

Examining The Acute And Subacute Toxicity Of Plectranthus Glandulosus Hook Essential Oil In Laboratory Rodents

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Abstract

Context, The fragrant plant of the *Plectranthus glandulosus* species, is widely used in traditional medicine in Africa, especially in Congo Brazzaville, to treat a variety of illnesses, including genitourinary and digestive diseases.

Objective, The aim of this study was to assess the acute and subacute toxicity of *P. glandulosus* leaf essential oil in laboratory rodents with the goal of adding to the value of medicinal plants.

Methods, The oral toxicity was conducted on mice and rats of the same sex were in a process that followed the 420 and 407 OECD guidelines for testing of chemicals.

Results, In terms of acute toxicity, the mice's overall behavior was only marginally altered by the 400 mg/kg single dose, and it did not result in their demise. However, when compared to control mice, the weight gain was significantly higher. The subacute toxicity study's findings demonstrated that giving rats 200 mg/kg of essential oil daily for two weeks significantly reduced their body weight and the mass of their internal organs (heart, spleen, lung, and left kidney). Certain biochemical parameters (Alanine Transaminase, Aspartate Transaminase, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and creatinine) showed a significant increase at this same dose. Therefore, in comparison with the rats in the group control (5 mg/kg), the treated rats (200 mg/kg) showed the appearance of inflammatory foci in their hepatocytes and the destruction of their pulmonary alveoli in terms of the histopathological parameters of their liver and lungs.

Conclusion Although there was no mortality observed throughout the study, the results suggest moderate consumption of *P. glandulosus* essential oil.

Keywords: *Plectranthus glandulosus*, toxicity, biochemical, hematological.

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I. Introduction

Medicinal plants have been used for their therapeutic properties in the field of human health for centuries (Petrovska, 2012). They are used worldwide in a variety of ways,

including topical application, ingestion, and inhalation. They can be found raw, crushed, or macerated. They have either curative or preventive effects on the human body. Every organ is used for profit, including the trunk, flowers, and every root and leaf. Nonetheless, the majority of their extracts and the more stable and effective synthetic versions of their active ingredients are used in industrial production and sold abroad (Fridlender et al., 2015; Gurib-Fakim, 2006)

In nations like the Congo, which boasts a great deal of floral diversity, medicinal plants represent untapped economic potential (Lachenaud, 2009). Up until that point, higher plants that are frequently used have been the main focus of research. Studies with other groups are still in their infancy. This holds true for aquatic plants, fungi, and algae, all of which are examined elsewhere (Ślusarczyk et al., 2021).

However, there is a risk of poisoning when consuming medicinal plant products (George, 2011; Röder/ Roeder, 2000), There have been sporadic reports of poisoning cases connected to the use of phytomedicine (Bnouham et al., 2006; Ghorani-Azam et al., 2018; Oliveira et al., 2020). Both naturally occurring toxicants and substances that are beneficial in certain amounts can cause poisoning.

The essential oil of *P. glandulosus* is derived from an aromatic plant belonging to the Lamiaceae family. The genus *Plectranthus* comprises several species that have demonstrated biological activities (Ahamed et al., 2023; Lukhoba et al., 2006; Rice et al., 2011). This plant is used to treat influenza, wounds, and postpartum pain by making herbal tea (Bertin et al., 2020; Lukhoba et al., 2006). Its oil's antibacterial and insecticidal qualities have been emphasized by recent research ((Leopold et al., 2002; Tatsadjieu et al., 2008).

II. Materials And Methods

Materials

Plant material

A sample of *P. glandulosus* leaves from the village of Douakani in the Lékoumou department, in the southwest of the Republic of Congo, made up the study material. These leaves were exposed to a seven-day drying process in a brick shelter and on a slatted platform locally. Every day, they were rotated and arranged in a single layer.

Animal material

We used 18-week-old Wistar strain rats weighing between 120 and 187 g and male albino mice, aged 14 ± 2 weeks, weighing between 23 and 26 g. The Marien NGOUABI University of Brazzaville, Republic of Congo, Faculty of Science and Technique's animal facility provided all of these animals. The animals were kept in standard conditions, which included $26 \pm 1^\circ\text{C}$ ambient temperature, 12 hours of light, and 12 hours of darkness. Every animal had unrestricted access to common food and drinking water.

Methods

Extraction of essential oil from *P. glandulosus* leaves for toxicity studies.

Traditionally, the essential oil from *P. glandulosus* leaves was extracted near Douakani through hydrodistillation using a handcrafted waterfall distiller. Three kilograms of dry leaves were added to an eight-liter container of water. A wood fire served as the source of heating, while a waterfall's 22°C water stream cooled the vapors. After being dried over anhydrous sodium sulfate, the extracted essential oil was used in toxicity tests.

Acute toxicity study.

To investigate acute toxicity, three mice of the same sex were used in a process that followed the 420 OECD guideline for testing of chemicals (Acute Oral Toxicity – Fixed Dose Procedure). The duration of the experiment was fourteen (14) days. Two (2) batches of three (3) mice each of the same sex, were assembled as follows for this purpose:

In Lot 1 (control group), mice were given a dose of 5 milliliters per kilogram of distilled water; in Lot 2 (treated group), mice were given a dose of 400 milligrams per kilogram per kilogram of *P. glandulosus* essential oil. The mice were placed in individual cages for observation. Gross symptoms observed included ptosis, piloerection, urinary excretion, reaction to external stimuli, stool status, and general animal behavior (aggression, mobility, vocalization, and convulsions). The animals were observed at $\frac{1}{2}$, 1, 2, 3, and 4 hours after administration of each product (essential oil or distilled water). Mortality was assessed within 48 hours of administration. The mice were left under observation for 14 days to detect the late appearance of signs of toxicity. Every other day, body weight, food, and water intake were recorded for 14 days.

Subacute toxicity

Subacute toxicity testing was carried out in compliance with the 407 OECD guideline for testing of chemicals (Repeated Dose 28-Day Oral Toxicity Study in Rodents), which has been slightly modified. Two batches of 5 rats (males) were distributed as follows:

- Lot 1 (control) of rats had received distilled water at a dose of 5 mL/kg;
- Lot 2 (treated) of rats treated daily with *P. glandulosus* EO (200 mg/kg).

For a duration of 14 days, the rats were given oral treatment consisting of distilled water or *P. glandulosus* essential oil. They were also weighed every day. The animals' body weights were recorded at the end of each week. All rats were put to sleep on the fifteenth day, after which they were sacrificed. Arteriovenous blood was drawn into EDTA tubes for hematological tests (white and red blood cell counts, hematocrits, and hemoglobin) and dry tubes for biochemical analyses (creatinine, transaminases including ASAT and ALT, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, urea, and uric acid). Subsequently, the organs (heart, liver, lung, spleen, left and right kidney) were extracted and examined under a microscope to detect any potential lesions. The relationship shown below was used to calculate relative organ weights:

$$Pr = \frac{PO}{100} \times 100;$$

Pa

Pr being the relative weight of the organ in grams per 100 g, P_O weight of the organ in grams, and P_a the body weight of the rat in grams.

Statistical analysis

Excel (Office 2016) was used for the statistical analysis, and the results were expressed as average \pm ESM. The Student's t-test was used to compare the average parameter values of the treated lots with the control lot ($p < 0.05$, $p < 0.01$, $p < 0.001$). The significance threshold has been set at $p = 0.05$. For values of p less than 0.05, the differences between the means have been deemed significant.

III. Results*P. glandulosus* leaf essential oil's acute toxicity

Impact on mouse's overall behavior

The effect of *P. glandulosus* leaf essential oil on mouse's general behavior is displayed in Table 1 below. When administered at a 400 mg/kg dose, the oil marginally altered the mouse's overall behavior, in contrast to the control group, which was given distilled water at a 5 mL/kg body weight. The increase in heart and respiratory rate was the most noticeable symptom.

Table 1: general condition of the animals after administration of water or *P. glandulosus* E.O

	Setting	
	Distilled water (5 mL/kg)	
	Treatments	
	EO (400 mg/kg)	
Number of animals	3	3
Mobility	N	N
Aggressiveness	N	N
Condition of stools	N	N
Tremor	A	A
Sleep	A	A
Pain sensitivity	N	N
Vomiting	A	A
Vocalization	A	A
Pilo -erection	A	A
Ptosis	A	A
Vigilance	N	N
Heart rate and rhythm and breathing	N	I
Number of deaths	0	0

ED: distilled water ; EO: essential oil ; A: absent ; N : normal ; I: increase.

Effect on the body weight of mice

The progression of the mouse's weight gain versus time is depicted in Figure 1. We note that the mean values of the treated mice in D4 (** $p < 0.01$), D6, and D14 (** * $p < 0.001$) were significantly different from the controls. One could argue that *P. glandulosus* essential oil stimulated weight gain in treated animals.

Impact on each mouse batch's food intake

Figure 2 shows the effect of *P. glandulosus essential oil* on food intake per batch of mice. In general, animals given 400 mg/kg of essential oil consumed less food than animals given controls.

Effect o on each batch of mice's water consumption

Figure 3 illustrates how *P. glandulosus* essential oil affects mice's fluid intake. Compared to controls, treated mice drank more fluids, as we saw. However, within the treated batch, this water intake progressively decreased.

Figure 3: Variation in water consumption of control and treated mice. Results are expressed as mean \pm standard error, $n = 5$ rats per batch

Subacute toxicity of the essential oil of the leaves of *P. glandulosus*

Effects on rat body weight.

The rats' weight changes over a 14-day period are displayed in Figure 4. The treated animals did not

significantly gain weight over the course of the two weeks, in contrast to the controls (**p > 0.01 and ***p > 0.001).

IV. Discussion

At a single dosage of 400 mg/kg, the acute toxicity study's findings demonstrated that EO from *P. glandulosus* leaves did not result in short-term death. However, there were some toxicity indicators (an increase in heart and respiratory rates) at this same dosage. These findings indicate that mice are able to tolerate this essential oil well because, when the dose given to humans is multiplied by ten, it does not result in death. In terms of the weight shift, it seems that the *P. glandulosus* leaves' essential oil (EO) significantly increased the animals' weight in comparison with those in the control group. The substantial water intake observed throughout the experiment may help to explain this weight gain.

When compared with control rats, the treated rats showed a significant weight loss (S 1: **P < 0.01; S 2: ***P < 0.001) at a daily dose of 200 mg/kg, as demonstrated by the subacute toxicity results. Additionally, the animals' metabolisms were altered. The decrease in food intake that has been noticed is unavoidably evidence of the cause of weight loss that has been brought about by giving 200 mg/kg of *P. glandulosus* leaves EO. This implies that the EO of *P. glandulosus* leaves would prevent rats from gaining more body mass by suppressing the appetite centers, which are found at the level of the hypothalamus.

In regard to the organs' weight, the administration of EO derived from *P. glandulosus* leaf at a dose of 200 mg/kg would affect the organs by significantly increasing the mass of the heart, spleen, lung, and left kidney. However, it results in a non-significant decrease in the mass of the right kidney and liver at the same dose. The expansion of the previously mentioned organs indicates that HE would work by causing proteins or even lipids to build up on noble organs. The non-significant reduction in the mass of the right kidney and liver that was seen indicates that *P. glandulosus* leaf extract (EO) may have hepatoprotective quality.

The hematological analyses performed in this study on rats treated with EO of *P. glandulosus* leaf and controls revealed a non-significant decrease in the number of red blood cells, white blood cells, platelets, CCMH, and MCV in addition to the hemoglobin level. This implies that the hematopoietic system is not affected by this essential oil.

Research shows that *P. glandulosus* leaf EO at a daily 200 mg/kg dose significantly increases ALT and AST activities (*p < 0.001) in terms of biochemical and histopathological parameters. Hepatocyte cell necrosis and liver inflammation are two potential explanations for this increase (Amacher, 1998; Kobayashi et al., 2020; P Sharma, 2014). The presence of

inflammatory foci at the hepatocyte level and the destruction of the pulmonary alveoli in rats treated with essential oils are shown by the histological sections that show the ultrastructure of the liver and lung cells, lending credence to this theory.

The triglyceride, total cholesterol, and LDL-cholesterol levels significantly increased (**P < 0.001) in the treated animals, indicating that the EO of *P. glandulosus* leaves may have caused liver damage (Honma & Suda, 1997). This increases the chance of atherosclerosis and subsequent cardiovascular illnesses.

V. Conclusion

The essential oil of *P. glandulosus* leaves does not result in the death of treated subjects at tested doses and times, according to the acute and subacute toxicity studies carried out during this work. On the other hand, in treated animals, it causes weight gain or loss, increases the mass of internal organs, elevates transaminases (ALAT and ASAT), creatinine, total cholesterol, HDL, LDL, and TG, and modifies general behavior slightly. Histological sections have shown that the pulmonary alveoli have been destroyed and that there are inflammatory foci at the hepatocyte level. *P. glandulosus* essential oil should be used extremely cautiously, just like any other essential oil.

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Conflicts Of Interest Statement

The authors whose names are listed immediately below certify that they have NO affiliation with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancy, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interests (such as personal or professional

relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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