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Comparative study of acute oral toxicity of essential oils of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* in wistar rats

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Abstract
The essential oils from *Ocimum gratissimum*, *Hyptis suaveolens* (Lamiceae) and *Psidium guajava* (Myrtaceae) have biological properties. The aim of this work is to compare the effect of essential oils of these three medicinal plants on weight growth, hematological and biochemical parameters of treated rats through the procedure of acute toxicity evaluation on Wistar rats. The doses applied are 100, 250 and 500mg/Kg bw for each of the essential oils From the results obtained, the effects of essential oils *of Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* leaves on biochemical, hematological parameters and weight growth of rats are dose dependent. At the dose of 500mg/Kg bw, they significantly increased the rate of white blood cells, urea, creatinemia, transaminases and reduced the weight growth of rats by more than 10% (p<0.05) compared to the control lot. This shows that these essential oils can have toxic effects when applied in high doses.

Keywords: Essential oils, Biochemical and Hematological parameters, Weight growth, Toxicity

Introduction
The use of medicinal plants in the treatment of diseases is a practice that has existed since ancient times [23]. A great importance is more and more given to this science because of the health problems that remain unanswered or that have difficulties, in particular, the resistance of pathogenic microorganisms to conventional drugs [24]. Many of these medicinal plants have proven biological properties. These scientific researches will have reached their goals when the population would have found relief and satisfaction through the use of medicinal plants, or its derivatives (Extracts, Essential Oils), and or TAMs (Traditionally Improved Medicines). Thus, it is important to conduct safety studies of plant species with biological properties to ensure that their uses will not have a negative impact on health. To this end, this work proposes to compare the toxicity of essential oils of three medicinal plants *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava. Psidium guajava* is a shrub of the Myrtaceae family whose fruits are edible and whose leaves are used in therapy. *Ocimum gratissimum* is an herb of the Lamiaceae family increasingly cultivated in gardens for its nutritional and therapeutic values. Found at the edge of the roads in the villages and qualified as weeds, *Hyptis suaveolens* of the Lamiaceae family is also solicited in traditional medicine. In sum, *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* are used in the treatment of dysentery, diarrhea vomiting, rheumatism, diabetes, and gastroenteritis, pulmonary problems, to cure wounds, ulcer, rheumatism, fever, malaria and intestinal parasitosis [1, 2]. They are used in the form of decoction, infusion or macerated but the dosage is often unclear. The method of preparation (decoction, maceration, infusion), the type of solvent used (water, ethanol, sugar beverages, carbonated water) can extract one or the other of the secondary metabolites responsible for the biological activities. However, essential oils, which are also secondary metabolites of medicinal plants, escape these methods and techniques of extraction. And yet, they possess interesting biological properties [25, 26] and act at very low quantities. It has been reported that essential oils extracted from the leaves of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* have Antioxidant, antibacterial, antifungal, insecticidal, anti-inflammatory, antidiarrheal and antiparasitic properties [3-8].

They can be used in the treatment of infectious diseases, as bioinsecticide and food industries. Essential oils are composed of several molecules that can act in synergy or the biological activity can only depend on the majority molecules [22, 26]. It is with regard to all these observations that the present study proposes to study the safety of essential oils of the leaves of Ocimum gratissimum, Hyptis suaveolens and Psidium guajava.

Material

Fresh leaves of Ocimum gratissimum, Hyptis suaveolens and Psidium guajava are collected in December 2020 in Abomey-calavi (Benin). Herbariums of each of the plants were authenticated at the National Herbarium of the University of Abomey-calavi and registeredunder the numbers YH 603/HNB, YH 604/HNB and YH 605/HNB respectively for *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava*. 30 healthy, non-pregnant, nulliparous female rats weighing between 150 and 200g and 8 to 12 weeks of age are used for acute toxicity tests. They were obtained at the animal house of the National University of Agriculture of Kétou and are kept in a room with artificial lighting with a sequence of 12 hours of light and 12 hours of darkness with a temperature between 23 °C and 25 °C and a relative humidity of 40 to 45. They were fed with Veto- service® pellets and had unlimited access to tap water.

Method

Preparation of essential oil extracts

The essential oils of the leaves are obtained through the extraction of a known mass of fresh leaves of each species by hydrodistillation for 3h using a Clevenger type device. The essential oils collected in vials are stored at 4 °C. For the evaluation of the tests, the essential oils were prepared in a hydroalcoholic solution.

Acute toxicity

The determination of acute toxicity was conducted according to OECD guideline $423_{(20)}$. The rats were divided into 10 groups of 03 each. The Control lot received 1 mL of the hydroalcoholic solution. Lots 1, 2 and 3 received the essential oil of *Ocimum gratissimum* leaves at doses of 100, 250 and 500 mg/Kg bw respectively. The essential oil of *Hyptis suaveolens* leaves was administered to the rats of lots4, 5 and 6 respectively at the doses of 100, 250 and 500 mg/Kg bw. And 100, 250 and 500 mg/Kg bw of *Psidium guajava* essential oil was given to lots 7, 8 and 9 respectively. Before the tests, the rats were deprived of food but not water overnight. Then they were weighed and the essential oils were administered through a stomach tube. The rats were fed and put under observation for 14 days. Weight monitoring was performed at D0, D7 and D14. At the end of the 14 days, blood samples were taken for hematological and biochemical analysis.

Statistical Analysis

To assess the effect of essential oils and dose on weight, biochemical and hematological parameters, an analysis of variance was performed with R 4.0.3 softwares (R Core Team, 2020) in case of normality. When normality was not acquired, the nonparametric Kruskal Wallis test was performed. Mean structuring was done with the SNK function of the "agricolae" package [21] when the probability was significant (p<0.05).

Results

No signs of toxicity such as agitation, convulsions, drowsiness and or diarrhea were observed in the different lots of rats treated with the essential oils of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* leaves. This indicates that the essential oils of the leaves of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* have an LD50 greater than 500mg/kg bw.

Effect of essential oils on the growth of treated rats

Table 1, 2 and 3 show the effect of administration of essential oils of *Ocimum gratisimum*, *Hyptis suaveolens*, and *Psidium guajava* respectively on the weight growth of rats. Treatment with the essential oil of *Ocimum gratissimum* leaves significantly reduced the growth rate of rats at the dose of 500mg/Kg bw after 14 days (p<0.05). Treatment with the essential oil of *Psidium guajava* leaves is associated with a significant reduction in the growth rate of rats (p<0.05). The essential oil of *Hyptis suaveolens* leaves stimulated the growth of rats betwen Day 1 and Day
7. However, the best growth rate is obtained with the doseof 250mg/Kg bw at the end of 14 days although in the interval of days D7-J14, there is no increase in the weight mass of rats.

Table 1: Effect of essential oil of *Ocimum gratissimum* leaves on weight growth of treated Wistar rats.

EO	Dose (mg/Kg bw)	Weight growth (g)		
		D1-D7	D1-D14	D7-D14
OG	500	0.03^{b}	0.03^{b}	0^{b}
	250	0.24^{a}±0.02	0.29^{a}±0.02	0.04^{c}±0.0003
	100	0.13^{a}±0.0001	0.16^{c}±0.01	0.03^{c}±0.000
Control	-	0.19^{a}±0.001	0.36^{a}±0.002	0.13^{a}±0.00003

a, b, c averages in the same row with different superscript as significantly different (P<0.05). OG: *Ocimum gratissimum*; EO: essential oil; D1: Day 1; D7: Day 7; D14: Day 14

Table 2: Effect of essential oil of *Psidium guajava* leaves on weight growth of treated Wistar rats.

EO	Dose (mg/Kg bw)	Weight growth (g)		
		D1-D7	D1-D14	D7-D14
PG	500	0.21^{a}±0.001	0.26^{b}±0.006	0.04^{b}±0.0005
	250	0.11^{b}±0.001	0.20^{b}±0.004	0.08^{b}±0.00005
	100	0.08^{c}±0.001	0.14^{c}±0.0003	0.06^{b}
Control	-	0.19^{a}±0.001	0.36^{a}±0.002	0.13^{a}±0.00003

a, b, c averages in the same row with different superscript as significantly different (P<0.05). PG: *Psidium guajava*; EO: essential oil; D1: Day 1; D7: Day 7; D14: Day 14

Table 3: Effect of essential oil of *Hyptis suaveolens* leaves on weight growth of treated Wistar rats.

EO	Dose (mg/Kg bw)	Weight growth (g)		
		D1-D7	D1-D14	D7-D14
HS	500	0.35^{b}±0.0008	0.37^{a}±0.006	0.02^{b}±0.002
	250	0.47^{b}±0.03	0.45^{a}±0.02	-0.01^{b}±0.0028
	100	0.26^{a}±0.0007	0.40^{a}±0.005	0.11^{a}±0.002
Control	-	0.19^{a}±0.001	0.36^{a}±0.002	0.13^{a}±0.00003

a, b, c averages in the same row with different superscript as significantly different (P<0.05). D1: Day 1; D7: Day 7; D14: Day 14

Effect of essential oils on hematological and biochemical parameters of treated Wistar rats

According to the analysis of hematological and biochemical parameters, the administration of the essential oil of *the* leaves of *Ocimum gratissimum*, is associated with an increase in the level of blood white blood cells, creatinemia, urea and transaminases compared to the control lot (Table 4). The effect of the essential oil on these parameters is dose dependent (p<0.05). Similarly, administration of the essential oil of *Hyptis suaveolens* leaves was associated with a significant difference (p<0.05) in white blood cell and urea levels compared to the control lot (Table 5). At doses 250 and 500mg/Kg bw, the essential oil of *Psdium guajava* leaves induced elevated white blood cell and urea levels in the treated rat lots compared to the control lot (Table 6). The elevated values of transaminases (AST and ALT) are obtained in rats treated with 500mg/Kg bw of the essential oils of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* leaves.

Table 4: Effect of essential oil of *Ocimum gratissimum* leaves on hematological and biochemical parameters of Wistar rats

OG (mg/Kg bw)	Urea (g/L)	Creat (mg/L)	AST (IU)	ALT (UI)	WBC ($x10^9$/L)	RBC ($x10^6$/mm^3)	Ht (%)	Hb (g/dL)
100	8.77^{b}±0.18	0.41^{b}±0.04	18.63^{d}±1.61	161.8^{c}±55.72	4.52^{c}±0.10	7.40^{b}±0.17	47.75^{a}±1.13	14.55^{a}±0.43
250	7.87b±0.54	0.36^{b}±0.01	21.83^{c}±0.51	221.42^{b}±171.47	5.48^{b}±0.17	7.42^{b}±0.05	45.55^{a}±1.01	14.25^{a}±0.14
500	9.88c±0.01	0.43^{b}	50.63^{b}±0.03	257.42^{b}±8.80	5.85^{b}±0.13	7.60^{b}±0.23	45.00^{a}±0,17	14.30^{a}
Control	5.85^{a}±0.38	0.23^{a}±0.03	55.00±28.70	116.10^{a}±16.60	3.15^{a}±0.26	5.25^{a}±0.05	44.65^{a}±0.43	15.30^{a}±0.29

a, b, c,d averages in the same row with different superscript as significantly different (P<0.05). OG: *Ocimum gratissimum*, AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; WBC: White Blood Cell; RBC: Red Blood Cells; Ht: Hematocrit; Hb: Hemoglobin.

Table 5*:* Effect of essential oil of *Hyptis suaveolens* leaves on hematological and biochemical parameters of Wistar rats

HS (mg/Kg/bw)	Urea (g/L)	Creat (mg/L)	AST (IU)	ALT (UI)	WBC ($x10^9$/L)	RBC($x10^6$/mm^3)	Ht (%)	Hb (g/dL)
100	4.49^{c}±0.14	0.22^{a}±0.05	28.81^{c}±8. 57	64.92^{d}±5,23	4.40c±0.12	4.59^{a}±0.34	39.00^{a}±2.89	14.25^{a}±0.84
250	5.75^{a}±0.11	0.25^{a}±0.03	67.80^{b}±35.50	220.00^{b}±26.20	5.20b±0.07	5.33^{a}±0.10	43.90^{a}±0.06	15.00^{a}±0.06
500	8.67^{b}±0.12	0.20^{a}±0.01	75.10^{b}±12.10	300.30^{c}±65,80	6.55d±0.78	7.60^{b}±0.46	44.25^{a}±0.61	15.55^{a}±0.43
Control	5.85^{a}±0.38	0.23^{a}±0.03	55^{a}.00±28.70	116.10^{a}±16.60	3.15a±0.26	5.25^{a}±0.05	44.65^{a}±0.43	15.30^{a}±0.29

a, b, c averages in the same row with different superscript as significantly different (P<0.05). HS: *Hyptis suaveolens*, AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; WBC: White Blood Cell; RBC: Red Blood Cells; Ht: Hematocrit; Hb: Hemoglobin.

Table 6: Effect of essential oil of *Psidium guajava* leaves on hematological and biochemical parameters of Wistar rats

PG (mg/Kg/bw)	Urea (g/L)	Creat (mg/L)	AST (IU)	ALT (UI)	WBC ($x10^9$/L)	RBC($x10^6$/mm^3)	Ht (%)	Hb (g/dL)
100	5.46^{a}±0.89	0.24^{a}±0.01	29.5^{c}±9.3	50.60^{c}±16.10	2.25^{d}±0.03	5.11^{a}±0.06	44.15^{a}±0.09	14.10±0.06
250	6.00^{a}±0.16	0.19^{c}±0.02	39.21^{a}±0.63	131.00^{a}±64.50	4.60^{c}±0.52	5.12^{a}±0.05	43.55^{a}±0.43	14.30±0.06
500	10.65^{b}±0.21	0.48^{b}±0.02	48.18^{b}±10.11	209.10^{b}±80.50	5.20^{b}±0.56	8.17^{b}±0.11	50.15^{b}±1.47	15.00±0.52
Control	5.85^{a}±0.38	0.23^{a}±0.03	55.00±28.70	116.10^{a}±16.60	3.15^{a}±0.26	5.25^{a}±0.05	44.65^{a}±0.43	15.30^{a}±0.29

a, b, c, d averages in the same row with different superscript as significantly different (P<0.05). PG*: Psidium guajava*, AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; WBC: White Blood Cell; RBC: Red Blood Cells; Ht: Hematocrit; Hb: Hemoglobin.

Comparison of the effects of essential oils of *Ocimum gratissimum, Hyptis suaveolens and Psidium guajava* on hematological and biochemical parameters of treated rats

Table 7 compares the effect of administration of essential oils of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* with each other on the renal parameters of treated lots of wistar rats. There was no significant difference between OG, HS and PG on transaminases and hemoglobin levels of treated batches of rats (p>0.05). However, the OG treatment increased urea, creatinemia and red blood cell levels more. PG, and HS treatments showed similar activity on blood urea and red blood cell levels. The OG and HS treatments showed similar activity on blood white blood cell count.

Table 7: Comparison of the effects of essential oils of *Ocimum gratissimum, Hyptis suaveolens and Psidium guajava* on hematological and biochemical parameters of treated rats

Parameters	Essential oils				p-value
	Témoin	HS	OG	PG	
Urea (mg/L)	5.85^{b}±0.38	6.30^{ab}±0.62	8.84^{a}±0.33	7.37^{ab}±0.87	0.030
Creatinemia (mg/L)	0.23^{b}±0.03	0.22^{b}±0.02	0.40^{a}±0.02	0.30^{ab}±0.05	0.002
AST (UI)	116.10±16.60	195.10±44.90	141.98±7.85	209.10±80.50	0.111
ALT (UI)	55.00±28.70	57.20±13.20	53.00±16.10	65.20±25.90	0.973
WBC($x10^9$/L)	3.15^{b}±0.26	5.38^{a}±0.39	5.28^{a}±0.21	4.02^{ab}±0.50	0.007
RBC($x10^6$/mm^3)	5.25^{b}±0.05	5.84^{ab}±0.48	7.53^{a}±0.10	6.13^{ab}±0.51	0.012
HB (g/dl)	15.30±0.29	14.93±0.33	14.37±0.14	14.47±0.20	0.133
Ht (%)	44.65±0.43	42.38±1.20	46.10±0.61	45.95±1.14	0.050

a, b, c, d averages in the same row with different superscript as significantly different (P<0.05). OG: *Ocimum gratissimum;* HS: *Hyptis suaveolens;* PS*: Psidium guajava*; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; WBC: White Blood Cells; RBC: Red Blood Cells; Ht: Hematocrit; Hb: Hemoglobin.

Discussion

Toxicity studies make it possible to evaluate the short, medium and long term impacts that the intake of a given substance could have on the organism. In the control of gastrointestinal parasitosis of small ruminants and humans, the leaves of *Ocimum gratissimum, Hyptis suaveolens* and *Psidium guajava* (three aromatic medicinal plants) are used. They could be an alternative in the fight against parasite resistance to synthetic anthelmintics and reduce ecotoxicity. In the present study, the acute toxicity of the essential oils of these three medicinal plants were evaluated on Wistar rats at different doses (100; 250 and 500 mg/Kg bw) in order to evaluate the impacts that their uses could have on the organism. According to the work of Bezerra [9], on *Artemia salina* larvae, the essential oil of *Hyptis suaveolens* leaves can be toxic. Similar results had been reported instead by Kpadonou [10] for the essential oil of *Ocimum gratissimum*. According to the Work of Fandohan [11], the LD50 of *Ocimum gratissimum* essential oil is 1750 mg/Kg bw. However, only the rats having received doses of 5-500 mg/Kg bw not shown any sign of toxicity like torpid and death. This justifies the choice of the doses used in this study.

In the present study, in general, it was observed that the essential oils of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* leaves influenced the growth of rats, their hematological parameters (White blood cell count) and biochemical parameters (Urea, creatinemia, transaminases) after 14 days of observations compared to the untreated lot. On the growth of rats, the essential oil of *Hyptis suaveolens* leaves stimulated a weight gain of the animals but with a low rate at the dose of 500mg/Kg bw. The essential oils of *Ocimum gratissimum* and *Psdium guajava* leaves, comparedto the control lot, significantly (p<0.05) decreased the growth rate of the rats with more than 10% reduction at the dose of 500mg/Kg bw. These results suggest that the essential oils of the leaves of *Psidium guajava* and *Ocimum gratissimum*, can be potentially toxic. Indeed, a significant weight loss of more than 10% is a sign of toxic effects caused by the intake of a substance [12, 13]. It could be explained by the presence in these essential oils of anti- nutritional compounds responsible for a decrease in food intake of treated rats [14]. According to the work of Efosa and Udoha [15, 16], repeated high dose administration of *Ocimum gratissimum* leaf extract, can be toxic. However, it followed from the work of [12, 17], that aqueous and methanolic extracts of *Psidium guajava* leaves are free from toxicity. However, from the dose 1200 mg/Kg bw, some signs of toxicity of the ethanolic extract were observed by Manikandan [18] who estimated 600mg/Kg bw as the non- toxic dose. The essential oil of *Ocimum gratissimum* leaves, acted on the hematopoietic system of rats by a significant (p<0.05) increase in the blood white blood cell count. These results corroborate [16]. The same phenomenon was induced by the essential oil of *Psidium guajava* and *Hyptis suaveolens* leaves at the dose of 500 mg/Kg bw. The increase in the number of blood white blood cells is observed in inflammatory processes [14, 19]. Urea an important nitrogenous metabolic waste product released during protein metabolism and creatinine produced and released in body fluids showed an increase in their levels in lots of rats treated with the essential oils at doses of 250 and 500mg/Kg bw. They are biomarkers for determining renal function. An increase in their concentration in the blood indicates an alteration of the renal function and also the toxic effects that a compound could have [18]. An increase in transaminase levels is observed at the dose of 500mg/Kg bwmay suggest liver damage. Essential oils from the leaves of *Ocimum gratissimum, Psidium guajava* and *Hyptis suaveolens* may have beneficial biolological effects on organisms however at high doses and this from 500mg/Kg bw they showed signs of toxicity. The high toxicity of essential oils can be explained by their lipophilic character which makes them capable of damaging the plasma membrane by affecting the structure of polysaccharides, the phospholipids [9]. The complexity of the chemical composition of the essential oils of the leaves of these three medicinal plants can explain the different results obtained. However, some factors such as metabolism and bioavailability of these essential oils which are not taken into account in the present work are important to explain the mechanisms of action of these essential oils in the organism of animals and human.

Conclusion

In sum, the essential oils of the leaves of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava* may show signs of toxicity above a level. Any abusive use should be avoided. Essential oils give satisfactory results at very small doses compared to the doses used in this study. The complexity of the chemical composition of the essential oils of medicinal plants and their mechanisms of action may explain the variations obtained in the results obtained from lots of rats treated with the essential oils of the leaves of *Ocimum gratissimum*, *Hyptis suaveolens* and *Psidium guajava*. Histological analyses of the organs and complementary studies of chronic and sub-chronic toxicity will be necessary for a good use of the essential oils of thesemedicinal plants.

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