

***In vivo* toxicity of essential oils of two medicinal plants from Burkina Faso: *Hyptis spicigera* Lam. and *Ocimum americanum* L.**

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doi: <https://doi.org/10.37745/irjns.13/vol13n12739>

Published November 18, 2025

Citation: Ouedraogo W.J., R., Yerbanga S., Boni I.S., Koama B.K., Meda R.N.T., Ouedraogo J.B., Ouedraogo G.A. (2025) In vivo toxicity of essential oils of two medicinal plants from Burkina Faso: *Hyptis spicigera* Lam. and *Ocimum americanum* L., *International Research Journal of Natural Sciences*, 13 (1),27-39

Abstract: The acute oral and subacute cutaneous toxicities of *Hyptis spicigera* and *Ocimum americanum* from Burkina Faso were evaluated in this study. In both experiments the concentration was 5000 mg/kg b.w., and control groups received corn oil. The acute oral toxicity consisted of administering by gavage each essential oil diluted in corn oil, to NMRI mice, which were monitored for 14 days. Blood samples were taken for hematological, biochemical analysis and organs were collected for macroscopic analysis after the follow-up. Wistar rats were used for the subacute cutaneous toxicity of *O. americanum*. For 4 weeks, diluted essential oil was applied daily to a shaved skin area and treatment was maintained by a bandage for 6h. The results of the acute oral toxicity showed that piloerection, drowsiness, itching and hyperactivity had a higher frequency of occurrence than salivation, aggressivity, diarrhea, convulsions, heavy breathing and coma. Animals weight gain and organs weight were normal, except for *H. spicigera* group and the heart in *O. americanum* group respectively, which had a statistical difference compared to the control. There was no statistical difference in the blood parameters analyzed (White Blood Cells, Red Blood Cells, Platelets, Hemoglobin, Aspartate aminotransferase, Alanine transaminase). For the subacute cutaneous toxicity, the Draize score was 0 for both edema and erythema.

Keywords: acute oral toxicity; subacute cutaneous toxicity; essential oils; *Hyptis spicigera*; *Ocimum americanum*

INTRODUCTION

Essential oils (EOs) are natural complexes of volatile and odorous molecules, synthesized by the secreting cells of aromatic plants (Duqu nois & Anton, 1968). They are present in only 10% of the plant kingdom, and there are about 3000 essential oils known. They are used in various areas such as pharmaceutical, chemical, cosmetic, perfume and food/beverage industries (Barbieri & Borsotto, 2018; Burt, 2004). Demand for natural products is continually increasing, and EOs are not left out, which enjoy a certain popularity with the public who only think of them as natural and therefore harmless products (Couderc, 2001). Despite their numerous benefits, EOs can be very toxic, depending on the dose and the exposition route.

Hyptis spicigera (*H. spicigera*) and *Ocimum americanum* (*O. Americanum*) are aromatic, herbaceous plants of the Lamiaceae family. These are aromatic medicinal plants commonly used in Burkina Faso. *H. spicigera* is used to treat toothaches (Tapsoba & Deschamps, 2006), in the preservation of cereals (Sanon et al., 2018; Savadogo et al., 2016). Bogninou-Agbidinoukoun et al. (2013) mentioned uses of the decoction, the powder, the fresh inflorescences as bath water and tea to treat bronchial secretions, migraines and headaches (Bogninou-Agbidinoukoun et al., 2013). The organic extracts have shown antimalarial and larvicidal activities (Uraku et al., 2015; Wangrawa et al., 2016) and hepatoprotective potential (Uraku, 2016). The essential oil of *H. spicigera* contains caryophyllene which is its major compound, with a content of 57 to 66% reported in Burkina Faso and 67.6% in Nigeria (Kini et al., 1993; Onayade et al., 1990). It has a protective effect against ulcers, antiproliferative activity against breast cancer cells, analgesic, antinociceptive effects as well as antioxidant and anti-inflammatory (Bayala et al., 2014; Bogninou-Agbidinoukoun et al., 2013; Sim es et al., 2017; Takayama et al., 2011). Other studies also highlight the insecticidal (Sanon et al., 2006) and antimicrobial (Moreira et al., 2010) activity of this essential oil.

As for *O. americanum*, studies have demonstrated antiviral, antibacterial, antihelmintic, antisickling properties (Dibala et al., 2016; Tshilanda et al., 2019). It is used for crops preservation (Savadogo et al., 2016), to treat colds, coughs, stomachaches, eye and ear infections; it can be consumed as tea or serve as a flavoring agent (Ali et al., 2021). The major constituent of the EO of this plant is 1,8-cineole (Bayala et al., 2014; Djibo et al., 2004). It is anti-inflammatory, antioxidant, antimicrobial (Bayala et al., 2014; Thaweboon & Thaweboon, 2009), (Yamada et al., 2013; Ilboudo, 2009). It also has insecticidal, larvicidal, ovicidal and repellent properties (Ilboudo, 2009; Sanon et al., 2018; Wangrawa et al., 2016).

The safe use of medicinal plants, and by extension their essential oils, requires toxicity studies. The evaluation of toxicity is important when considering application in public

health, because exposure to any chemicals, natural or not, can cause adverse effects (Jothy et al., 2011). In our study, we investigate the acute oral toxicity of the EOs of *H. spicigera* and *O. americanum*, and the subacute cutaneous toxicity of the EO of *O. americanum* from Burkina Faso.

MATERIALS AND METHODS

Plant material

Aerial parts of *H. spicigera* and the whole plants (with roots) of *O. americanum* were collected in the Guiriko region (ex- Hauts-Bassins). The plants were identified by a botanist from Institut de la Recherche en Sciences de la Santé in Bobo-Dioulasso (IRSS-DRO) and specimens were preserved in the herbarium of Institut des Sciences et Techniques de Bobo (INSTech-Bobo) under voucher number BiTech22. *O. americanum* plants were dried on racks in an aerated and ventilated room for approximately 2 weeks. Fresh parts of *H. spicigera* were used immediately after collection.

Essential oils extraction

Hydrodistillation of the dried plants of *O. americanum* and the fresh aerial parts of *H. spicigera* was each carried out using an artisanal alembic, according to the Clevenger method. EOs were extracted using vapors generated after boiling plants material in water and passing through a cooling column where they condense to form droplets of essential oil. The plant material mixed with water was boiled for approximately 3 hours. Each essential oil obtained was decanted using a separating funnel, then stored in a glass bottle covered with aluminum foil at 2-8°C until use (Dabiré et al., 2009).

Animals

Animals were obtained from the Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES). They were acclimated for a minimum of 5 days and kept in cages under controlled conditions ($T = 22(\pm 3)^{\circ}\text{C}$; $30\% < \text{RH} < 70\%$) with an alternation of 12 hours of light and 12 hours of darkness, and food and water ad libitum. Six (6) NMRI mice (3 males and 3 females) aged 8 to 10 weeks and weighing between 25g to 38g were used per treatment for oral toxicity. The day before the test they were weighed, allocated to treatment ~~into six (6)~~ groups, and identified by marking a part of the body with picric acid (head, tail, flank, back). At the end of each test, the animals' remains were incinerated using specific ovens and procedures that comply with ethics and current legislation on animal waste management and experimentation of Burkina Faso veterinarians' services.

Ten (10) WISTAR rats (5 males and 5 females) with an average weight of 131g and aged 8 to 10 weeks were used per treatment for dermal toxicity. The rats were shaved with a depilatory cream (SIVODERM hair removal cream with aloe vera; manufacturer= SIVOP; lot#137-6-22) (approximately 6 cm²) and weighed 24 hours before the first application,

then every weekend. They were anesthetized once a day before each manipulation with ketamine hydrochloride (Brand: Swiss; lot# 2321241; concentration: 50mg/ml), at a dose of 30 mg/kg bw.

Assessment of toxicity

The criteria of the Organization for Economic Cooperation and Development (OECD) were used in our methodology, in its guidelines 410 and 420 (OECD 1981, OECD 2008). The tests were carried out in 3 replicates, for each toxicity and each treatment.

Acute oral toxicity of *H. spicigera* et *O. americanum*

Essential oils were administered in a single dose of 5000 mg/kg b.w by gavage, diluted in corn oil. The control group received the solvent corn oil only (200 µl). Mice were weaned for 2 hours and observed for 5 minutes immediately after administration and then 2 hours later; from the second day they were observed twice a day until the fourteenth day. Initial and final weights were recorded for each mouse. Behavioral monitoring concerned piloerection, salivation, aggressiveness, diarrhea, convulsion and coma. After 14 days of follow-up, the mice were euthanized by ether inhalation. A blood sample was taken by cardiac puncture for the evaluation of haemato-biochemical parameters, and organs (heart, liver, kidneys, lungs, spleen) were taken and examined macroscopically.

Subacute cutaneous toxicity of *O. americanum*

Essential oil of *O. americanum* was applied to the shaved skin of the rats once a day, 5 days a week, for 4 weeks, at 5000 mg/kg bw diluted in 200 µl of corn oil. The control group received the solvent, corn oil, only. The EO was applied using a soaked compress placed on the test site, then maintained with an adhesive bandage for 6 hours. All treated rats were observed immediately after application of the EOs for approximately 5 minutes; 6 hours later the bandage was removed, and the treated skin area cleaned with cotton soaked in water. The follow-up concerned the general condition (piloerection, salivation, aggressiveness, diarrhea, convulsion and coma) and the aspect of the skin (erythema and edema). After 28 days, the skin aspect was evaluated through the Draize score described in Table 1. At the end of the test rats were euthanized.

Table 1. Draize dermal irritation scoring system (DDISS) (Hemmati. *et al*, 2016)

Erythema and Eschar Formation	Value	Edema Formation	Value
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well-defined erythema	2	Slight edema (edges of area well defined by definite raising)	2

Moderate to severe erythema	3	Moderate edema (raised approximately 1mm)	3
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Statistical analysis

At the end of the acute oral toxicity experiments, only the data for surviving animals was included in the analysis, which differ for each group. The general behaviour data was reported as frequency; the body weight data were expressed as mean value $\pm$ standard error (SE). The data were analyzed using the Kruskal-Wallis method, with a comparison with Dunn's test and a Bonferonni adjustment. The significance threshold was 5%. As for the subacute cutaneous toxicity, results were expressed as frequency.

RESULTS

Acute oral toxicity

The behavior patterns of the animals were monitored during the acute toxicity study. The results have been presented in Table 2. Salivation did not occur in any group; aggressivity, diarrhea, convulsions, and coma were rare and happening in only one of the 3 groups as follow: aggressivity was noticed 3 times in the control group, diarrhea 1 time in *H. spicigera* group, and convulsion 1 time as well in *O. americanum* group; heavy breathing was not noticed in the control group; as for piloerection, drowsiness, itching and hyperactivity, they were more frequent, from most to least respectively. Animals weight was also recorded before the administration of the EOs, and at the end of the monitoring period of 14 days. All the animals gained between 15% and 24% of their initial weight, and the mean body weight variation has been presented in Table 3.

Table 2. Frequency of behavior occurrence of mice

Behavior/ Treatment	<i>H. spicigera</i>	<i>O. americanum</i>	Control
Piloerection	29	21	11
Salivation	0	0	0
Aggressivity	0	0	3
Diarrhea	1	0	0
Convulsions	0	1	0
Drowsiness	13	23	1
Itching	4	1	11
Hyperactivity	4	3	5
Heavy breathing	3	1	0
Coma	0	1	0
Death	1	4	1

Table 3. Mean body weight of all replicates of mice in grammes (g) (* = p-value<0.05)

Treatment	Mean initial weight (g)	Mean final weight (g)	Difference
<i>H. spicigera</i> *	29.6	34	4.40
<i>O. americanum</i>	32	37.2	5.20
Control	27.6	34.2	6.60

The observation of organs revealed no apparent change. The organs' weights were also all comparable, except the heart for *O. americanum* where the difference was statistically significant compared to the control (Figure 1).

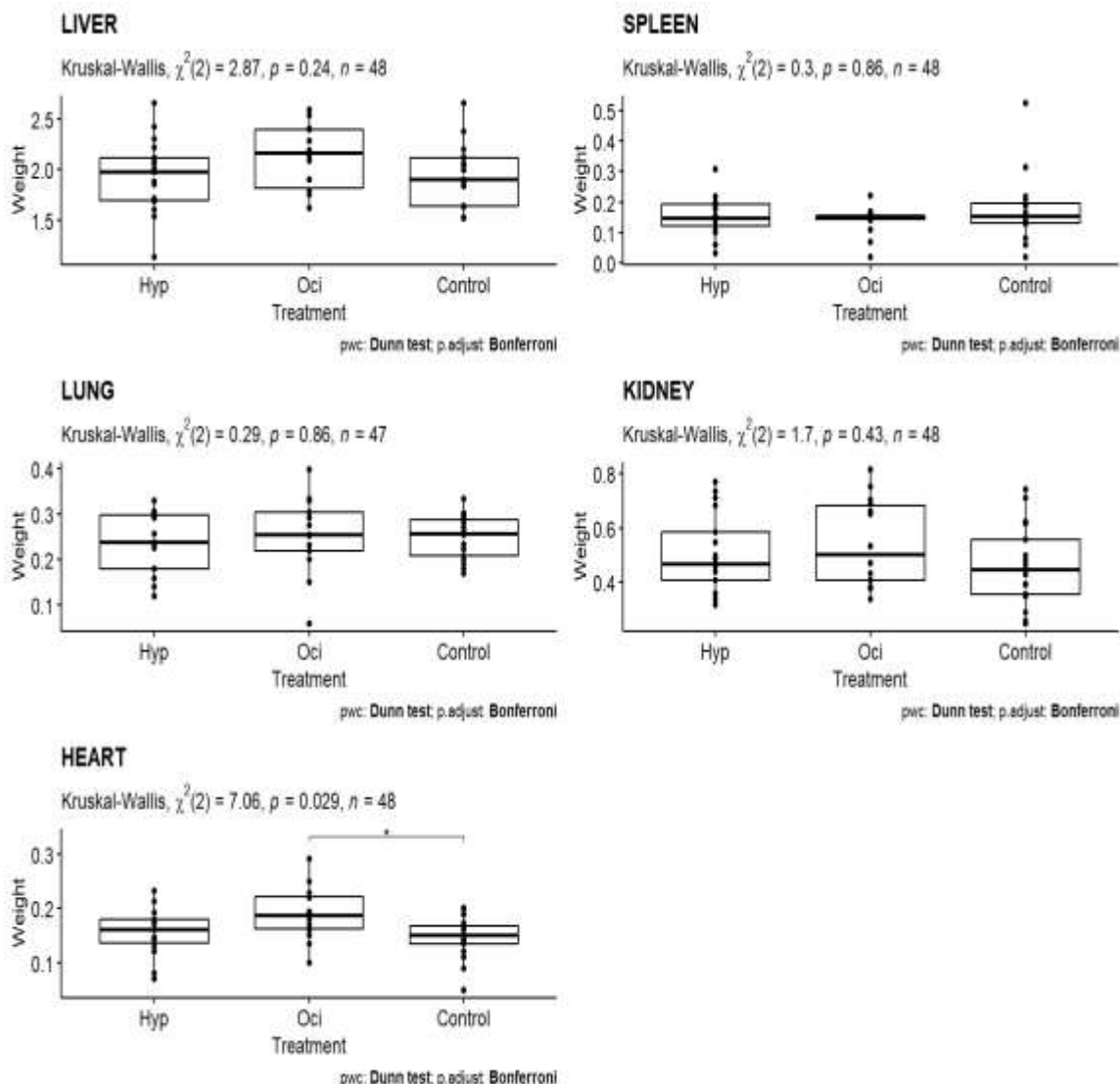


Figure 1. Mean weight of mice organs in grammes (g) (Hyp =*H. spicigera*; Oci = *O. americanum*)

The blood sample analysis revealed no abnormalities among the tested parameters; there was no statistical difference between the EOs groups and the control group (Figure 2).

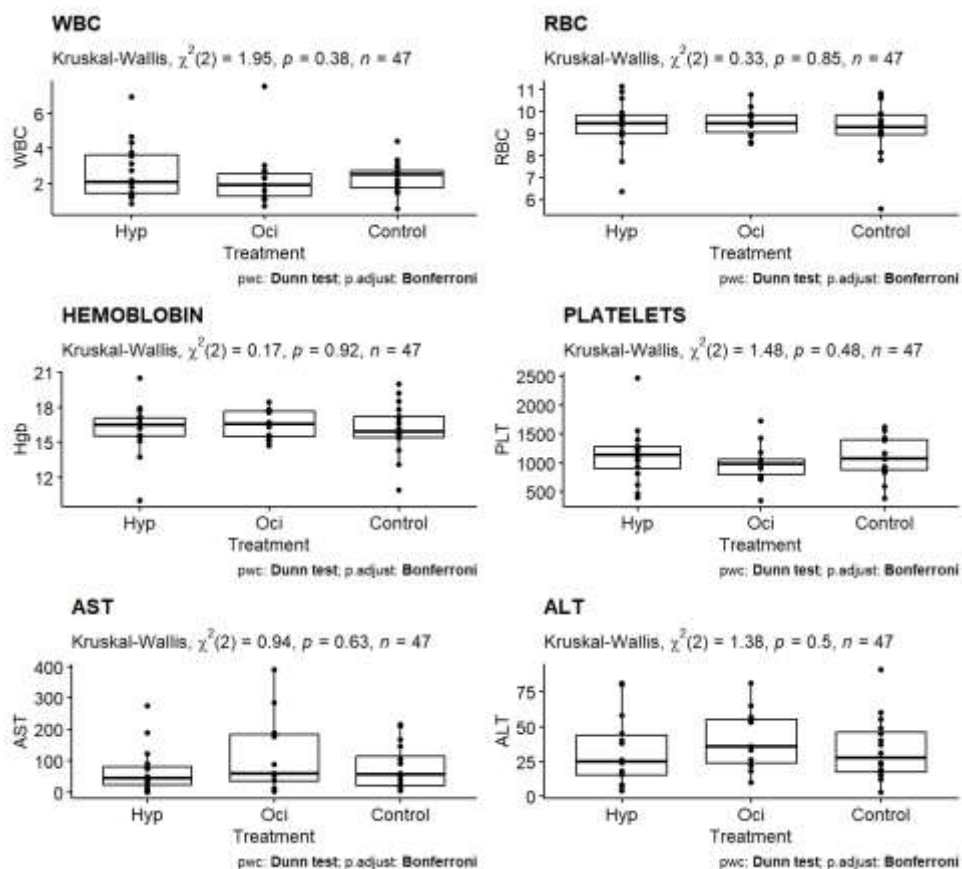


Figure 2. Hematologic and biochemical parameters of mice 14 days after EO administration (WBC= $10^3/\mu\text{L}$; RBC= $10^6/\mu\text{L}$; Hemoglobin=g/dL; Platelets= $10^3/\mu\text{L}$; AST=U/L; ALT=U/L) (Hyp = *H. spicigera*; Oci = *O. americanum*; WBC= white blood cells; RBC=red blood cells; AST= aspartate aminotransferase; ALT=alanine transaminase)

Subacute cutaneous toxicity of *O. americanum*

The results of the cutaneous toxicity have been presented in Tables 4 and 5. Piloerection and diarrhea were the only parameters of general condition that occurred. The proportion of occurrence in the male group was 5 to 6 times higher than in the female group. As for the skin aspect, there was no noteworthy change.

Table 4. Frequency of reactions after cutaneous application of EO

Behavior	Ctrl	Males	Ctrl	Females
Piloerection	16	28	3	5
Diarrhea	59	89	11	12

Table 5. Skin aspect-Draize scoring after 28 days of EO application

Aspect	Ctrl	Males	Ctrl	Females
Oedema	0	0	0	0
Erythema	0	0	0	0

DISCUSSION

The goal of the present study was to evaluate the acute oral toxicity of the EOs of *H. spicigera* and *O. americanum*, and the subacute cutaneous toxicity of *O. americanum*. The single dose of 5000 mg/kg bw was used for both EOs and in both experiments.

In the acute oral toxicity experiment, the general condition, the body weight, the weight of vital organs, and blood parameters of the animals were analyzed.

Piloerection and drowsiness had higher frequencies in the EOs treated ~~mice~~ groups compared to the control ~~mice~~. More specifically when comparing both EOs, the frequency of piloerection was higher in the *H. spicigera* group while drowsiness was higher in the *O. americanum* group. These observations suggest that the EO of *O. americanum* could have a more pronounced sedative effect than *H. spicigera*. On the other hand, the frequency of itching was higher in the control ~~group~~ than the EOs treated groups. The other behavioral parameters and general condition had similar proportions in the three groups of mice, suggesting that no effect was induced by the oral administration of the EOs. A total mortality of 11% was observed, all three replicates' tests combined: the highest frequency corresponded to the *O. americanum* treated mice. *H. spicigera* treated mice and control mice had equal mortality frequency. However, there was no evidence that the mortality was related to the treatments. These results indicate that the two EOs have a minimal effect on general condition. The weight evolution of the mice was normal overall; however, there was a significant difference in the *H. spicigera* treated mice compared to the control, denoting a slightly lower weight gain than the control. This EO might have induced a satiety effect in the mice, therefore limiting their food intake. The diet of the other mice as well as their metabolism were not affected by the administration of EOs. Macroscopic observation of the different organs revealed no visible signs of toxicity, and the comparison of their weights was not statistically significant, except the weight of the heart for the *O. americanum* treatment which was slightly higher than the control. There was also no statistical difference in the hematological and biochemical parameters analyzed, namely white blood cells, red blood cells, hemoglobin level, platelets, and transaminases. The EOs therefore did not alter the production of blood cells or cause inflammation; liver function was also not affected. Relatable studies have had the same conclusions with the EOs tested. Indeed, Ayenew et al. (2022) investigated the subacute toxicity of *Cymbopogon martini* EO in mice, at 500 and 1000 mg/kg b.w, and found no significant difference in body weight variation, in hematological and biochemical parameters (Ayenew et al., 2022); in another study, broiler chickens were fed with added licorice EO in their diet for 42 days, and there was no significant difference in weight gain compared to the control group (Dheyauldeen

Salahdin et al., 2023); on the contrary, Adaszyn'ska-Skwirzynska & Szczerbinska found a significant difference in body weight gain in broiler chicken given lavender EO infused drinking water, compared to the control (Adaszyńska-Skwirzyńska & Szczerbinska, 2019). Lastly, treatment of quails with 150 and 300 mg/kg b.w of oregano EO did not significantly affects their body weight gain, liver, heart and spleen weight, and leukocyte count, but inversely increased AST, ALT levels at the end of the 42 days treatment (Farouk et al., 2020).

As for the subacute cutaneous toxicity of the EO of *O. americanum*, no reaction was observed in the skin of rats throughout the 28 days duration of the study. There was no inflammation or redness, indicating the safety of the EO. The Draize Score was 0 for edema and erythema, for all animals. However, piloerection and diarrhea were noted in all rats, even the control group, with a much higher proportion in males. Ketamine has been found to affect the gut function, which might explain the diarrhea observed after daily injection (Sun et al., 2004).

CONCLUSION

The results of this study indicate that the essential oils of *H. spicigera* and *O. americanum* diluted in corn oil do not present any apparent toxicity *in vivo* at the concentration of 5000 mg/kg bw, neither by oral intake for the two essential oils, nor by cutaneous administration for the essential oil of *O. americanum*.

Therefore, these essential oils seem safe to use with traditional remedies, or as additives in food, beverages, supplements. The incorporation of the essential oil of *O. americanum* in a cosmetic formulation would also be safe at a dose of 5000 mg/kg bw.

To corroborate current findings, the investigation of additional blood parameters could be beneficial and allow for a broader application, similarly to previous studies. Including parameters such as creatinine, D-dimer, C-Reactive Protein, Total bilirubin would inform on the renal function, reflect the cardiac state and evaluate the liver function or determine if the rate of red blood cells destruction is normal, respectively.

Ethical Aspect

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85-23, revised 1985). Animals were cared for by trained technicians.

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