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Antiradical and Sedative Non-Hypnotic Effect of Tikoni Tea Extract from Leaves of *Vitex madiensis* Oliv. (Lamiaceae) Locally Used in Congolese Villages

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Abstract

The present study aimed to contribute to the valorization of Congolese medicinal drugs and food plants. This study shows that the aqueous extract of Tikoni tea (200 and 400 mg/kg) significantly reduces motor activity and increases the duration of barbiturate-induced sleep in mice compared to diazepam (10 mg/kg). However, diazepam decreased time to sleep onset, whereas the extract increased it. The extract therefore has no hypnotic effect; the increase in latency time could be a stimulating effect. This study also revealed that this tea extract possesses free radical scavenging properties with an IC50 of 8.54 mg/mL, which are thought to be linked to the presence of secondary metabolites in this plant.

Keywords

Vitex madiensis, Tikoni Tea, Aqueous Extract, Antioxidant, Sedative

1. Introduction

Oxidation is an essential process in the digestion of aerobic cells in the body. It involves the oxygen molecule, whose production through uncontrolled metabolic pathways leads to the formation of reactive oxygen species (ROS) such as superoxide ($O_2^{-}\cdot$), hydroxyl ($HO\cdot$), alkoxyl ($RO\cdot$), and peroxyl ($ROO\cdot$). These radicals are implicated in oxidative stress, which is characterized by an imbalance between the production of ROS (prooxidants) and the elimination of these species

by the antioxidant defense mechanism [1].

Oxidative stress can damage molecules such as lipids, carbohydrates, proteins, and DNA. Free radicals are also implicated in the development of numerous diseases, including obesity, cancer, diabetes, inflammation, atherosclerosis, and neurodegenerative diseases [2] [3].

Antioxidants are a family of substances that can neutralize free radicals and thus prevent the onset of diseases associated with oxidative stress. Among the best-known natural antioxidants are alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), and phenolic compounds [4].

Insomnia is the most common sleep disorder encountered in sleep medicine. It is estimated that nearly 20% of the population suffers from chronic insomnia and 30% from occasional insomnia. Insomnia can be primary or secondary, and in both cases, transient (up to 3 months) or chronic (https://www.cenas.ch). Sedatives are a class of drugs used to treat anxiety and nervous tension. They soothe pain and facilitate sleep in cases of insomnia. They also help to relax agitated individuals. The person experiences a sense of calm; their breathing and reflexes slow down, and their muscles relax.

The genus *Vitex* is one of the largest genera in the Lamiaceae family, with approximately 250 species. It is widely distributed, but is found primarily in tropical zones with some in subtropical regions [5] [6]. Among these species is *Vitex madiensis*, which, due to its secondary metabolites. Indeed, this plant is widely used in traditional medicine to relieve or cure certain illnesses [7]-[9]. In Congo, people use it daily in the form of a tea locally called Tikoni, which means aches in the local language. Thus, the present study is undertaken to evaluate the antiradical and sedative effects of the aqueous extract of Tikoni, a preparation made from the leaves of *V. madiensis*, in laboratory mice.

2. Material and Methods

2.1. Animal Material

Swiss strain male mice, supplied by the animal facility of the National Institute for Research in Health Sciences (IRSSA) and transported to the Laboratory of Animal Pathophysiology and Experimental Pathophysiology to be reared under standard conditions (25˚C ± 5˚C, 40 - 70 HR), with a 12-hour light and 12-hour dark cycle and free access to water and standard food. Published ethics rules from the International Association for the Study of Pain (Zimmermann, 1983) were respected [10].

These male Swiss mice, aged 6 - 8 weeks and weighing 25 - 30 g, were randomly selected for the study. Animals showing signs of illness, abnormal behavior, or weight outside the specified range were excluded from the experiments to ensure uniformity of experimental conditions.

2.2. Vegetal Material: Tikoni Tea

The leaves of *Vitex madiensis* were collected in Brazzaville, in the Mayanga district of the 8th arrondissement (Madibou), in July 2020. Identification was carried

out by Dr. KAMI Emile, a systematic botanist and research professor at the Faculty of Sciences and Technologies (FST). Subsequently, these leaves were washed with water and dried in the shade at room temperature for 30 days. Once dried, they were then ground using a wooden mortar until a homogeneous powder was obtained (Tikoni), from which the extract was prepared.

2.3. Extraction

The Tikoni tea aqueous extract was prepared by maceration. 50 g of leaf powder was dissolved in a beaker containing 500 ml of distilled water and left to stand for 72 hours. After 72 hours, the mixture was filtered through absorbent cotton and then evaporated using a rotary evaporator until the dry extract was obtained. The dry extract, weighing 20 g and yielding 8%, was collected and stored at room temperature in a tightly sealed glass jar to prevent denaturation. The solution was dissolved in distilled water to prepare the solutions for administration at doses of 200 and 400 mg/kg for pharmacological testing [11].

2.4. Antioxidant Test

The DPPH radical reduction method, as described by Auniq *et al.* (2020), involves reducing DPPH with radical substances presumed to be present in Tikoni tea extract. 40 mg of the dry extract was weighed and then diluted 50/50 to the following concentrations: 20, 10, 5, and 2.5 mg/ml. Activity was then measured at 517 nm after 40 minutes of incubation in the dark using a UV-visible spectrophotometer [12] [13].

The percentage of inhibition was calculated using the following formula:

$$\mathrm{Ir} = \frac{\left[\mathrm{D.Oc} - \mathrm{D.Oe}\right]}{\mathrm{D.Oc}} \times 100$$

where D.Oc = Optical density of the negative control;

D.Oe = Optical density of extract/inhibitor;

Ir: Inhibition rate in %.

2.5. Solution Volume Given

The volume of each solution to be administered was calculated based on the dose and the animal's mass according to the following formula

$$V_{SD} = \frac{MA \times D}{C}$$

where V_{SD}: Volume of solution to be administered (mL);

MA: Mass of the animal (kg);

D: Dose to be administered (mg/kg);

C: Concentration of the solution (mg/mL).

Minimizing environmental factors on experimentation

Motricity test and barbiturate-induced sleep test were conducted systematically between 9 a.m. and 2 p.m. to minimize the influence of circadian rhythms. All tests were performed by the same experimenter under standardized light-

ing and temperature conditions. Minor environmental fluctuations were minimized.

2.6. Motricity Test

The Tikoni aqueous extract on locomotor activity was evaluated in mice housed in the cage with a gridded floor, following the experimental protocol described by Boissier and Simon in 1967, adapted by Nkundineza *et al.* (2020) [14]. The test consisted of counting the number of squares traversed by each mouse in a cage with a gridded floor containing 16 equally spaced holes, measuring 40 × 40 cm and 1.8 cm thick, after a fixed time. Four groups of five mice each were formed and treated orally as follows:

1) Group 1 (negative control) received distilled water at a dose of 0.5 mL/100 g per mouse;

2) Group 2 (positive control) received the reference substance, diazepam, at a dose of 10 mg/kg per mouse;

3) Groups 3 and 4 were treated with an aqueous extract of *Vitex madiensis* at doses of 200 mg/kg and 400 mg/kg per mouse, respectively.

One hour after administration, the animals were placed in a cage with a gridded floor. The number of squares traversed by each animal after five minutes was thus determined.

2.7. Barbiturate-Induced Sleep Test

According to the method described by Lechat *et al.* in 1964 and reported by Nkundineza *et al.* (2020) [14], this test consists of inducing sleep in mice by intraperitoneal injection of phenobarbital (Gardenal® injectable 40 mg/kg) at a dose of 70 mg/kg in mice, one hour after administration of the various products.

The administration of any substance with hypnotic properties potentiates phenobarbital-induced sleep by increasing the time to sleep onset and/or its duration. Four groups of five mice each were formed and treated orally as follows:

1) Group 1 (negative control) received distilled water (0.5 mL/100 g per mouse);

2) Group 2 (positive control) was treated with diazepam at a dose of 10 mg/kg per mouse;

3) Groups 3 and 4 received aqueous extracts of Tikoni at doses of 200 and 400 mg/kg, respectively.

After 1 to 49 minutes, the sleeping mice were placed on their sides. The time to onset and duration of sleep for each animal were determined. Sleep duration corresponds to the time elapsed between the moment the mouse lost the righting reflex and the moment this reflex reappeared. The loss or onset of the reflex was measured by tickling the tail or ear of each mouse with the pen. An awake mouse would react by raising its head or moving along the cage.

2.8. Data Processing

Statistical analysis was performed using Excel software 2016, and the compar-

ison of means between groups was carried out using Student's t-test. The values are presented as means ± standard error. The significance level was set at $p < 0.01$.

3. Results

Tikoni Tea aqueous extract antiradical effect

Figure 1 shows the quantitative measurement of the antiradical effect of the aqueous extract of TIKONI tea. We observe that the percentage of inhibition increases with concentration. The percentages of free radical inhibition are 14.81%, 33.10%, 58.53%, and 74.81% for concentrations of 2.5, 5, 10, and 20 mg/ml, respectively.

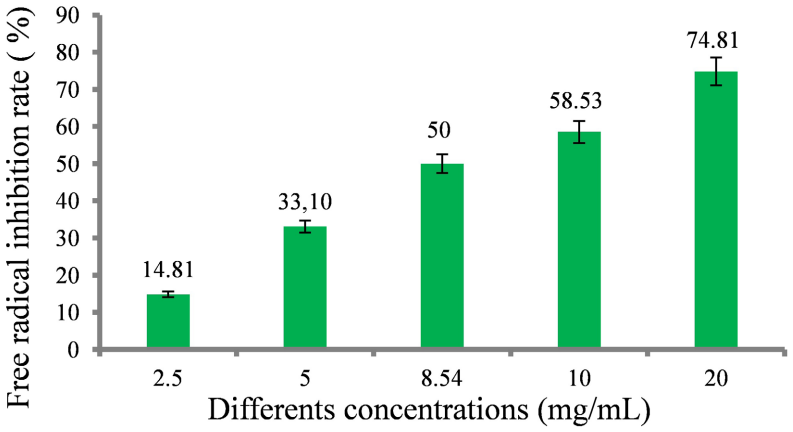

Figure 1. Antiradical effect of Tikoni tea aqueous extract.

Sedative effect of Tikoni aqueous extract

-Tranquilizing effect of Tikoni aqueous extract

Results of motor activity test

Table 1 presents the results of the motor activity test in mice treated with aqueous extract of Tikoni tea at doses of 200 and 400 mg/kg. Aqueous extract of Tikoni tea significantly reduces motor activity at doses of 200 and 400 mg/kg. These results also show that the animals receiving the extract at a dose of 200 mg/kg crossed fewer squares compared to those receiving the extract at a dose of 400 mg/kg and the reference molecule, diazepam (10 mg/kg).

Table 1. Tikoni tea aqueous extract effect on the motor activity of the mouse.

Product	Dosage	Number of squares crossed after five minutes
Distilled water	0.5 (a)	160.00 ± 3.74
Diazepam	10	123.40 ± 4.84***
Tikoni	200	117.20***
	400	122.20***

(a): In mL/100 g. Values are means ± SEM, with n = 5; ***$p < 0.001$ significant difference compared to mice that received distilled water.

Hypnotic effect of Tikoni tea aqueous extract

Table 2 presents the results of the barbiturate sleep test in mice under the effect of Tikoni tea aqueous extract, administered at doses of 200 and 400 mg/kg. These results show that Tikoni tea aqueous extract significantly decreases (***$p < 0.001$) the time to onset of barbiturate sleep in mice and significantly increases (***$p < 0.001$) the duration of barbiturate sleep in mice.

Table 2. Tikoni tea aqueous extract effect on barbiturate sleep in mice.

Product	Dosage (mg/kg)	Time to sleep onset (min)	Sleep duration (hours)
Distilled water + Gardenal (b)	0.5 (a)	16.40 ± 0.24	12.76 ± 0.19
Diazepam + Gardenal (b)	10	7.00 ± 0.31***	19.76 ± 0.19***
Tikoni + Gardenal (b)	200	30.80 ± 3.05***	19.56 ± 2.12***
	400	21.60 ± 1.13***	23.4 ± 1.66***

(a): In ml/100 g. (b): At 70 mg/kg. Values are means ± SEM, with n = 5; ***$p < 0.001$ significant difference compared to mice that received distilled water.

4. Discussion

This work, which contributes to the valorization of Congolese medicinal and food plants, was initiated to evaluate the antiradical and sedative effect of the aqueous extract of Tikoni tea made from *Vitex madiensis* leaves.

The DPPH test provides information on the reactivity of compounds with a stable free radical, DPPH, which exhibits a strong absorption band at 517 nm in the visible region. When the unpaired electron becomes paired in the presence of a free radical scavenger, absorption decreases and the DPPH solution is decolorized, changing from dark purple to light yellow. The degree of absorption reduction reflects the radical scavenging (antioxidant) capacity of compounds [15].

The results obtained show that the aqueous extract of Tikoni tea has free radical scavenging activity. Based on these results, a concentration of 8.54 mg/ml is required to inhibit 50% of free radicals. The inhibitory concentration (IC50) is inversely proportional to the antioxidant capacity of a compound. Indeed, if the IC50 is low, the plant has a high antioxidant potential [16] [17].

The antioxidant activity of the aqueous extract of Tikoni tea can be attributed to the presence of identified phenolic compounds, flavonoids, and tannins found in *Vitex madiensis* [12] [18] [19]. Flavonoids and tannins are phenolic compounds and plant phenolics constitute a major group of compounds that act as primary antioxidants or free radical scavengers [20].

The extract's free radical scavenging properties may be an important contributing factor to the plant's applications in the management and treatment of various diseases. Antioxidants prevent oxidative stress caused by free radicals that damage cells and vital biomolecules. They terminate the chain reactions triggered by free radicals by eliminating free radical intermediates and inhibiting other oxidation reactions.

Previous work on the leaves of other *Vitex* species has also shown the presence of phenolic compounds that could contribute to free radical scavenging activity [21] [22]. Our results are consistent with those of Boungou-Tsona *et al.* (2023) [12], who also demonstrated, with the methanolic extract of *Vitex madiensis*, a significant free radical scavenging activity correlated with a decrease in the production of reactive oxygen species (ROS), justified by the presence of ecdysteroids and flavonoids.

Similar activity has also been significantly demonstrated with the ethanolic extract and its hexane and ethyl acetate fractions on *Vitex donania*, another species of the same family and genus as *Vitex madiensis*, by Sarr *et al.* (2015) [22]. Using organic acid and ethanol extracts from the leaves of *Vitex madiensis* Oliv., Ngbolua (2020) also demonstrated interesting antioxidant activity [8]. The unique aspect of our work is the demonstration of this activity in an aqueous extract within the real-world context of this plant's use by farmers, without the use of an organic solvent.

To evaluate the sedative effect of aqueous Tikoni tea extract, two pharmacological tests were conducted: the tranquilizing effect by assessment of the extract's effect on the motor activity of mice and the hypnotic effect by the potentiation of barbiturate-induced sleep.

At doses of 200 and 400 mg/kg, aqueous Tikoni tea extract significantly reduced motor activity compared to distilled water. Specifically, a marked reduction in motor activity was observed in animals receiving the extract at a dose of 200 mg/kg. It was also observed that mice that received the extract at a dose of 200 mg/kg had an average of 117.20 squares traversed compared to an average of squares traversed by mice that received distilled water (160.00) at 0.5 mL/100 g and diazepam (123.40) at 10 mg/kg. The effect of the extract on motor activity does not appear to be dose dependent.

At a dose of 400 mg/kg, the extract appears to have comparable effects to that observed with diazepam on reducing motor activity, with an average of 122.20 squares traversed by mice receiving the aqueous extract of Tikoni tea and an average of 123.40 squares traversed by those receiving diazepam at 10 mg/kg. Chemical screening performed by Ntandou *et al.* (2018) revealed the presence of flavonoids [1].

Thus, the effect of Tikoni tea on motor activity appears to be linked to the presence of secondary metabolites, particularly flavonoids, found in this plant. These results are similar to those obtained by Auniq and his colleagues, who evaluated the anxiolytic, sedative and antioxidant activity of *Vitex peduncularis* and showed that flavonoids and tannins were at least partly responsible for the anxiolytic and sedative effects [13].

Tikoni tea significantly increased the onset and duration of barbiturate sleep, respectively, at doses of 200 and 400 mg/kg. Diazepam at 10 mg/kg was used as a reference. Diazepam decreased the onset of barbiturate sleep from 16.40 minutes (in negative controls) to 7 minutes. Diazepam increased the duration of barbiturate sleep from 12.76 minutes in negative controls to 19.76 minutes in positive

controls. However, the aqueous extract of Tikoni tea appeared to be more effective than diazepam in potentiating barbiturate sleep at a dose of 400 mg/kg, with an increase of 23.4 minutes. The potentiation of sleep would be linked to the presence of flavonoids in this plant. Indeed, Marder (2012) demonstrated a selective affinity of natural flavonoids for the benzodiazepine binding site, with a broad spectrum of effects on the central nervous system [23]. However, unlike diazepam, the extract does not decrease sleep latency, but rather significantly increases it. It is possible that the extract acts as a mild sedative or an allosteric modulator of the GABAA receptor, slowing neuronal activity without inducing immediate hypnosis. This corresponds to a decrease in motor activity and an increase in sleep duration, but without the direct hypnotic effect of diazepam. Furthermore, the significant increase in sleep latency would indicate a stimulating effect [24]. This paradoxical profile could be explained by competition between different neurotransmitters stimulated or blocked by the extract. Indeed, an extract is a mixture of several chemical groups and molecules capable of acting simultaneously on different types of receptors and inducing different pharmacological effects [24].

Our results on the potentiation of barbiturate-induced sleep are similar to those obtained by Nkundineza *et al.* (2020), the potentiation of barbiturate-induced sleep is linked to the presence of phenolic compounds, specifically flavonoids [1] [14].

5. Conclusion

The study found that the aqueous extract of Tikoni tea has antioxidant, sedative (tranquilizing and hypnotic), and significant effects, which could be attributed to its secondary metabolites, particularly phenolic compounds. However, this study used a crude extract, and the specific bioactive compounds responsible for these effects were not identified. Future work should focus on isolating and characterizing these active constituents to better understand the mechanisms underlying the observed activities.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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