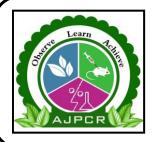
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STUDY OF THE ACUTE TOXICITY OF PERSEA AMERICANA IN WISTAR STRAIN RATS (RATTUS NORVEGICUS)

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ABSTRACT

Persea Americana is a plant known for its medicinal properties. Its use is therefore unregulated. This raises the problem of uncontrolled use of this plant. This study aims to evaluate the acute toxicity of the aqueous extract of Persea Americana leaves on witsar rats. Methodology: The phytochemical study of Persea Americana was carried out and the toxicity was evaluated by orally administering a single dose of 2000mg/kg of body weight to groups of rats and distilled water to two other groups of control rats. The toxicological parameters were observed compared to the control groups for 14 days. Results: The phytochemical study identified sterols, tannins, quinones, flavonoids, polyphenols, terpenes and saponins. The single dose of 2000mg/kg did not induce death in the animals, no signs of apathy, breathing problems, or bleeding etc. were observed. On the other hand, a decrease in the quantity of food and water ingested as well as in weight was recorded but this was not significant compared to the control groups. Conclusion: The results obtained from this study confirm the usefulness of *Persea Americana* in traditional medicine. Although this plant would be non-toxic at the dose of 2000mg/kg bw, further studies are necessary to define the dose necessary for longer exposure.

KEYWORDS

Persea Americana, Aqueous extract, Phytochemical and Toxicity.

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INTRODUCTION

The use of Persea Americana leaves as an antidiabetic and anti-inflammatory treatment is no longer a myth. Research has shown the interest of this plant for the medicinal treatment of several 2011)¹. Previous work pathologies (Koane, mentions an anti-diabetic effect (Adiko et al, 2014)². However, the mechanism of action is not sufficiently elucidated (Ojo et al, 2022)³. In 72

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addition to its nutritional and cosmetic qualities, Persea Americana is highly valued for its medicinal benefits (Lima et al, 2012)⁴. So much so that depending on the cultures and beliefs of the people, it is no less used in traditional medicine. In developing countries, around 80% of populations use traditional medicine, particularly those from rural areas (Zeggwagh et al, 2013)⁵. This practice is also spreading in cities, where residents are increasingly using this medicine (Guele and Koffi, 2021)⁶. Indeed, traditional plant-based medicine seems more accessible to populations and due to several other factors, medicinal plants are therefore the first treatment option (Kati-Coulibaly et al, 2021)⁷. Despite efforts by states to facilitate access to medical care, opinions are controversial. A study conducted by Chaachouay (2020)⁸ in Morocco showed that users prefer to resort to traditional medicine because it would have proven beneficial effects and would not carry any health risks. Thus, medicinal plants are widely administered either in infusion or decoction for therapeutic or preventive purposes of certain pathologies, particularly metabolic diseases (Kati -Coulibaly et al, 2021)⁷. On the other hand, the work of Sekkat et al, 2021⁹ showed the existence of adverse effects associated with medicinal plants. In a word, the use of medicinal plants is not without danger. Because research studies mention a problem of toxicity that can cause death with plant-based treatments. This is why measures have been taken at the global level through the WHO to guide the use of this type of medicine (WHO, 2013)¹⁰. The West African Health Organization has joined this initiative by publishing a book on the West African pharmacopoeia. This is a contribution to the promotion of medicinal plants which informs on the basis of the results of scientific research the benefits and risks associated with their use (WAHO, 2020)¹¹. Regarding Persea Americana, its therapeutic effects are currently being studied and the recent discoveries of its antidiabetic and anti-inflammatory properties pose the problem of uncontrolled use. It is therefore appropriate to direct research on this plant. This study aims to evaluate the toxicity of the aqueous extract of *Persea Americana leaves* on witsar rats. It aims to provide additional answers on the risks associated with the use of this plant.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Persea Americana* were harvested in the town of Azaguie (Agneby region) Tiassa, in the south of Ivory Coast, 40km from Abidjan. The ground material obtained after drying was diluted in 1 liter of water and filtered. The aqueous extract of the leaves of *Persea Americana* (ETA) is obtained by drying the filtrate in an oven.

Animal material

Nulliparous and virgin female rats of the same species were selected. Their weight varied between 100 and 125g. These animals were kept under the same breeding conditions.

Phytochemical screening

The tube staining method was used to identify the active ingredient of Persea Americana leaves. Phytochemical tri-analysis through staining and precipitation reactions was specific to each chemical compound (Kouakou et al, 2021)¹². Thus, sterols and polyterpenes were highlighted by the Liebermann reaction with the solution of 1ml of acetic anhydride and 0.5ml of sulfuric acid. Polyphenols were characterized by the reaction with ferric chloride (FeCl3). Flavonoids were revealed by the reaction with cyanidin and catechic tannins using STIASNY reagents. Quinonic substances were highlighted using BORNTRAEGEN reagents hydrochloric acid. The reagents DRAGENDORFF (potassium iodobismuthate reagent, BOUCHARDAT (iodide-iodine reagent) and VALSEN-MAYER (potassium iodomercurate reagent) were used to identify the alkaloids. As for the saponids, they were revealed by the foam test.

Toxicological study of Persea Americana

For this study, the method chosen is that described by OECD guideline 423 (OECD, 2002). Thus, we chose an initial dose of 2000mg/kg BW above the doses of 5, 50, 300mg/kg BW. To do this, two batches of three fasting rats (batch 1 and batch 2)

were formed, these were weighed before the treatment which was carried out as follows:

Batch 1: Rats treated with 2000 mg/kg of PC of ETA from P. americana

Batch 2: Rats treated with distilled water (10ml/kg of BW).

After administration, the animals were again kept fasting for 4 hours and the behavior of each animal was observed each in 30-minute episodes daily for 14 consecutive days. Observation focused on the following signs: Apathy; excitement, breathing difficulties; refusal of food; oral and/or nasal bleeding; abdominal pain (writhing); convulsion; tremor; diarrhea; coma; death.

Assessment of food and water intake and weight gain

This study consisted of evaluating the toxicity of ETA from *Persea Americana* through the change in the feeding behavior of rats after administration of *Persea Americana* at a dose of 2000mg/kg BW. The criteria studied were: the amount of water absorbed, the amount of food ingested and the weight. These data were measured every two days for 14 days. Thus, two other batches of female rats (batch 3 and batch 4) were maintained in conditions similar to the first stage.

Statistical analysis

Statistical processing of the data was performed using Graph Pad Uninst_Prism7 software. The results are presented as means followed by the standard error of the mean (mean \pm SEM). Multiple comparisons and determination of significance levels were performed by the one- way ANOVA test and the differences were significant at the 0.05 level (p < 0.05).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical analysis of the aqueous extract of *Persea Americana* leaves identified the following chemical compounds: sterols, polyterpenes, catechin tannins, alkaloids, polyphenols, flavonoids and saponins. The results are shown in Table No.1.

Acute oral toxicity of *Persea Americana* leaves

The single dose administration of 2000 mg/kg BW of the oral extract of *Persea Americana ETA* did not cause death of the treated rats. Furthermore, no clinical signs of toxicity were recorded (Table No.2).

Effect of Persea Americana ETA on body mass

Slow decrease in the weight of the treated animals was observed compared to the control rats. However, no significant difference was observed between the weight gains of the rats receiving the single dose of extract and the control rats (p>0.05) during the 14 days of observation (Figure No.1).

Effect of *Persea Americana* ETA on water consumption

Water consumption of control animals and animals receiving a single dose of 2000 mg/kg/bw of the extract was respectively $20\pm1.15\text{ml}$ and $19\pm1.15\text{ml}$ during the first 2 days of the experiment. These quantities increased respectively to $46.37\pm5.89\text{ml}$ and $43.33\pm6.09\text{ml}$ on the 7^{th} day and then to $82.67\pm1.76\text{ml}$ and $76\pm9.84\text{ml}$ on the 14th day. No significant difference was observed in the water consumption of control and treated rats (p>0.05) (Figure No.2).

Effect of ETA Persea Americana on feed consumption

Regarding the quantities of food ingested, there were no significant differences between the animals receiving the single dose of 2000mg/Kg/BW of *Persea Americana extract* compared to the control rats (p>0.05). The quantities consumed by the controls were 42.26 ± 4.75, 49±6.35, 55.56±6.36 and 81.67±7.26 grams on the second, fourth, seventh and fourteenth days, respectively. Whereas, the rats receiving these doses had consumed 39.33±5.23, 46.67±6.64, 50.88±6.35 and 77±6.35 grams on days 2, 4, 7 and 14, respectively, as shown in Figure No.3. However, these quantities, although lower than those of the controls, were not significant during the study period.

Discussion

Phytochemical screening of *Persea Americana* showed similar results to those of Mamadou *et al*, $(2016)^{13}$ who evaluated the content of the

compounds identified in this study to those obtained from three different extracts. Furthermore, the catechic tannins present in this extract were rather identified in the ethanolic and methanoic extracts of Mamadou et al, (2016)¹³. They identified gallic tannins in their aqueous extract. According to Biaye, (2022)¹⁴, the dissolution of tannins would be in colloidal form in water and; they would be very soluble in alcohol. Thus, the aqueous extract would highlight more gallic tannins as observed by these authors. Tannins, thanks to their antibacterial, antiviral and anti-inflammatory properties (Biaye, 2022)¹⁴ give *Persea Americana* its modulating effects on the immune system. Similarly, Ayodeji et al, 2025¹⁵ found high concentrations of phenols and flavonoids in the aqueous extract of Persea Americana. According to Lima et al in 2012, flavonoids would be at the origin of the antidiabetic effect of this plant. Masengo et al, 2024¹⁶ confirm the presence of quinone in this extract. The diversity of chemical compounds identified in the aqueous extract of *Persea Americana* is proof of its use against several pathologies (Al- Otaibi et al, 2023¹⁷, Nascimento et al, 2025)¹⁸.

The study of the acute toxicity of ETA from *Persea Americana leaves* showed no significant impact on the observed parameters. In addition, the weight, quantities of food and water ingested experienced a decrease which was not significant during this study. This then indicates in accordance with the work of Mamadou *et al*, $(2016)^{13}$ that *Persea Americana* is not toxic. However any substance is toxic and this is relative to the administered dose. According to wallet $(2012)^{19}$, the observability of low-dose toxicity of substances depends on the measurement tools. It should take into account the notion of temporality relating to the latency time and the occurrence of pathological effects.

Furthermore, Zeggwagh et al. 2013⁵ during their work showed that out of a sample of 48 medicinal plants identified as non-dangerous, five of them were potentially toxic. As for the aqueous extract of Persea Americana, other works such as Padilla-Camberos et al, 2013²⁰ mention a toxic activity of the ethanolic extract of *Persea Americana* kernel at a concentration of 500mg/kg in rodents. While the literature review by Agunloye et al, 2025²¹ also supports that *Persea Americana* would be non-toxic and that, on the other hand, its toxic effects should be evaluated over the long term. Indeed, the short duration of the acute toxicity test would not be sufficient to assess the chronology of toxicity based on the dose-effect. Thus, the single dose of 2000mg/kg of PC administered to rats not being harmful implies that the LD 50 is higher than the dose studied (OECD, 2008)²². Furthermore, this extract at this dose could interfere in the long term with the nutritional balance and behavior of rats.

Table No.1: Chemical composition of the aqueous extract of Persea Americana leaves

C	Nia	Sterols and	Polyphenols	flavonoids	Tannins		Owinanaa	alkaloids		Cononing
3	S.No	Polyterpenes			Cat	Gal	Quinones	D	В	Saponins
	1	+	+	+	+	-	+	+	+	+

Gal: gallic (+): presence (-): absence, Cat: catechism, D: Dragendroff B: Bouchardat

Table No.2: Results of clinical signs of the acute toxicity test after 14 days of observation

S.No	Lots of animals Clinical sign	Lot 1: witness (Distilled water)	Lot 2: Treated (2000mg/kg of PC)
1	Apathy	-	-
2	Excitement	-	-
3	Breathing problems	-	-
4	Refusal of food	-	-
5	Mouth and/or nose bleeding	-	-
6	Abdominal pain (writhing)	-	-
7	Convulsion	ŀ	-
8	Trembling	ŀ	-
9	Diarrhea	-	-
10	Coma	-	-
11	Refusal of food	-	-
12	Apathy	-	-

(+): presence de signe clinique, (-): absence de signe clinique

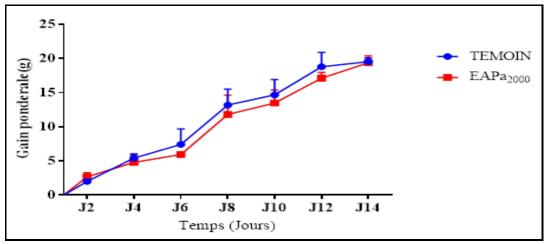


Figure No.1: Evolution of the weight of rats over time

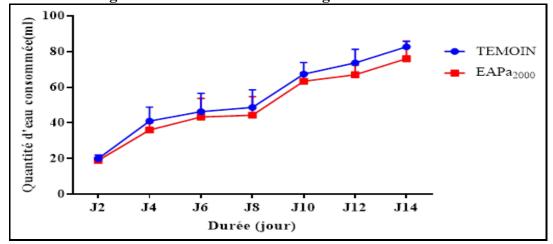


Figure No.2: Evolution of the weight of rats over time

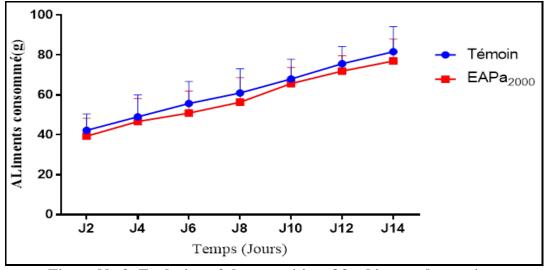


Figure No.3: Evolution of the quantities of food ingested over time

CONCLUSION

Conducting a screening of the chemical compounds of Persea Americana ETA is an asset for a better understanding of its mechanism of action on the pathologies it treats. The photochemical study of the aqueous extract of Persea Americana showed the presence of Sterols, Polyphenols, Flavonoids, Tannins, Quinones, Alkaloids and saponosides, giving it its various medicinal properties. No mortality and no major clinical signs of deficiency were observed during the toxicological study. Similarly, no major impact was observed on the feeding habits and nutritional balance of the rats. The studied dose of 2000mg/kg of *Persea American* PC would then be safe but it should be the subject of a complementary study to evaluate the necessary and safe dose for longer exposure.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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