

PHYTOCHEMICAL SCREENING AND SAFETY OF A POLYHERBAL TEA BASED ON LIPPIA MULTIFLORA, ZINGIBER OFFICINALE AND MENTHA PIPERITA

ABSTRACT

SUMMARY

Aims: *Lippia multiflora*, *Zingiber officinale*, and *Mentha piperita* are aromatic plants known for their health benefits. This study aims to determine the phytochemical composition and evaluate the safety of a tea made from them for safe human consumption.

Methodology: For tea formulations, powder of each plant was made and combined in specific proportions to formulate five polyherbal teas. A hedonic test was conducted by a panel of tasters on each formulation to evaluate consumer appreciation. Phytochemical composition was carried out by coloration and precipitation tests, then safety were also evaluated by acute and subacute tests.

Results: Among the five formulations, formulation 4 consisting of "55% *L. multiflora*, 30% *Z. officinale* and 15% *M. piperita*" was the most appreciated tea with a value of 8.01. Regarding safety of the formulation 4, no deaths or signs of intoxication were observed. Subacute toxicity tests showed that tea has no adverse effects biochemical parameters. Apart from white blood cells for which there is an increasing of their number from $14.40 \pm 0.50 \times 10^3/\mu\text{L}$ to $19.30 \pm 0.40 \times 10^3/\mu\text{L}$ in comparison to the control group ($10.60 \pm 0.90 \times 10^3/\mu\text{L}$), the others haematological parameters were not significantly modified. This Tea formulation is rich in compounds such as alkaloids, polyphenols, flavonoids, saponosides, sterols and polyterpenes, leucoanthocyanins, and mucilages, which makes its consumption more attractive.

Conclusion: The polyherbal tea 4 which was the most appreciated, was rich in secondary metabolites and was no toxic in wistar rats.

Keywords: Tea, polyherbal, powder, aromatic, hedonic test.

1. INTRODUCTION

INTRODUCTION

Medicinal and aromatic play a prominent role in traditional health care practices across the world. As reported by the World Health Organization, approximately 80% of the population in sub-Saharan Africa relies on plant-based remedies for their primary health needs (1). This practice is justified by the cultural habits of the populations living in these areas, and by the region's high biodiversity. In addition to their therapeutic applications, certain plant species are also incorporated into the dietary practices of local populations (2,3). Such consumption is not merely for the purpose of meeting energy requirements, it is a means of ensuring growth and consumer protection. A diverse array of these plants offers a combination of medical and nutritional benefits (4). These include the species *Lippia multiflora* Mold., *Zingiber officinale* Rosc., and *Mentha piperita* L.; three aromatic plants, widely distributed in West Africa, and free to access on local markets (5,6).

In Côte d'Ivoire, *L. multiflora* known as “savannah tea or Gambian tea”, is utilized in the form of herbal tea for the treatment of respiratory diseases, fever, malaria, microbial infections, inflammations, pain, diabetes, and asthenia (7,8). *Zingiber officinale*, commonly known as ginger or “Gnamankou”, is a widely utilized plant in Côte d'Ivoire for its medicinal and culinary properties. It is employed as a beverage due to its anti-fatigue, digestive, antimicrobial, analgesic, anti-inflammatory, immunomodulatory and mucolytic properties (9,10,11,). With regard to *M. piperita*, commonly known as peppermint, it is a plant widely used in the culinary field for the purpose of flavoring dishes and juices (12). However, it is also known to possess beneficial properties for human health when consumed in the form of tea. These properties include the treatment of gastrointestinal disorders, inflammation, microbial infections, pain, and diabetes (13).

Among plant foods utilized as medicinal agents, aromatic plants have garnered the most attention (14,15). The use of preparations from these plants, typically prepared through soaking or heating plant organs, has been prevalent for centuries. In this present era, health care providers in Europe and Asia, frequently prescribe herbal teas for general well-being (16,17). It is regrettable that despite their presence within the flora, these plant species of interest as previously described, are not yet well developed in ready-to-use commercial products such as teas or herbal teas in Côte d'Ivoire. It is therefore recommended that local aromatic plants be valorized by offering an innovative tea formulation based on palatability and acceptability with a view to enriching the market for natural wellness products in line with the Sustainable Development Goals (SDG3). In a previous study conducted by N'DRI et al. (2019), it was demonstrated that the combination of *Z. officinale* and *L. multiflora* was more accepted than other combinations by tasters following a hedonic test. The aforementioned result demonstrates that specific combinations of plant species may be more accepted by consumers than others. Building on this, the present study proposes the introduction of *Mentha piperita*, a food plant widely used in cooking to flavor dishes, into the previous formulation with the aim of improving the acceptability.

The combination the aforementioned plant species in a unique tea formulation has the potential to offer a synergistic effect, resulting in a product with enhanced therapeutic properties. However, to optimize the benefits associated with the use of this product and ensure consumer safety, it is imperative to characterize the phytochemicals that constitute it and to evaluate its safety in an animal model.

2. MATERIAL AND METHODS

I MATERIALS AND METHODS

1 Material

1.1. Plant material

The composition is derived from the leaves of *Lippia multiflora*, the rhizomes of *Zingiber officinale*, and the leaves of *Mentha piperita*. The harvesting of the different organs was conducted in the Poro region (Korhogo, Côte d'Ivoire).

1.2. Animal material

The animal experiments were conducted using albino rats (*Rattus norvegicus*) of the Wistar variety. The rats weighed between 94 g and 145 g. They were provided by the animal facility of the UFR of Pharmaceutical and Biological Sciences of the Félix Houphouët-Boigny University (UFHB). All animals were acclimated to the GeRProPhaT (UJLoG) facility within a controlled environment, maintained at $24 \pm 1^\circ\text{C}$ with a 12-hour light/dark cycle. They were fed a complete standard pellet diet provided by the company “IVOGRAIN” and had constant access to tap water via feeding bottles.

2 Methods

2.1 Preparation of plant powders

Following the harvest, the leaves of *L. multiflora*, the rhizomes of *Z. officinale* and the leaves of *M. piperita* were harvested, washed, sorted and dried in a location free from sunlight for a period of 7 days. Subsequently, the dry organs were individually pulverized and filtered to produce a fine powder.

2.2 Tea formulation

To formulate the polyherbal tea, the plant powders were combined in varying proportions to obtain 5 combinations, the basis of which was the previous result published (18):

Tea formulation 1: 70% *L. multiflora* +30% *Z. officinale*

Tea formulation 2: 65% *L. multiflora* + 30% *Z. officinale* + 5% *Mentha piperita*

Tea formulation 3: 60% *L. multiflora* + 30 % *Z. officinale*+ 10% *M. piperita*

Tea formulation 4: 55% *L. multiflora* + 30% *Z. officinale* + 15% *M. piperita*

Tea Formulation 5: 55% *L. multiflora* + 25% *Z. officinale* + 20% *M. piperita*

2.3. Sensory analysis

The acceptability of the formulated tea was assessed using hedonic tests. These tests were conducted over the course of several sessions, with approximately sixty untrained individuals selected based on their availability and familiarity with herbal teas. The samples, coded with 5 random numbers corresponding to the different tea formulations, were presented individually to each taster to each taster. The acceptability of each sample was evaluated on a hedonic scale of 1 to 9 (19).

1: Extremely unpleasant; 2: Very unpleasant; 3: Unpleasant; 4: Quite unpleasant 5: Neither unpleasant nor pleasant; 6: Quite pleasant; 7: Pleasant; 8: Very pleasant; 9: Extremely pleasant. The taster is required to rinse his mouth after each snack.

2.4-Evaluation of toxicity

2.4.1. Preparation of the extract to be administered

The extract to be administered was separately prepared according to the method described by (20) using each tea formulations. A mass of 100 g of plant powder of the formulation was infused in 1 L of boiling water for a period of 15 min. Subsequently, the infusion was filtered twice on filter cotton, and once on Whatman paper No. 3 with the objective of removing fibers. The resulting filtrate was subjected to 50°C drying process in an oven for 3 days. The residual material was then recovered and constituted the dry extract of the formulation.

2.4.2. Assessment of acute toxicity

The experiment was conducted with the best formulation according to OECD 423 guidelines. Two doses including 2000 mg/kg and 5000 (mg/kg) were tested on 9 female Wistar rats divided into 3 groups of 3 rats. The rats were fasted during the night preceding the experiment. Before starting the experiment, the animals are weighed. Thereafter, the extract is administered via gavage in a single dose using a gastric tube. The dose to be administered is calculated based on the fasting body weight of each rat. After administration of the extract, the animals are deprived of food for 1 to 2 hours. The treated animals are observed for 14 days for signs of acute poisoning (21)

2.4.2 Evaluation of subacute toxicity

The study was carried out with the best formulation in accordance with OECD 407 guidelines. It involved 24 albino Wistar rats, divided into four equal groups, each comprising 3 males and 3 females. Three doses were tested: 17 mg/kg bw, 85 mg/kg bw and 425 mg/kg bw. The test substance was administered by daily oral gavage for 28 days. The dose to be administered was adjusted according to the fasting body weight of each rat. Before starting the experiment, the rats were fasted with free access to water. The concentrations 17 mg/kg bw, 85 mg/kg bw and 425 mg/kg bw were tested on groups 2, 3, and 4 respectively at a rate of 1 mL per 100 g bw. Group 1 received only distilled water. During this period, the animals were carefully observed every day for possible signs of toxicity. Finally, the rats were weighed every 7 days for 28 days (22).

2.4.5 Determination of hematological and biochemical parameters

At the end of the treatment period, the rats were anesthetized via inhalation of Cooper's solution. Blood samples were obtained by puncture of the retro-orbital sinus. Blood samples were collected in two tubes: one containing ethylenediaminetetraacetic acid (EDTA) and the other containing heparin. Tubes containing EDTA were used for hematological analysis whereas heparinized tubes were subjected to centrifugation at 4000 rpm for a period of 10 minutes. The serum was stored at -20°C for subsequent biochemical analysis (23). The impact of the administered dose was evaluated based on the results of blood analysis (complete blood count), as well as measurements of specific hepatic markers (AST and ALT transaminases, alkaline phosphatase: PAL), renal (creatinine and urea), lipid (total cholesterol, HDL, LDL and triglycerides) and carbohydrate metabolism (glycemia).

1.2.3 Phytochemical screening

Phytochemical tests were conducted using colorimetry and precipitation techniques. The tests offer a comprehensive overview of the diverse range of secondary metabolites present in plants.

The search for alkaloids was carried out using Dragendorff's reagent, which produces an orange precipitate; and Valser-Mayer reagent (iodine and mercury), which forms a milky white precipitate (24,25).

The search for polyphenolic compounds was carried out by the test with ferric chloride solution (FeCl₃) which in the presence of the latter gives a blue-blackish or green coloring. The search for tannins was carried out using Stiasny's reagent which precipitates the latter. Subsequently, the addition of a 2% FeCl₃ solution allows for the highlighting of gallic tannins which appear as a blackish-blue color (26).

The search for flavonoids was conducted using the cyanidin reaction, wherein the addition of magnesium shavings results in the emergence of an orange-pink or purplish color, indicative of the presence of flavonoids (27).

The search for sterols and polyterpenes was carried out using Liebermann- Burchard reagent. The formation of a purple or violet ring, which subsequently changes to blue then green, serves as an indicator of their presence (28).

The search for quinones was carried out using Borntraeger reagent, which produces a cherry red color in the presence of quinones (24).

The reaction with cyanidin without the addition of magnesium chips enabled the detection of leucoanthocyanins by the formation of a red color (25). Pour the aqueous extract into a test tube, then add the absolute alcohol. The formation of a flaky precipitate after shaking suggests the presence of mucilages (29).

Finally, the saponosides were identified by the very high foam index test (1 cm).

3. Statistical analysis

GraphPad.Prism version 8.0 and Excel ® software was used for data processing. The mean and standard deviation were calculated for each parameter evaluated. Data were analyzed with one-way ANOVA. Comparison of the means of the different parameters of the groups treated with the control group was carried out using the Tukey's test. The difference between the values of a parameter whose *p value* < 0.05 was estimated to be statistically significant.

3. RESULTS AND DISCUSSION

II RESULTS AND DISCUSSION

1. Results

1.1. Acceptability of formulations

The data relating to the hedonic test were presented in **Figure 1**

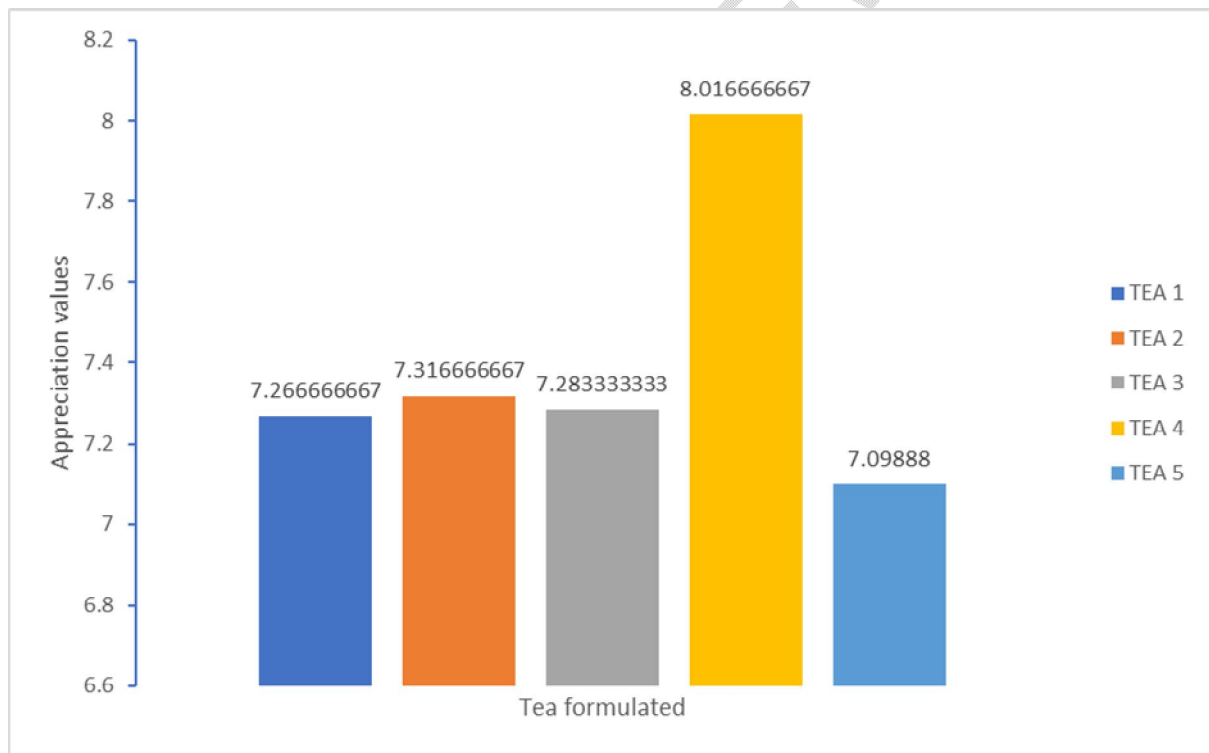


Figure 1: Histograms of the appreciation values of the different THES

During this study, five distinct tea formulations were developed. All of the formulations were evaluated positively with a value greater than 7. However, formulation 4 stood out with a higher rating (8.01) and thus was selected for further investigations.

1.3. Toxicity

1.3.1 Acute toxicity

1.3.1.1. Signs of poisoning

The acute oral toxicity study demonstrated that administration of doses of 2000 mg/kg and 5000 mg/kg body weight (bw) of Tea 4 did not result in mortality after 14 days of observation. Furthermore, the treated animals did not exhibit any signs or symptoms, including drowsiness, writhing, diarrhea, excitement, refusal to feed or straightening of hair at different concentrations of the dry extract.

1.3.1.2. Effect of tea on animal body weight

Throughout the entirety of experimental period, the body weight of the animals was meticulously monitored on daily basis (**Figure 2**). The animal's weights exhibited a general increase over time.

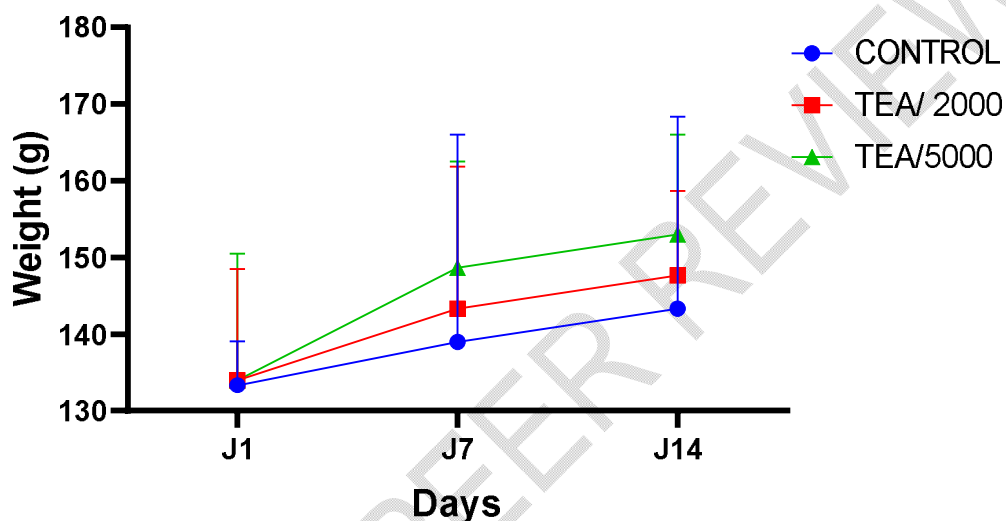


Figure 2: Evolution of the weight of rats after administration of Tea

In comparison to the control group, the weight of rat treated with Tea 4 at varying doses demonstrated a slight increase over the course of the 14-day experimental period. However, these weight differences were not statistically significant.

1.3.2 Subacute toxicity

During the 28 days of experimentation, no signs of poisoning and no mortality were recorded.

1.3.2.1 Effect of tea on animal body weight

Throughout the experiment, the animals body weight was monitored daily (**Figure 3**). Overall, there was a notable increase in the animal's weights over time (A vérifier pour vous rassurez).

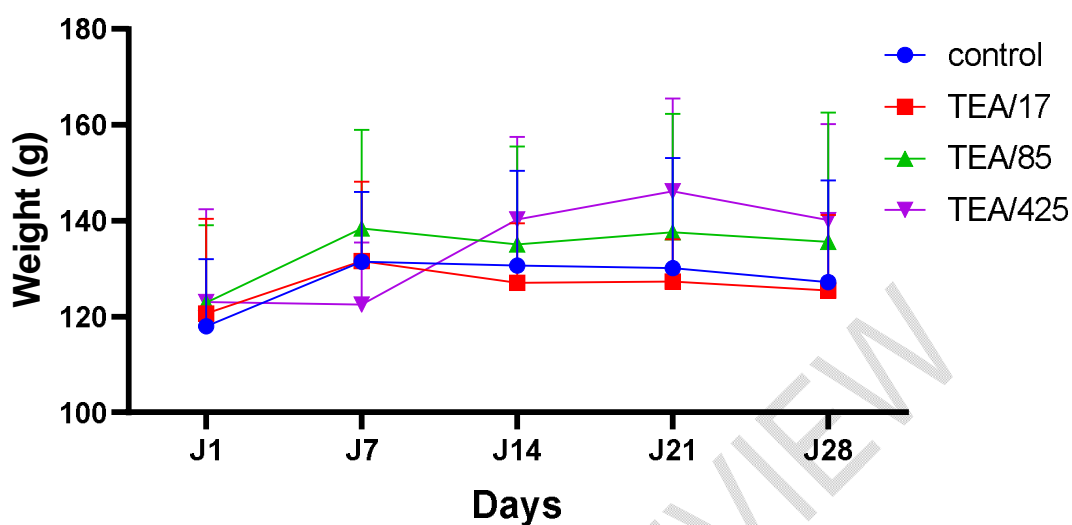


Figure 3: Evolution of the weight of rats after administration of Tea 4

1.3.3 Effect of Tea 4 on hematological parameters

The hematological analysis carried out on experimental rats with different doses of tea 4 revealed an increase in white blood cells in the treated groups compared to the control group. However, other parameters such as hemoglobin, platelets, hematocrit and MCV did not show significant variations. The data are reported in **Table I**

Table I: Hematological parameters

Hematological parameters	Group1 (control)	Group 2 (17mg/kg)	Group 3 (85 mg/kg)	Group 4 (425 mg/kg)
White blood cells ($10^3/\mu\text{L}$)	10.60 ± 0.90	$14.40 \pm 0.50^*$	$19.30 \pm 0.40^*$	$15.45 \pm 0.45^*$
Red cells ($10^6/\mu\text{L}$)	7.29 ± 0.87	7.72 ± 0.73	5.59 ± 0.49	6.66 ± 0.33
Hematocrit (%)	41.10 ± 6.20	41.70 ± 1.10	37.90 ± 2.20	40.40 ± 1.70
Hemoglobin (g/L)	12.55 ± 1.45	13.30 ± 0.30	12.65 ± 1.35	13.00 ± 1.30
TCMH (pg)	17.25 ± 0.05	17.45 ± 2.05	18.65 ± 1.15	19.50 ± 1.00
CCMH (g/dL)	30.70 ± 1.10	31.90 ± 0.60	36.25 ± 3.75	34.25 ± 2.35
Blood platelets (mm^3)	456.0 ± 116.0	409.0 ± 18	405.5 ± 51.50	497.5 ± 90.50
VGM (fL)	56.20 ± 1.80	54.50 ± 5.30	64.95 ± 7.85	56.95 ± 1.05

1.3.4 Effect of Tea 4 on biochemical parameters

During this work, biochemical parameters such as blood sugar, creatinemia, urea, AST and ALT were evaluated. The results are illustrated in Figures 4, 5, 6, 7,8.

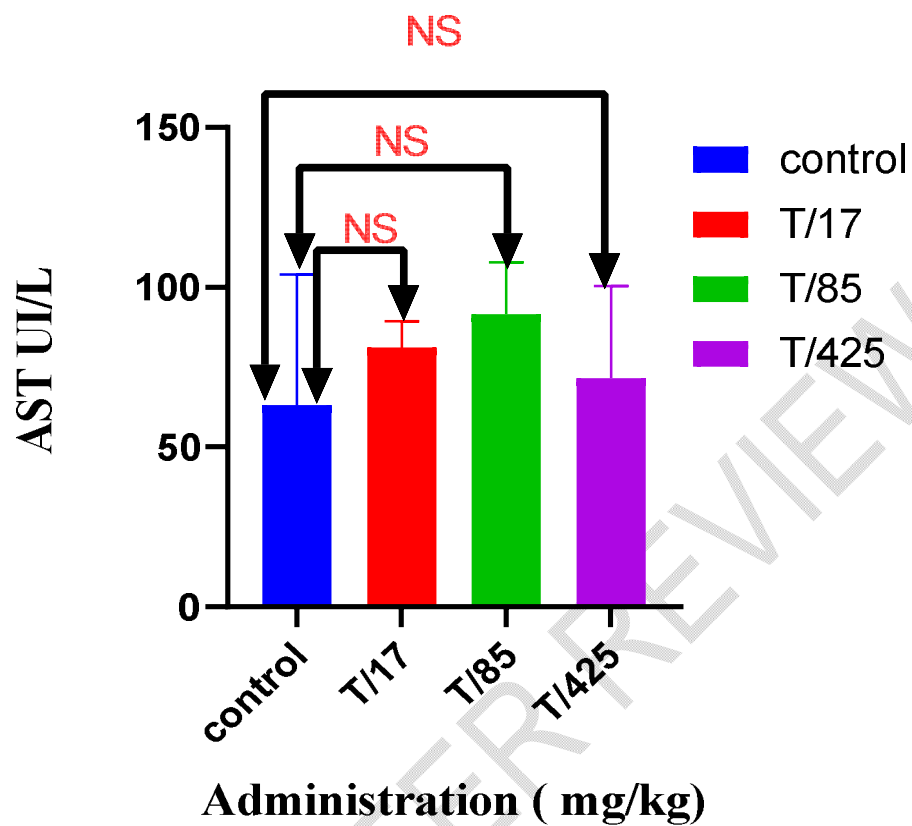


Figure 4 Histograms of AST concentration after administration of Tea 4

No significance difference was observed in the AST concentrations between the control and treated groups;

T/17: Rats treated by 17 mg/kg bw; T/85: Rats treated by 85 mg/kg bw; T/425: Treated by 425 mg/kg bw

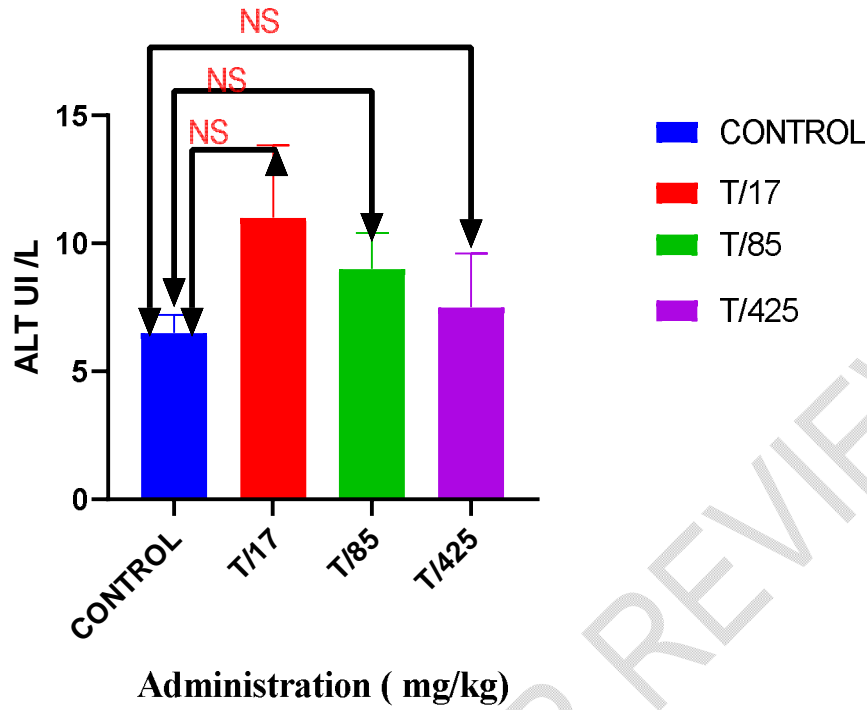


Figure 5: Histograms of ALT concentration after administration of Tea 4

No significance difference was observed in the ALT concentration between the control and treated groups; T/17: Rats treated by 17 mg/kg bw; T/85: Rats treated by 85 mg/kg bw; T/425: Treated by 425 mg/kg bw

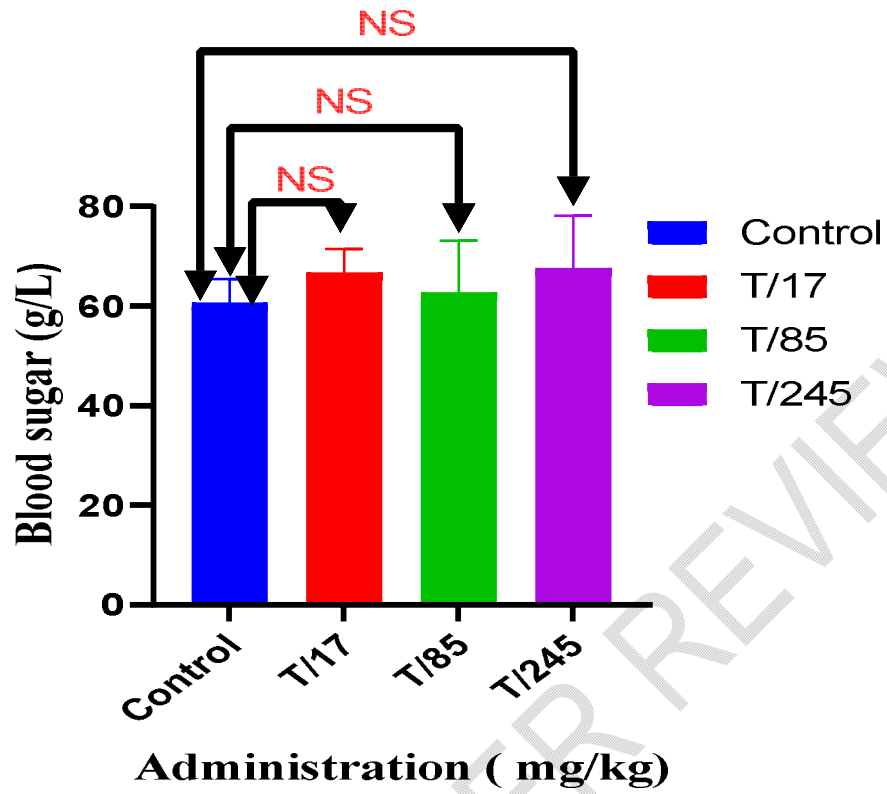


Figure 6: Blood sugar histograms after administration of Tea 4

No significance difference was observed in Blood sugar concentration between the control and treated groups;

T/17: Rats treated by 17 mg/kg bw; T/85: Rats treated by 85 mg/kg bw; T/425: Treated by 425 mg/kg bw

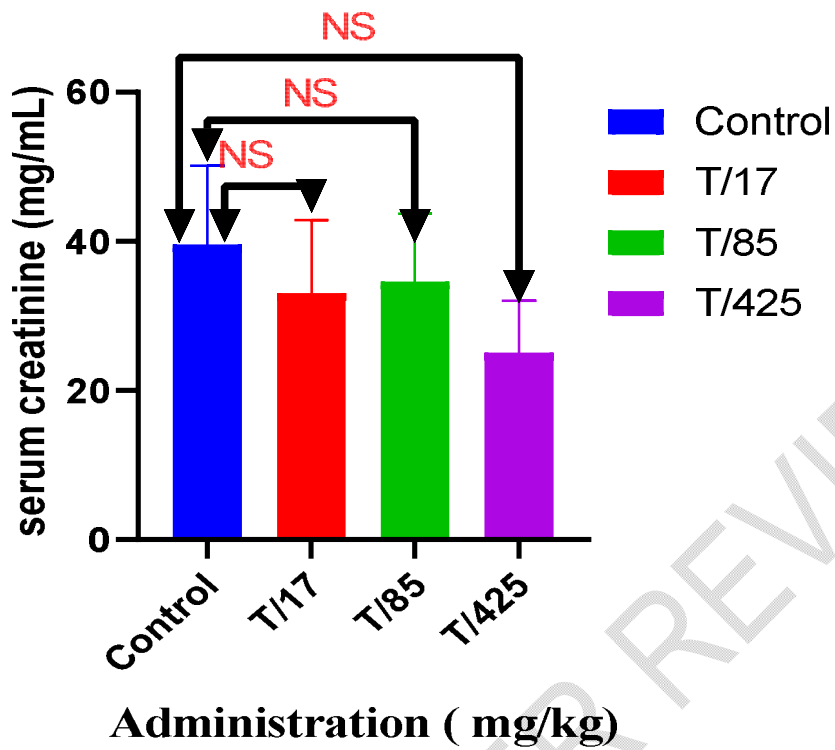


Figure 7: Histograms of creatinemia after administration of Tea 4

No significance difference was observed in serum creatinine concentration between the control and treated groups; T/17: Rats treated by 17 mg/kg bw; T/85: Rats treated by 85 mg/kg bw; T/425: Treated by 425 mg/kg bw

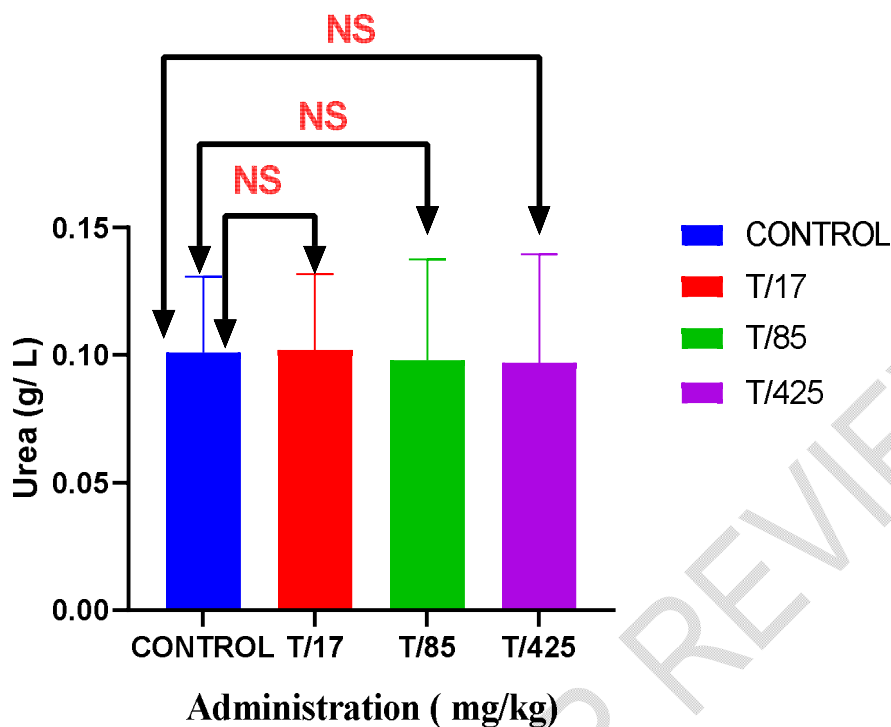


Figure 8: Histograms of Urea after administration of Tea 4

No significance difference was observed in Urea concentration between the control and treated groups; T/17: Rats treated by 17 mg/kg bw; T/85: Rats treated by 85 mg/kg bw; T/425: Treated by 425 mg/kg bw

1.4 Chemical screening

Table III gives the major chemical groups contained in the tea formulation 4. The chemical screening in solution carried out on the tea made it possible to identify compounds such as alkaloids, flavonoids, polyphenols, leucoanthocyanins, saponosides, sterols and polyterpenes, tannins and quinones. However, it should be noted the absence of quinones (Table II)

Table II: Identification of secondary metabolites

Secondary metabolite families	Tea formulation 4
Alkaloids (Valser-Mayer Reagent)	+
Alkaloids (Dragendorff Reagent)	+
Polyphenols	+
Catechic tannins	+
Gallic tannins	+

Flavonoids	+
Quinones	-
Saponosides	+
Sterols and Polyterpenes	+
Leucoanthocyanins	+
Mucilages	+

2-Discussion

In terms of the hedonic test, five (5) teas were formulated from three aromatic plant species: *Lippia multiflora*, *Zingiber officinale*, and *Mentha piperita*. The choice of these plants was guided by their aromas, their common use in the Ivorian diet in the form of herbal teas or juices, and the specific health benefits that each could provide as added value for consumers (7,10,30). In terms of overall acceptability of the formulations, the scores range from 7.09 to 8.01 on a scale of 9, indicating that all the formulations were well accepted by the participants. The highest score 8.01 was assigned to Tea 4, indicating that it is the most popular formulation those tested. Tea 5 exhibited the lowest score (7.09), yet it remained within the acceptability zone, albeit slightly lower than the other formulations. The scores for Teas 1, 2, and 3 are nearly identical, with a slight preference for Tea 2 (7.31) over Teas 1 (7.26) and 3 (7.28). The difference between Tea 4 and other teas is more pronounced, suggesting that Tea 4 possesses a distinctive quality or attribute that sets it apart from other formulations. This formulation has the highest score and should be considered the optimal basis for potential optimizations or market release. Tea 5, although well-accepted exhibits, a slightly lower score, indicating potential for acceptability enhancement through adjustments. Teas 1, 2, and 3, while also well-accepted, exhibit a near-equivalent score. Understanding the specific consumer perceptions of these teas could inform improvement strategies.

The considerable approval of formulation 4 (Tea 4) demonstrates its marketability and could serve as a valuable decision-making tool in the development of marketing strategies for the product (31,32).

In general, the results demonstrated a high level of overall acceptability for all formulations, with a notable preference for Tea 4. Formulations 1, 2, and 3 also exhibited a favorable reception; although the scores were relatively similar. Tea 5 while still deemed acceptable, was slightly less preferred.

With the regards to the acute toxicity study, the absence of mortality at doses as high as 2000 mg/kg and 5000 mg/kg suggests that the substance has low acute toxicity. This indicates that the administered doses are not lethal for Wistar rats under the conditions

of the study. Moreover, the absence of clinical signs (such as lethargy, behavioral changes, weight loss, etc.) at these doses indicates that the substance is well tolerated by rats and does not induce visible toxic effects in the short term. The absence of mortality or clinical signs at doses of 2000 mg/kg and 5000 mg/kg allows for the reasonable conclusion that the LD50 of the substance is greater than 5000 mg/kg. This classification aligns with the WHO 214 and OECD 425 guidelines, which define a practically non-toxic substance (33).

The insignificant increase weight observed in rats treated with the tea suggests that tea may possess beneficial effects on general health. Indeed, the bioactive compounds of *Lippia multiflora*, *Zingiber officinale*, and *Mentha piperita* can work together to stimulate metabolism. Ginger, with its thermogenic properties, when combined with the lipid-regulating effects of *Lippia multiflora* and the ability of peppermint to improve digestion, can create a beneficial combined effect for weight management (34,35).

The absence of clinical signs of intoxication or mortality at doses of 17 mg/kg bw, 85 mg/kg bw, 425 mg/kg bw at the subacute toxicity test level is an important indicator of the safety of tea during use over 28 days. The safety of Tea 4 is contingent upon the absence of individual toxicity of each of the plants comprising tea formulation 4. Indeed, the LD50 values of the plant species involved in the formulation exceed 5000 mg/kg bw (36,37). Therefore, the tea can be consumed without risk of intoxication at the doses tested over a period of 28 days.

The absence of significant variations in biochemical parameters (glycemia, creatinemia, urea, AST, ALT) in the treated rats, when compared to the controls, demonstrates that renal, hepatic, and pancreatic functions were not altered by the consumption of tea (38). With regard to the hematological parameters (WBC, GR, Hb, Hte, VGM, TCMH, CCMH, and blood platelets), only white blood cells (WBC) exhibited notable alterations, this may be attributed to the potential immunostimulatory effect of formulation 4 (tea 4), as the utilized species recognized for their antioxidant and immunomodulatory properties (39,40).

The results of phytochemical sorting indicate the presence of flavonoids, polyphenols, alkaloids, tannins, saponosides, sterols, polyterpenes, leucoanthocyanins, and mucilages in formulation 4 (Tea 4). These compounds are known for their antioxidant, anti-inflammatory, analgesic, and immunostimulating properties, among others, and are beneficial for health (41,42). These compounds contribute to the reduction of oxidative stress

and may confer cardiovascular and neuroprotective benefits, as well as potential anticancer effects (43).

4. CONCLUSION

Of the five tea formulations produced from the aromatic plants *Lippia multiflora*, *Zingiber officinale*, and *Mentha piperita*, formulation 4 (Tea 4) was the highly rated in a hedonic test, with a score of 8.01. The safety study of this formulation in Wistar rats demonstrated no mortality and no clinical signs of poisoning. Furthermore, analysis of biochemical and hematological parameters revealed no significant modifications. Based on these findings, it can be concluded that Formulation 4 (Tea 4) does not present a potential risk of poisoning for users over a period of 28 days. Additionally, the presence of certain phytochemicals in formulation 4 (Tea 4) suggests that it may enhance the well-being of users.

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