterogeneous group of chemicals used against plants and pests (insects, rodents, mould, weeds, etc.) (Cherin et al., 2012). They are used in rural areas to fight against crop pests, but also to preserve foodstuffs such as millet, corn, rice, cowpeas, etc. (Waongo et al., 2013; Zongo et al., 2015). However, nowadays, these chemical pesticides are a source of problems because they are responsible for acute and chronic intoxication with harmful effects on the health of populations and their environment (Blanc-Lapierre et al., 2012; Cherin et al., 2012; Ilboudo et al., 2014; Multigner, 2005). According to the World Health Organization (WHO) in 2004, there are between one to five million poisonings of chemical pesticides each year worldwide, which are responsible for several thousand deaths per year (OMS, 2004). Face to these public health and environmental problems linked to synthetic pesticides; research is underway to formulate pesticides that are more effective against pests and less harmful to humans and the environment. Biopesticides are presented as an alternative to synthetic pesticides. Biopesticides are living organisms or products from these organisms with the particularity of suppressing or limiting the proliferation of pests. These pesticides of natural origin are distinguished in three main categories according to their microbial, plant or animal origin (Deravel et al., 2014).

Cymbopogon nardus (L.) Rendle (*C. nardus*) or Ceylon citronella is a plant native to southern India and Sri Lanka and introduced to other countries including Burkina Faso. It is a herbaceous plant of the Poaceae family which is commonly cultivated. It is perennial, green, forming dense tufts of 1 to 1.8 m in high (Prelude medicinal plants database, 2019). In Burkina Faso, the essential oil (EO) of this plant is used to formulate biopesticides. A study carried out in Ivory Coast, showed that the EO of *C. nardus* contains molecules such as citronellol, limonene, with the citronellal as the majority chemical molecule (42.20%) (Kanko et al., 2017). Studies carried out showed the insecticidal properties of *C. nardus* EO. In India, a study carried out have shown the effectiveness of this EO on some fungi which contaminate cereals and legumes (Kroet al., 2017). In Morocco, another study showed the antimicrobial efficacy of *C. nardus* EO on bacteria responsible for nosocomial infections (Kamari et al., 2018). There are several studies showing the pesticidal properties of the EO of *C. nardus*, but few authors have been interested in studying its toxicological profile.

The aim of this study was, therefore, to study the toxicological profile of *C. nardus* EO. It was more specifically a question of determining its acute and subacute oral toxicity and its skin and eye irritancy power in order to provide additional data on the safety of users of this plant EO.

Material And Methods:-**Plant material:**

Fresh leaves of *C. nardus* were collected from the botanical garden of the "Institut de Recherche en Sciences Appliquées et Technologies" (IRSAT), Bama, Burkina Faso (Latitude Nord 11 22' 00", Longitude Ouest 4 25' 00"). After collection, the plant sample was identified and authenticated at "Herbier National du Burkina (HNBU)" of the "Centre National de Recherche Scientifique et Technologique (CNRST)", Ouagadougou (Burkina Faso). The fresh leaves were washed with tap water and kept according to good conservation practices and used later for the extraction of EO.

Essential oil:

A portion of fresh leaves (40 kg) was submitted to hydrodistillation using an alembic/Clevenger-type apparatus for 3 h. After that, anhydrous sodium sulfate was used to remove water from the extract. The essential oil obtained was stored in an airtight container in a refrigerator at 4°C until toxicological tests (Bassolé et al., 2018).

Animals:

Healthy female and male Wistar rats weighing respectively between 110-140 g and 127-140 g were used for acute and sub-acute toxicity studies. They were provided from the pet shop of the "Institut de Recherche en Science de la Santé" (IRSS), Ouagadougou, Burkina Faso. They were housed in the animal cage with free access to water and standard laboratory pellet enriched with protein (29%). All animals were kept in laboratory conditions (temperature room of 22°C (± 3) with a humidity rate of 60% (± 5) and 12 h light/dark cycle).

Healthy female and male rabbits weighing between 1.67-2.09 kg were used for eyes and skin irritation tests. They were procured from breeders in the city of Ouagadougou and acclimatized during two weeks in a ventilated room with free access to water and standard granula before use.

The experimental protocol was carried out following to the international standard protocols [Guidelines set by the European Union on the Protection of Animals (CEC Council 86/609)] and adopted by IRSS, Burkina Faso (Ilboudo et al., 2019; Konaté et al., 2016).

Acute orale toxicity test:

The acute oral toxicity test of *C. nardus* EO was performed on female rats according to the Organization for Economic Cooperation and Development (OECD) test guideline 423 (OCDE, 2001). After a fasting period (4 h of starvation only), *C. nardus*, was administered orally by gavage in a single dose to the rats according to the sequential procedure. In the conducting of the test, the starting dose of 2000 mg/kg b.w. of EO was administered to treated group. A control group received a single dose of distilled water orally. All animals were observed during the first 2 hours post-treatment before the restoration of food. They were then observed at least once a day for two weeks. The mortality and signs of toxicity such as changes in skin and fur, eyes, mucus membranes, salivation, convulsion, diarrhoea, lethargy, sleep and coma were notified during this observation period.

Sub-acute oral toxicity test:

The sub-acute oral toxicity study of *C. nardus* EO was performed in accordance with OECD guideline 407 (OCDE, 2008). A total of 40 Wistar rats of both sexes were randomly selected for the sub-acute toxicity studies. The rats were divided into 4 groups of 10 animals each (5 per sex). Males and females rats were kept separately in cages. The control group (group 1), received distilled water. The others groups (groups 2 to 4) received a daily dose of 100, 500 and 1000 mg/kg b.w. of *C. nardus* EO at the same hour for 28 consecutive days respectively. Doses were prepared by dispersion of *C. nardus* EO in distilled water. All animals were closely observed the 4 first hours post-treatment to notified any signs of toxicity and at least once a day for mortality and morbidity. Water intake was monitored daily, while food and body weight was recorded once a week throughout the 28 days study period (Ilboudo et al., 2019; OCDE, 2008).

Blood analysis:

All animals fasted on the evening of the 28 days. On day 29th, the animals were anesthetized with ketamine (150 mg/kg), and blood samples were collected from each animal via cardiac puncture into dry vacutainers. Blood samples were then centrifuged at 3000 rpm for 10 min using a table centrifuge (ROTOFIX 32A, Mettich Zenfrifugen, Germany) and the serum obtained was used for serum biochemical analysis. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CREAT), glucose, total cholesterol (TC) and total protein (TP) levels were determined using a semi-automatic biochemistry analyzer (MINDRAY BA-88A, China) (Ilboudo et al., 2019).

Effects on vital organs:

After blood collection, vital internal organs such as heart, liver, lungs, kidneys and spleen were removed, observed macroscopically for any lesions and weighed on a sensitive balance. The Relative Organ Weight (ROW) ratio of each organ was calculated according to the following formula:

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

Skin irritation test of *C. nardus* EO:

Skin irritation test was inspired by those of Draize (Draize et al., 1944) and adapted by the OECD guideline 404 for product testing using acute skin irritation/corrosion method (Draize et al., 1944; OCDE, 2015). The test was conducted using three adult male rabbits. Animals were shaved on the flanks 24 h before the application of the EO. A single dose of *C. nardus* EO (0.5 mL) is applied for 4 h to a small area ($\approx 6 \text{ cm}^2$) of one flank, and the untreated skin area of animal had used as the control. At the end of the exposure period, patches were removed. Observations and rating of erythema and oedema were made after 1, 24, 48 and 72 h after the removal of the patch. The observations were continued up to 14 days for a possible change in behaviour, general condition, posture and reflexes (Ilboudo et al., 2019; OCDE, 2015). The reactions, defined as erythema and oedema, were evaluated according to the scoring system for skin reactions (OCDE, 2015). The Dermal Irritation Score (DIS) was calculated using the following formula:

$$DIS = \frac{\text{Value (erythema + edema)}}{\text{Number of animal} \times \text{Number of observations}}$$

The EO's DIS was classified according to the scale proposed by Draize, adapted by Dutok and adopted by OECD for determining the degree of acute skin irritation/corrosion (Draize et al., 1944; OCDE, 2015; Dutok et al., 2015).

Eye irritation test of *C. nardus* EO:

The study was performed according to an adaptation of the OECD guidelines 405 for determining the degree of acute eye irritation/corrosion (OCDE, 2017). Three rabbits per group were constituted for experimental assays. Prior to test, they were subjected to a rigorous observation of the ocular structures such as the cornea, iris, and conjunctiva. After that, 0.1 mL of the EO was instilled in a single dose to the bottom of the right conjunctival sac of one eye of each animal and, keeping eyelids together over the next 30 s. The other left eye (untreated one) served as control. 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. Ultraviolet light was used for observation (OCDE, 2017). The individual irritation score was determined using the scale of weighted scores for grading the severity of ocular lesions from Draize (Draize et al., 1944). The observations were allowed to continue up to 21 days to evaluate the reversibility or irreversibility of the effects. The Maximum Mean Total Score (MMTS) was determined using the following formula:

$$MMTS = \frac{\text{Sum of total maximum score (rabbit 1 + rabbit 2 + rabbit 3)}}{3}$$

The MMTS obtained were used for classification of eye irritability in the system of Kay and Calandra (Kay JH., Calandra JC., 1962).

Data analysis:

Results were expressed as mean \pm standard deviation SD (n = 5). The statistical significance of the difference between treated and control groups was analyzed by One-way analysis of ANOVA, using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $p < 5\%$.

Results:-

Acute oral toxicity study:

Acute oral toxicity study was indicated that the *C. nardus* EO at a single dose of 2000 mg/kg b.w did not cause any remarkable behavioural changes or mortality of rats in the two steps. According to the acute toxicity class method of OECD 423 guideline, the *C. nardus* EO has an LD₅₀ value estimated to 5000 mg/kg b.w. and is classified to the 5th toxicity class (OCDE, 2001).

Sub-acute oral toxicity study:

During the subacute oral toxicity study period (28 days), no mortality was recorded. In addition, daily administration of *C. nardus* EO resulted in no behavioural changes in the treated rats compared to the control one.

Effect on water and food consumption:

Daily mean water and food consumption are presented in Tables 1 and 2, respectively. The averages of water and food consumption of treated groups were found to be similar to the control groups. However, a non-significant increase in water consumption during the 28 days study was observed to at 500 and 1000 mg/kg b.w.

Bodyweight gain:

Figure 1 shows the weekly mean weight changes of females and males rats, which received 100, 500 and 1000 mg/kg b.w. EO of *C. nardus* for 28 days. These results show an increase in body weight of all animals (control and treated groups) during the 28 days of study. However, this increase was not significant to be attributed to the product.

Effect of *C. nardus* EO on vital organs:

Macroscopic examination in the fresh state of vital organs such as heart, lungs, liver, kidneys and spleen of control and treated rats with *C. nardus* EO showed that there was no change in colour and aspect of different organs extract

does not affect vital organs as there was. Also, no significant change was noticed in relative organ weights (ROW) of control and treated animals with EO at doses of 100, 500 and 1000 mg/kg b.w. ($p > 0.05$) (table 3).

Effect of *C. nardus* EO on biochemical parameters:

The results of serum biochemical analysis of treated and control groups are presented in table 4. These results show that the daily oral administration of *C. nardus* EO at doses of 100, 500 and 1000 mg/kg b.w. on rats for 28 consecutive days did not cause significant changes in blood serum biochemical parameters such as ALT and glycemia levels in treated compared to control groups ($p > 0.05$). However, a significant increase in AST level of male rats was observed in treated groups at doses of 1000 mg/kg b.w. when compared to control ($p < 0.05$). Also, a significant decrease in total protein and creatinine levels in male rats and cholesterol level in female rat treated with EO at doses of 100, 500 and 1000 mg/kg, were observed in treated groups compared to control ones ($p < 0.05$).

Acute skin irritation test of *C. nardus* EO:

In the acute skin irritation study, the results obtained indicate that *C. nardus* EO didn't cause any signs of erythema or oedema during the 72 h of observation after patch removal. The Dermal Irritation Score (DIS) for *C. nardus* EO found was "0.00" (Table 5). This product is classified as not irritant for skin according to the scale proposed by Draize (Draize et al., 1944).

Acute ocular irritation test of *C. nardus* EO:

The results of eye irritation scores of *C. nardus* EO are presented in Table 6. These results indicated that the Maximum Mean Total Score (MMTS) was 5.33 for the group of rabbits treated with the EO. According to classification in the system of Kay and Calandra (Kay JH., Calandra JC., 1962), *C. nardus* EO is classified as a substance with minimal irritation on the rabbit's eye.

Table 1:- Mean daily water consumption (mL/day/rat) during 28 days treatment with *C. nardus* EO.

Week	Sex	Doses			
		Control	100 mg/kg	500 mg/kg	1000 mg/kg
Week 1	F	33.57 ± 3.41	29.00 ± 2.65	37.86 ± 4.84	34.29 ± 4.27
	M	37.86 ± 3.34	37.71 ± 1.38	38.71 ± 3.45	41.57 ± 5.88
Week 2	F	38.71 ± 6.05	36.00 ± 2.94	41.43 ± 4.61	40.43 ± 2.29
	M	42.86 ± 3.72	42.00 ± 3.61	41.57 ± 3.55	46.14 ± 7.43
Week 3	F	39.57 ± 2.44	36.43 ± 3.21	43.29 ± 4.27	43.86 ± 3.63
	M	46.71 ± 5.47	46.29 ± 2.36	48.14 ± 5.82	51.57 ± 7.02
Week 4	F	40.57 ± 6.24	38.71 ± 3.82	47.29 ± 5.02	41.00 ± 5.97
	M	49.00 ± 5.77	50.71 ± 3.90	48.71 ± 4.35	54.86 ± 7.67

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

Table 2:- Mean weekly food consumption (g/day/rat) during 28 days treatment with *C. nardus* EO.

Week	Sex	Doses			
		Control	100 mg/kg	500 mg/kg	1000 mg/kg
Week 1	F	25.90	22.09	30.14	28.43
	M	26.06	33.55	29.54	34.49
Week 2	F	25.61	20.00	25.48	17.33
	M	28.54	30.20	27.53	31.43
week 3	F	28.06	21.51	26.23	25.51
	M	31.43	27.37	27.00	31.43
Week 4	F	26.86	18.66	20.17	21.06
	M	28.57	25.94	27.14	28.57

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

Table 3:- Mean relative organ weights (%) of rats after 28 days of treatment with *C. nardus* EO.

Organs	Sex	Doses			
		Control	100 mg/kg	500 mg/kg	1000 mg/kg
Heart	F	0.37 ± 0.02	0.42 ± 0.05	0.42 ± 0.02	0.39 ± 0.04

Lungs	M	0.37 ± 0.03	0.37 ± 0.04	0.38 ± 0.02	0.39 ± 0.02
	F	0.62 ± 0.05	0.58 ± 0.05	0.80 ± 0.39	0.65 ± 0.03
Liver	M	0.52 ± 0.07	0.58 ± 0.12	0.60 ± 0.07	0.60 ± 0.07
	F	3.37 ± 0.22	3.28 ± 0.31	3.84 ± 0.50	3.74 ± 0.40
Kidneys	M	3.13 ± 0.13	3.05 ± 0.33	3.05 ± 0.28	3.47 ± 0.41
	F	0.72 ± 0.06	0.76 ± 0.05	0.80 ± 0.07	0.78 ± 0.05
Spleen	M	0.70 ± 0.02	0.75 ± 0.06	0.76 ± 0.08	0.76 ± 0.01
	F	0.26 ± 0.05	0.28 ± 0.04	0.30 ± 0.03	0.25 ± 0.04
	M	0.22 ± 0.01	0.25 ± 0.06	0.23 ± 0.02	0.23 ± 0.02

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

Table 4:-Biochemical parameters for rats after 28 days of treatment with *C. nardus* EO.

Parameters	Sex	Doses			
		Control	100 mg/kg	500 mg/kg	1000 mg/kg
ALT (UI/L)	F	32.40 ± 6.19	35.80 ± 4.32	44 ± 8.54	37.60 ± 8.65
	M	40.40 ± 2.07	39.20 ± 6.42	39.60 ± 4.93	45.40 ± 5.37
AST (UI/L)	F	135.0 ± 6.21	144.2 ± 12.62	147.8 ± 11.19	149.4 ± 11.10
	M	150.6 ± 8.56	162.00 ± 7.18	153.00 ± 4.95	164.80 ± 10.99*
TP (g/L)	F	64.14 ± 4.27	60.60 ± 2.36	59.72 ± 5.88	63.98 ± 5.25
	M	62.32 ± 2.08	55.58 ± 1.15**	55.66 ± 3.12**	55.64 ± 3.42**
CREAT (µmol/L)	F	62.98 ± 4.84	59.54 ± 3.28	57.14 ± 3.15	58.24 ± 3.91
	M	60.44 ± 6.93	54.92 ± 3.64	55.30 ± 4.17	51.18 ± 1.32*
GLY (mmol/L)	F	4.38 ± 0.32	4.23 ± 0.78	4.02 ± 0.88	4.49 ± 0.30
	M	4.04 ± 0.57	3.53 ± 0.31	3.73 ± 0.15	3.58 ± 0.23
TC (mmol/L)	F	2.27 ± 0.08	1.55 ± 0.12***	1.48 ± 0.10***	1.66 ± 0.14***
	M	1.70 ± 0.10	1.63 ± 0.09	1.60 ± 0.13	1.59 ± 0.11

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

ALT: alanine aminotransferase; AST: aspartateaminotransferase; CREAT: creatinine; GLY: glycemia ; TP: total protein; TC: total cholesterol.

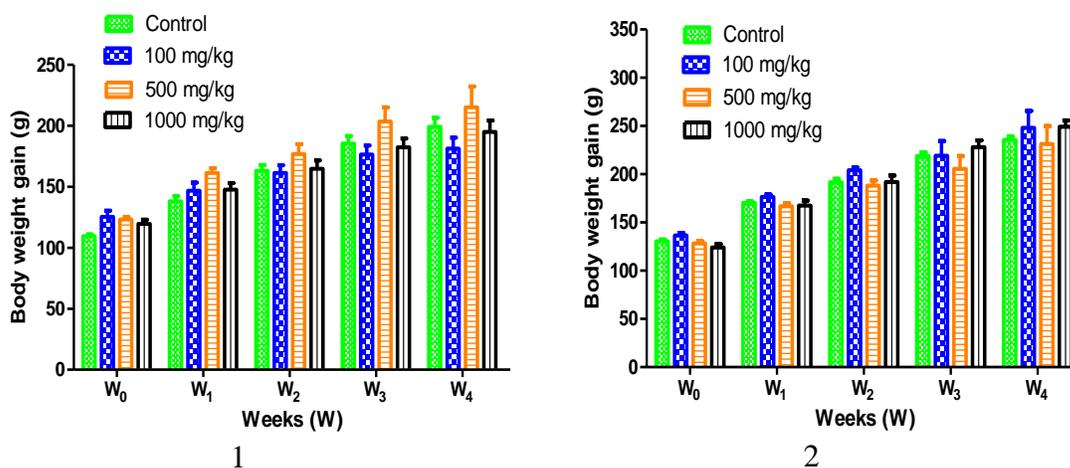
*** p < 0,001; ** p < 0,01 ; * p < 0,05 ; p > 0,05 vs controls

Table 5:- Score of erythema and oedema after application of *C. nardus* EO.

Rabbit N°	Flanks	24 hours		72 hours		Total (24 + 72 H) Erythema + Edema
		Erythema	Oedema	Erythema	Oedema	
1	Control	0	0	0	0	0
	Treated	0	0	0	0	0
2	Control	0	0	0	0	0
	Treated	0	0	0	0	0
3	Control	0	0	0	0	0
	Treated	0	0	0	0	0
Total (T)						0
DIS = T/12 = 0/12 = 0.00		Conclusion : 0 < DIS = 0.00 < 0,4 ; => Not irritant				

Table 6: -Score of cornea, iris and conjunctiva after application of *C. nardus* EO

	Rabbit N° 1				Rabbit N° 2				Rabbit N° 3			
	Hours				Hours				Hours			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A × B × 5 (≤ 80)	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A × 5 (≤ 10)	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctiva												
(A + B + C) × 2 (≤ 20)	6	2	0	0	4	0	0	0	6	2	0	0
Total	6	2	0	0	4	0	0	0	6	2	0	0
MMTS	5.33											
	Conclusion: 2.6 < MMTS = 5.33 < 15 => Minimal irritant											

**Figure 1:** - Mean body weight gain of control, females (1) and males (2) rats treated with *C. nardus* EO (n = 5/Sex).**Discussion:-**

EOs are odorous, volatile substances and generally liquid at room temperature. They are variable and complex mixtures of different chemical compounds, dissolved in one another, forming homogeneous solutions (Samaté, 2002). The study of the acute oral toxicity of *C. nardus* EO gave an LD₅₀ estimated to 5000 mg/kg b.w. According to OECD guideline 423, the EO tested is classified in the 5th class of toxicity, that means in the class of substances with a low acute oral toxicity according to the General Harmonized System (GHS) United Nations (United Nations, 2011). This result is similar to that of US-Environmental Protection Agency (EPA) which classified *C. nardus* EO in the class of substances with a low acute oral toxicity with an LD₅₀>5000 mg/kg b.w. (EPA, 1997). An acute oral toxicity study carried out on mice gave 2511.89 ± 791.5 mg/kg b.w. as an LD₅₀ of this EO (Yoewono et al., 2016). Acute oral toxicity studies of EO of other species of *Cymbopogon* have also shown the absence of a toxic effect for the EO of *Cymbopogon schoenanthus* (LD₅₀ > 5000 mg/kg b.w.) and the EO of *Cymbopogon citratus* (LD₅₀ ≈ 3.5 mg/kg b.w.) (Costa M., 2011; Ilboudo et al., 2019).

In the sub-acute oral toxicity study, daily administration of *C. nardus* EO to 100, 500 and 1000 mg/kg b.w. during the 28 consecutive days not showed any mortality or signs of toxicity. The results showed a non-significant increase (p > 0.05) in mean water consumption while the average food consumption of treated groups was similar to the control ones. According to other studies, the insignificant increase in mean water consumption could be explained by the progressive weight gain in animals and therefore, is not linked to the extract (Dawoud, 2015). These results show that the EO had no toxic effect on water and food average consumption during the four weeks study period. However, another study showed that inhalation of β-citronellol, one of the molecular components of the EO led to a decrease in the average consumption of food, after a treatment of 5 weeks (Batubara et al., 2015). In order to determine the toxic effect of the EO on animal body weights, we evaluated the bodyweight evolution of the treated

rats compared to control ones. Results showed a non-significant increase in the bodyweight of treated animals compared to control ones. These results show that the EO did not have a toxic effect on the body weight of treated rats compared to the control ones, during the study period up to 1000 mg/kg b.w. However, studies have shown that the β -citronellol in the EO of *C. nardus* was able to induce a decrease in body weight in rats after five weeks of study in treated compared to control groups. It is the same for the EO of the *schoenanthus* species in a study of sub-acute oral toxicity (Batubara et al., 2015; Ilboudo et al., 2019). The difference between these results and this one could be explained by the difference between the species. For to evaluate the effect of the EO on certain organs, a macroscopic examination of the organs was carried out, and the results did not show any lesions, colour or shape change in the organs of the test compared to control groups. On the other hand, a non-significant increase in the relative organ weights of animals treated with EO was noted compared to the controls. This result shows that *C. nardus* EO did not have any toxic effect on the relative organ weights of the rats treated in the sub-acute toxicity study up to 1000 mg/kg. Other studies have also shown the absence of lesions and histopathological signs on the vital organs of animals with the *schoenanthus* and *citratus* species after 28 days of treatment with their EO (Ilboudo et al., 2019; Lulekal et al., 2019). Certain toxins, once in the organism, can cause disturbances in biochemical parameters, thus identifying the organs concerned by intoxication. Indeed, several studies have reported that EOs of plant origin can cause, for example, hepatotoxicity, nephrotoxicity and cardiotoxicity (Doriane, 2018). However, the 28 days sub-acute toxicity study showed a slight increase in ALT and AST, while total protein, creatinine, glucose and total cholesterol levels decreased slightly in treated groups compared to control ones. The significant increase to 1000 mg/kg of blood serum AST and creatinine levels only in males treated compared to control groups cannot be linked to the extract. However, a sub-acute toxicity study carried out on mice treated with EO of *C. nardus* showed a significant increase in the level of AST at 600 mg/kg b.w. (early liver damage) in the test groups compared to control ones. However, the level of creatinine in the test groups remained similar to control groups at the same dose (600 mg/kg b.w.) (Yoewono et al., 2016). The significant decrease ($p < 0.05$) in total protein and total cholesterol levels in all males and females respectively in treated compared to control groups, could be related to the sympathetic nerve activity in brown adipose tissue (BAT) (Shen et al., 2005). A sub-acute toxicity study carried out on rats showed that citronellal and citronellol also contained in *C. nardus* EO could cause lower triglyceride and total cholesterol levels, respectively (Batubara et al., 2015).

The skin and eye irritancy of *C. nardus* EO has been evaluated. Its DIS is between 0.00 and 0.4. According to the classification of Draize (Draize et al., 1944), the plant's EO is classified in the first class. This means that it is classified as not irritating to the skin. Erythema and oedema being warning signs of inflammation, a study showed that *C. nardus* EO had no anti-inflammatory properties (Naidoo et al., 2009). Other studies have also shown that EOs from the *schoenanthus* and *citratus* species did not cause irritation on the skin of the rabbit (Ilboudo et al., 2019; Lulekal et al., 2019). On the other hand, the ocular irritant power gave an MMTS equal to 5.33. This value is, therefore between 2.6 and 15. According to the classification system of Kay and Calandra (Kay JH., Calandra JC., 1962), this EO is classified in the third class, that is to say among the substances with minimal irritant power on the eyes. This result is similar to that of the US-Environmental Protection Agency (EPA, 1997) which classified *C. nardus* EO as a category III eye irritant, that means in the class of substances with weak eye irritancy.

Conclusion:-

This study allowed us to assess the toxicological profile of *C. nardus* EO. This consisted of evaluating its oral LD₅₀, the parameters of its subacute oral toxicity as well as its skin and eye irritant power. The study of acute oral toxicity has shown that this EO has relatively low toxicity by the oral route with an LD₅₀ estimated at 5000 mg/kg b.w. The sub-acute toxicity study of *C. nardus* EO showed low toxicity on the physical and biochemical parameters of rats. The study of irritant power has shown that it has a weak irritant effect on the skin and the eye. *C. nardus* EO, therefore, has low oral toxicity with very low skin and eye irritant power. This essential oil has a low toxicity and therefore presents a less toxicological risk in the event of exposure. However, its use as biopesticides should be accompanied by precautions for use for the applicator.

Conflicts Of Interests:-

The authors have not declared any conflicts of interests.

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