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An Herbal Tea Blend of Hibiscus *sabdariffa*, *Zingiber officinale*, and *Mentha spicata*: A Potent Source of Antioxidant and Anti-Obesity Properties

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Abstract

Background: There is an urgent need to find safer and more sustainable solutions to tackle the rising global epidemic obesity and associated complications. The main objective of this study was to develop formulations of herbal tea blend from three plant species and to assess the antioxidant and antiobesogenic properties of the best formulation. Methods: The best formulation (FX) obtained (80% *Hibiscus sabdariffa*, 10% *Zingiber officinale* and 10% *Mentha spicata*) was validated by their better sensory acceptability and antioxidant properties. *In vivo* study using high-fat diet Wistar rats revealed that FX alleviated oxidative stress and metabolic disorders including those affecting hepatic and renal functions caused by high-fat died. Results: The administration of FX resulted in a reduction in food intake, body weight gain and metabolic efficiency index alongside lower blood content in triglycerides, total cholesterol, LDL-cholesterol, glucose, and atherogenic index when compared to the control groups. The results were comparable if not better than those obtained from the reference groups treated with a standard obesity treatment medicine, Orlistat. Conclusion: The developed herbal blend showed promising results for use as a safer product for obesity prevention and management as well as other oxidative stress-related health issues.

More Information

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Keywords:

Herbal tea formulation,
Hibiscus sabdariffa,
Zingiber officinale,
Mentha spicata,
Obesity,
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Introduction

Obesity represents an abnormal or excess fat aggregation in adipose tissues characterized by an increase in the body mass index (BMI) that may have a negative impact on health and well-being due to various associated comorbidities. Its worldwide rate has nearly tripled since 1975. Moreover, although obesity is preventable, most of the world's population live in countries where overweight and obesity kill more people than underweight [1]. It is now recognized that obesity is associated with metabolic complications such as lipids profile impairment and dysfunction of redox status. Indeed, an increase in the amount of accumulated fats contributes to oxidative stress.

Oxidative stress is essentially an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to counteract or detoxify their harmful effects. ROS are by-products of metabolism that play an important role in the development of obesity and its metabolic complications [2]. Thus, obesity management should fully consider oxidative stress.

There are many different options for obesity treatments, including dietary control, exercise, life-style modification, weight-loss medication, and weight-loss surgery. However, finding a risk-free and efficient weight management treatment is still a real challenge whilst obesity rates continue to rise around the world. Lifestyle modification, changing in diet and reducing sedentary behavior are currently considered as best alternatives but they are difficult to implement by many people. Phytotherapy is a promising option that could complement some of the current approaches to weight management since plants constitute a source of many bioactive compounds, including polyphenols, alkaloids, terpenoids, and carotenoids with potential synergistic effects. These natural compounds have therapeutic potential for diseases mediated by oxidative stress and obesity [1-2]. Moreover, natural plant supplementation can cause important weight loss and improve health through the neutralization of ROS and regulation of adipogenesis. For example, *Hibiscus sabdariffa* calyxes have been reported to increase the level high-density lipoprotein and significanly reduce low-density lipoprotein concentration and blood presure [3]. Jamous et al. showed that *Mentha spicata* leaves have promising antioxidants and antiobesity properties [4]. Similarly, *Zingiber officinale* rhizomes reduce body weight, glucose, insulin and regulate lipids profil [5].

H. sabdarifa, *M. spicata* and *Z. officinale* are readily in most African's countries where they are commonly consumed as spices, vegetables, drinks, or herbal teas. Our recent study confirmed the antioxidant and antiobesogenic properties of their aqueous extracts and suggested their combined use to optimize their properties [6]. It is hypothesized that the formulated herbal tea blend from the mixture of the three plants would have better efficacy for the prevention and treatment of obesity and associated diseases. Herbal tea or tisane is a generic term for tea made from herbs instead of *Camellia sinensis* leaves. There is a renewed interest in tea and herbal tea because of the growing consumer's awareness of the health benefits derived from their consumption. Herbal teas are often a blend of many plants or different parts of the same plant [7]. For example, De-Heer et al. formulated herbal teas from *H. sabdariffa, Moringa oleifera,* and *Cymbopogon* citratus [8]. Caleja et al. developed herbal tea blends with *M. spicata*, *Erica australis*, *Genista tridentata*, *Melissa officinalis*, and *Prunella vulgaris* [9] whereas Alakali et al. produced herbal teas from *Z. officinale* and *Pavetta crassipes* [10]. Similarly, Ajayi and Oyerinde formulated herbal teas with *H. sabdariffa* and *Citrus limon* peels or *Z. officinale* [11]. Suseno et al. produced blend herbal teas from lemongrass, roselle, and ginger and found that the formulation with 25% lemongrass, 50% roselle and 25% ginger was the best with higher total phenolic compound properties and antioxidant activity [12]. Teye et al. reported better acceptability and higher micronutrient content for herbal tea blends formulated 50% roselle, 25% ginger and 25% turkey berry [7].

To the best of our knowledge, no study on blend herbal teas formulated from the three plants of interest, *H. sabdariffa* calyxes, *M. spicata* leaves and *Z. officinale* rhizomes have been reported. Moreover, very few studies on herbal tea blends focused on their biological properties, especially their effects on metabolic diseases with *in vivo* study. To this end, the main objective of the research presented here was to evaluate the antioxidant and antiobesogenic properties of an herbal tea blend formulated from these three plants to harness any potential synergistic effect for better efficacy. Different herbal teas were formulated and the best sample used for *In vivo* study were selected based on sensory properties, total phenolic content (TPC) and *In vitro* antioxidant activity.

Material and Methods

Material

Dried calyxes of *H. sabdariffa* were purchased at Garoua whereas *M. spicata* leaves and fresh *Z. officinale* rhizomes were obtained at Douala. The collected samples were transported to the Biochemistry Laboratory of the University of Douala where they were washed, cleaned, and oven dried at 45°C (BINDER) and blended in a food processor (MOULINEX). The resulting powders were packed in plastic bags and stored in a dry place protected from light until formulation. Twenty male *Wistar* rats at three months of age weighing 200 ± 15 g were used in this study. They were obtained from the Laboratory of Physiology and Animal Biology of the University of Douala.

Methods

This study was approved by the University of Douala Institutional Ethics Committee (N°2714CEI-Udo/06/2021/M) and informed consent was obtained from each participant for sensory attributes evaluation. All animal experiments were complied with the ARRIVE guidelines and carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Design and Formulation Approach

Different herbal tea formulations were made by mixing dried powder of *H. sabdariffa*, *M. spicata* and *Z. officinale* at different proportions. The minimum

proportion of each plant was fixed at 10% and the maximum at 80% and these values were informed by the local practice and recommendations from literature. A total of 10 samples (F1-F10) with different proportions of each plant were formulated (Table 1 – Appendix 1).

Each herbal tea formulated was prepared by infusion in hot water (100°C) for 10 min using the ratio sample to water 1:100 (w:v). The obtained sample was then divided in two parts for sensory properties evaluation and determination of TPC and *In vitro* antioxidant activity, especially free radical scavenging and reducing power activities.

- *Sensory attributes of the formulated herbal teas*

Sensory attributes were evaluated by an untrained panel of 70 students (36 men and 34 women) recruited from the University of Douala. About 35 ml of each of the ten coded herbal freshly prepared as indicated in section 2.2.1 were presented in a porcelain cup to each judge. The color, aroma, flavor, aftertaste and overall acceptability of the supplied teas were evaluated using a 9-point hedonic scale where 9 = like extremely and 1 = dislike extremely. One sample was served at a time in randomized and balanced order among subjects and were evaluated at room temperature. The sample infusions were approximately 40-50°C at the time of tasting. Panelists were required to rinse their mouths with warm water (~40°C) before starting the test. They were also required to rinse their mouths with warm water after each tasting and wait 10 min before tasting the next sample. They were allowed to repeat the tasting where necessary.

- *TPC and In vitro antioxidant activity of the formulated herbal teas*

TPC, free radical scavenging and reducing power activities were assessed on herbal tea extract according to the methods previously used by Nkepndep et al. [6]. Extraction was done by infusion as indicated in section 2.2.1. Whatman N°1 filter paper was used to filter the infusion and the filtrate was concentrated under vacuum by rotary evaporation at 40°C. The concentrated samples were then stored at 4°C until the analysis. TPC was determined by Folin-Ciocalteu's method, free radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and reducing power by ferric reducing antioxidant power (FRAP) assay based on Fe(III) reduction to Fe(II). Ascorbic acid was used as reference.

- *Selection and confirmation of the best herbal tea formulation*

The sensory properties, TPC and *In vitro* antioxidant activity results from the analysis of the formulated herbal teas were used to determine the best herbal tea formulation, i.e. which with good sensory attributes, high TPC and antioxidant activity. Then, the sensory attributes, TPC and antioxidant activity of individual plants were analyzed and compared with the best formulation. The same sensory panel was used for the best formulation and individual plants.

Phytochemical Screening of the Best Herbal Tea Formulation

This extract of the best formulation was subjected to qualitative phytochemical screening according to methods described by Harborne to identify the presence or absence of the bioactive compounds of interest, particularly saponins, flavonoids, anthocyanins, coumarins, tannins, steroids, glycosides, alkaloids and anthraquinones [13].

***In vivo* Experiment with the Best Herbal Tea Formulation**

Rats were acclimatized to the environmental conditions for one week at the animal handling facility of the laboratory. They were then randomly divided into four groups of five animals. Two control groups with no treatment, one fed with normal standard diet (NSD) and the other with high-fat diet (HFD). The experimental group was fed with HFD and treated with herbal tea extract whereas the last group considered as reference was fed with HFD and treated with Orlistat, a standard treatment for obesity. NSD consisted of Standard Laboratory Animals Diet (SLAD) while HFD was 'cafeteria' diet composed of 50% of SLAD and 50% of a mix containing salami, cookies, cheese, sausage, chips, chocolate and almonds in a proportion of 2:2:2:1:1:1:1 [6]. The animals of the experimental group were orally administered herbal tea extract at 1 g/kgbw dosage per day while the reference and controls groups were respectively administered Orlistat and distilled water at 1 mg/kgbw dosage per day. Throughout the experiment, which lasted 28 days, food and water were given *ad libitum*. Food intake was recorded daily while the animals' weight and length (nose-to-anus length) were monitored weekly. At the end of the experimental period (on the 29th day), animals were sacrificed after 12 hours of fasting.

The blood was collected by cardiac puncture and some organs (liver, heart, spleen, kidneys, lungs, and adipose tissue) were removed. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes and sera were stored at -20°C until analysis. The liver and kidneys were rinsed with physiologic water, wrung out, weighed and observed, macroscopically. A part of these organs was crushed and homogenized in 50 mM TrisHCl buffer (pH 7.5) using a weight/volume ratio of 1:5. The homogenates obtained were centrifuged at 3000 rpm for 10 minutes and the supernatants were collected and stored at -20°C until analysis. Other parts of the organs to be used for the histology test were kept in the formalin and stored at room temperature.

- *In vivo antioxidant activity*

Markers of oxidative stress lipid peroxidation (evaluation of the level of thiobarbituric acid reactive

substances as well as malondialdehyde/MDA), superoxide dismutase/SOD and catalase activities were evaluated in the serum and homogenates obtained from the liver and kidneys according to the methods previously used by Nkepndep et al. [6]. In parallel, the total protein content was determined via the Biuret method using a commercially kit (www.biolabo.fr, Les Hautes Rives 02160, Maizy, France).

- *Antiobesogenic properties*

They were evaluated through *In vivo* experiment described above alongside the determination of the animals' food consumption pattern, anthropometric parameters (BMI and organs relative weight), metabolic efficiency index, blood lipids profile and glucose content.

Food consumption pattern

Food consumption behavior of rats was estimated weekly using data from food intake recorded daily. Practically, each day 250 g of feed were given to groups of rats in their cages every morning and food intake was recorded by evaluating the remaining quantity (mass) after 24 hours.

Anthropometric parameters and *metabolic efficiency index*

Animal weights and lengths, monitored weekly were used to determine the BMI of rats which is defined as:

$$BMI\ (g/cm^2) = \frac{Weight}{(Nose-to-anus\ length)^2} \quad (1)$$

Metabolic efficiency index was calculated as following equation:

$$Metabolic\ efficiency\ index = \frac{Body\ weight\ gain}{Food\ intake} \quad (2)$$

Relative weight of organs was evaluated using the formula:

$$Relative\ weight\ (\%) = 100x\ \frac{Organ\ weight}{Animal\ weight} \quad (3)$$

Blood lipids profile and *glucose*

Serum contents in triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol and glucose were measured by spectrophotometric methods with test kits from BIOLABO (BIOLABO S.A.S, Paris-France) as previously used by Nkepndep et al. [6].

Low density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula:

$$LDL\text{-}cholesterol = [total\ cholesterol - (HDL\text{-}cholesterol + triglycerides/5)] \quad (4)$$

The atherogenic index was calculated using equation:

$$Atherogenic\ index = \frac{Total\ cholesterol}{HDL-cholesterol} \quad (5)$$

- *Effect of the best herbal tea formulation on liver and kidney functions*

Serum biomarkers of liver functions (blood alanine aminotransferase/ALAT and aspartate aminotransferase/ASAT activities) and renal functions (blood creatinine and urea content) were evaluated by spectrophotometric methods using test kits from BIOLABO as previously used by Nkepndep et al. [6]. The histopathological examination of these tissues was also done. Briefly, the tissue was sliced, and pieces were fixed in 10% buffered formaldehyde solution for histological study. The fixed tissues were processed by an automated tissue processing machine and further embedded in paraffin wax by conventional methods. Sections of 5 µm in thickness were prepared and stained with hematoxylin-eosin. These sections were then observed under a microscope (Leitz Wetzlar, Germany) to ascertain any histopathological changes.

Statistical analysis

The statistical significance was assessed using Student test and one-way analysis of variance (ANOVA) followed by Turkey's post hoc tests for pairwise separation and comparison of means using GRAPHPAD PRISM software version 5.9 (GraphPad Software, La Jolla California USA). Pearson correlation was used to establish the associations between the parameters. The values of $P<0.05$ were considered significant.

Results and Discussion

Formulation

Table 1 (Appendix 1) shows the sensory attributes (color, aroma, flavor, aftertaste and overall acceptability), TPC and the antioxidant activity (free radical scavenging activity and reduction power) of the ten samples of herbal teas formulations. It is apparent that sensory attributes are generally improved with a higher proportion of *H. sabdariffa* and a lower percentage of *M. spicata* and *Z. officinale* in the formulation. In fact, a significant positive correlation is noted with H. *sabdariffa* percentage and color (r = +0.88), aroma (r = +0.79), flavor (r = +0.64), aftertaste (r = +0.76) and overall acceptability (r = +0.82). This correlation was negative for all these sensory attributes with both *M. spicata* (r varying from -0.35 to -0.70) and *Z. officinale* (r varying from -0.13 to -0.73).

Furthermore, we noted that TPC concentration significantly increased with an increasing percentage of *M. spicata* (r = +0.41) and decreasing concentration of *H. sabdariffa* (R = -0.30) and *Z. officinale* (r = -0.11). Contrary, free radical scavenging and reduction power activities increased with an increasing *H. sabdariffa* concentration (r = +0.68 and +0.71 respectively) and decreasing *M. spicata* (r = -0.26 and -0.46 respectively) and *Z. officinale* (r = -0.51 and -0.46 respectively) proportion. These results corroborate with those reported by Teye et al. [7], Ajayi and Oyerinde [11],

Suseno, et al. [12] who studied herbal tea formulations from *H. sabdariffa* and *Z. officinale* and found that teas with high proportion of *H. sabdariffa* and low proportion of *Z. officinale* were the most preferred. *H. sabdariffa* is characterized by its imposing red color and high aromatic content which justified the observed good color, aroma and flavor preference for samples containing a higher concentration of *H. sabdariffa*. Likewise, Suseno et al. revealed that samples with a high proportion of *H. sabdariffa* had the highest antioxidant activity [12]. *H. sabdariffa* has a high content of anthocyanins which are well known for their oxidative stress reduction properties [14].

From these results, one noted that the best formulation (named FX), i.e. which with good sensory attributes, high TPC and antioxidant activities, is that with 80% *H. sabdariffa,* 10% of *M. spicata* and 10% of *Z. officinale*. Fig.1 shows the sensory properties results of FX and of each plant. FX showed sensory attributes relatively higher than those obtained with individual plant. A possible explanation for this result is the existence of a synergy between the organoleptic properties of these plants, thus giving a more pronounced taste and aroma to FX [15]. These findings are in accordance with those reported by Suseno et al. who showed that the combination of several plant species in the formulation of an herbal tea improves their organoleptic properties [12].

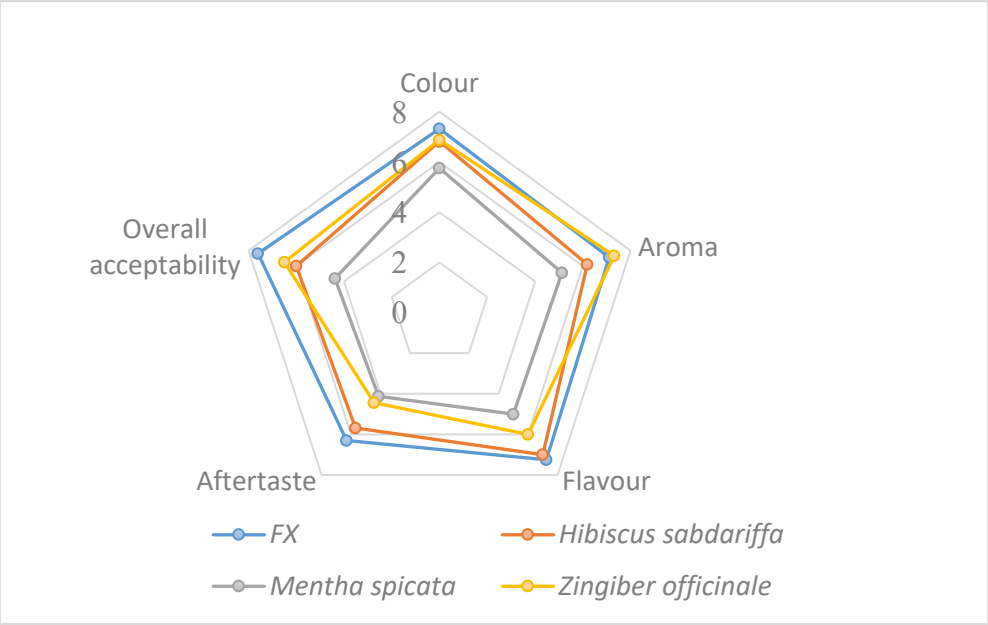

Figure 1: Sensory Attributes of FX and Individual Plants

TPC (A) and the antioxidant activity expressed in terms of DPPH free radical scavenging (B) and reducing power (C) activities of FX and of each plant depicted in Fig. 2 (Appendix 2) showed similar trends to those obtained with the sensory evaluation. Indeed, TPC and antioxidant activity of FX were relatively higher when compared to individual plant. Similarly, Preciado-Saldaña et al. reported an increase in antioxidant activity of a blend of *H. sabdariffa* and *C. sinensis* based teas [16]. FX has a TPC of 5.15 ± 0.41 mgGAE/g, an antioxidant potential for DPPH free radical scavenging of 18.25 ± 4.01 10^{-5} mol DPPH/g of extract and a FRAP reducing power IC_{50} of 1997.15 ± 231.25 ppm. It is noted that TPC was significantly lower in FX when compared to *M. spicata*, but was similar to those values obtained from *H. sabdariffa* and *Z. officinale*. DPPH antioxidant potential of FX was significantly higher compared to each of the three plants. The FRAP reducing power of FX was comparable to *M. spicata* value and significantly higher than those of *H. sabdariffa* and Z*. officinale*. However, DPPH free radical scavenging and FRAP reducing power of FX were significantly higher when compared to the values from ascorbic acid.

Phytochemical Screening of Aqueous Extract of FX

The phytochemical profile of FX based on a qualitative screening, showed the absence of saponins but the presence of many bioactive compounds at different levels from moderate concentration of flavonoids, coumarins and alkaloids to more abundance anthocyanins, tannins, steroids, glycosides and anthraquinones. Our previous study also showed the presence of these compounds in each of the plants used in FX formulation [6]. The higher concentration of these compounds presumably explains the antioxidant activity of FX.

***In vivo* Antioxidant Activity of Aqueous Extract of FX**

As shown in Table 2, this study confirmed with control groups that HFD-fed induces oxidative stress in rats' tissues when compared with NSD-diet as highlighted by an increase in MDA content and decrease in SOD and/or catalase activities. The administration of FX extract remedied these disorders. In fact, among HFD-fed rats, animals receiving FX had a significantly lower MDA content, high SOD and catalase activities in almost all tissues examined when compared with the control group. Such effects reflect the antioxidant activity of FX, which is attributed to its high content in bioactive compounds. Many studies have confirmed this hypothesis which suggests that plants' phenolic compounds are responsible for their antioxidant activities. These compounds and their metabolites can trap and neutralize free radicals, inhibit the enzymes responsible for their formation and chelate certain metal ions [17]. Also, the plants used contain vitamins, minerals and other nutrients. *H. sabdariffa* calyces contain vitamins such as thiamine, riboflavin, niacin and vitamin C, alongside carotenoids and minerals including potassium, sodium, calcium, iron, magnesium, phosphorus, manganese, zinc and copper [18]. Equally, it has been reported the presence of as thiamine, riboflavin, niacin, panthenic acid, vitamin B6, folate, vitamin C, vitamin E, carotene, calcium, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc in *Z. officinale* rhizomes [19]. Alam et al. noted that *M. spicata* leaves are good sources of vitamins and minerals [20]. Some of these water-soluble nutrients would naturally be present in the investigated plant extracts and could prevent lipid peroxidation induced by the HFD [21-23]. Minerals can also contribute to restore the catalytic activities of antioxidant enzymes acting as cofactors in enzymatic reactions. The results obtained with FX were comparable and even higher than those noted with the reference. Moreover, the observed effects were similar and even more intense than those noted with plants individually according to our previous study [6]. These findings evidence potential synergistic effect between compounds from different plants.

Antiobesogenic Activity of Aqueous Extract of FX

The antiobesogenic activity of FX were assessed by monitoring rat subjects' food consumption patterns (Fig. 3A - Appendix 2) and evaluating the anthropometric parameters (Fig. 3B - Appendix 2 and Table 2) and metabolic efficiency index (Fig. 3C- Appendix 2) alongside their blood glucose and lipid profile (Table 2).

Food Consumption Pattern, Anthropometric Parameters and Metabolic Efficiency Index

The type of diet showed a significant ($P<0.05$) effect on the subjects' feed intake (Fig. 3A - Appendix 2) with higher food consumption observed in the NSD-fed groups when compared to HFD-fed ones from the 2nd week of the experiment. Among rat subjects fed with HFD, those treated with FX showed a significant ($P<0.05$) lower food intake when compared to the control groups and those receiving Orlistat. As shown in Fig. 3B (Appendix 2), BMI of NSD-fed rats did not significantly change under the experimental conditions for control groups whereas the group fed with HFD showed a significant increased ($P<0.05$) in BMI from the 1st week of the experiment. Interestingly, BMI did not significantly change during the experiment with rat subjects treated with FX even though they were fed with HFD. These findings suggest that FX extract induced a reduction in food intake and body weight gain. These observations could be explained by several mechanisms including an increase in the mandatory energy expenditure and adaptive thermogenesis through regulation of thermogenin expression and suppression of appetite by certain herbal tea ingredients able to provide favorable effects to satiety (minerals, phytocompounds, etc.) and the presence of minerals such as Mg^{2+} with ability to increase lipid excretion by forming complexes between insoluble salts and fatty acids, thereby preventing their intestinal absorption [24]. Also, HFD-feeding resulted in high conservation of fats as reserves characterized by a significant ($P<0.05$) increase in the metabolic efficiency index with time (Fig. 3C- Appendix 2). Similarly, the administration of FX significantly led to the mobilization of these fats into other forms of energy characterized by a decrease in the metabolic efficiency index from the second week. Indeed, the greater the metabolic efficiency, the more built-up reserves in the body [25]. All these observations noted with FX were comparable to those of reference groups. As previously mentioned, the effects were higher than those noted with individual plants reported by Nkepndep et al. [6].

Conversely neither the diet nor the treatment showed an effect on the rat subjects' heart, spleen, kidneys and lungs based on these organs weight (Table 2). The liver weight was considerably higher for the HFD-fed control animals when compared with NSD-fed groups. These results evidence that HFD-feeding induced body weight increase, associated with an accumulation of adipose tissue. Similarly, the increase in the liver weight of the HFD-fed control animals suggests possible accumulation of fat in that organ. Among HFD-fed rats, those treated with FX extract presented a lower liver and adipose tissue relative weight when compared with the control groups. Bioactive compounds present in FX could then explain these results. Indeed, they are likely to influence satiety (and weight control), resulting in fat reserves loss [21, 24]. Results obtained with FX were similar to those from the standard treatment using Orlistat as medicine and better than the results from the individual plants reported by Nkepndep et al. [6].

Table 2: Effect of Aqueous Extract of FX on Rats' Organs Oxidative Markers, Organs Relative Weight, Blood Lipid Profile and Glucose Levels, Liver and Kidney Markers

	Controls		FX + high-fat diet	Reference + high-fat diet	P
	Normal diet	High-fat diet			
MDA					
Blood (µmol/L)	15.79 ± 4.03^{a,b}	19.48 ± 1.69^{a}	14.19 ± 2.87^{b}	17.76 ± 1.89^{a,b}	0.0088
Liver (µmol/g)	0.047 ± 0.002^{b,c}	0.049 ± 0.007^{a}	0.043 ± 0.002^{b}	0.036 ± 0.003^{c}	<0.0001
Kidneys (µmol/g)	0.054 ± 0.006^{b}	0.059 ± 0.004^{a}	0.050 ± 0.003^{c}	0.058 ± 0.002^{a}	<0.0001
SOD (µmol/min/mg proteins)					
Blood	23.85 ± 5.98^{a}	24.07 ± 7.73^{a}	28.07 ± 3.65^{a}	27.75 ± 9.19^{a}	ns
Liver	43.11 ± 5.50^{a,b}	39.38 ± 5.22^{c}	52.30 ± 4.20^{a}	40.97 ± 4.56^{b,c}	0.0033
Kidneys	34.76 ± 7.13^{a}	38.46 ± 4.75^{a}	54.99 ± 7.63^{b}	43.39 ± 3.87^{b}	0.0042
CATALASE (nmolH_2O_2/min/mg proteins)					
Blood	54.47 ± 6.88^{c}	32.31 ± 2.19^{d}	128.02 ± 9.34^{a}	106.95 ± 12.74^{b}	<0.0001
Liver	53.88 ± 12.75^{a}	99.02 ± 26.91^{b}	66,23 ± 9,88^{b}	98.71 ± 14.16^{b}	<0.0001
Kidneys	23.38 ± 5.67^{c}	64.01 ± 8.52^{a}	43.02 ± 5.94^{b}	68.06 ± 10.75^{a}	<0.0001
RELATIVE ORGANS WEIGHT (%)					
Liver	1.53 ± 0.18^{c}	2.35 ± 0.23^{a}	2.24 ± 0.09^{b}	2.51 ± 0.10^{b}	<0.0001
Heart	0.25 ± 0.02^{a}	0.24 ± 0.02^{a}	0.24 ± 0.11^{a}	0.26 ± 0.01^{a}	ns
Spleen	0.30 ± 0.07^{a}	0.32 ± 0.07^{a}	0.34 ± 0.06^{a}	0.31 ± 0.02^{a}	ns
Kidneys	0.56 ± 0.06^{a}	0.47 ± 0.06^{a}	0.45 ± 0.04^{a}	0.51 ± 0.04^{a}	ns
Lungs	0.70 ± 0.20^{a}	0.48 ± 0.21^{a}	0.51 ± 0.02^{a}	0.55 ± 0.05^{a}	ns
Adipose tissue	0.90 ± 0.09^{b}	4.77 ± 0.69^{a}	1.22 ± 0.13^{b}	1.25 ± 0.23^{b}	<0.0001
BLOOD LIPID PROFILE AND GLUCOSE					
Triglycerides (g/L)	1.38 ± 0.20^{b}	2.06 ± 0.16^{a}	0.89 ± 0.21^{b}	1.21 ± 0.63^{b}	0.0007
Total cholesterol (g/L)	1.26 ± 0.03^{b}	1.51 ± 0.12^{a}	0.96 ± 0.17^{b}	1.01 ± 0.19^{b}	<0.0001
HDL-cholesterol (g/L)	0.23 ± 0.04^{a}	0.21 ± 0.06^{b}	0.24 ± 0.04^{a}	0.12 ± 0.03^{b}	<0.0001
LDL-cholesterol (g/L)	0.76 ± 0.07^{b,c}	1.07 ± 0.11^{a}	0.62 ± 0.09^{c}	0.85 ± 0.05^{b}	<0.0001
Atherogenic index	5.72 ± 1.19^{b}	7.60 ± 2.24^{a}	4.12 ± 1.22^{b}	8.66 ± 2.19^{a}	0.0006
Glucose (g/L)	0.70 ± 0.13^{a}	0.77 ± 0.08^{a}	0.51 ± 0.12^{b}	0.72 ± 0.04^{a}	0.0041
BLOOD LIVER AND KIDNEY MARKERS					
ALAT (UI/L)	21.75 ± 5.04^{a,b}	27.04 ± 4.76^{a}	10.23 ± 2.33^{c}	14.45 ± 2.82^{b,c}	<0.0001
ASAT (UI/L)	18.71 ± 6.72^{b}	39.06 ± 9.79^{a}	25.27 ± 6.76^{a,b}	23.34 ± 7.51^{b}	0.0050
Creatinine (mg/L)	2.91 ± 0.74^{c}	20.99 ± 3.27^{a}	12.63 ± 2.66^{b}	14.17 ± 5.13^{a,b}	<0.0001
Urea (mg/dL)	46.67 ± 10.32^{b}	73.96 ± 5.71^{a}	48.89 ± 9.89^{b}	43.03 ± 9.88^{b}	0.0002

Note: Values of the same line with different letters are significantly different; ns: not significant difference at P<0.05

Blood Lipid Profile and Glucose

HFD-fed groups showed an increase in the blood glucose and a dyslipidemia characterized by a significant increase in blood triglycerides, total cholesterol, LDL-cholesterol contents and atherogenic index while a significant decrease in HDL-cholesterol was recorded when compared to NSD-fed subjects (Table 2). Among animals under HFD feeding, those receiving FX extract had a lower blood content in triglycerides, total cholesterol, LDL-cholesterol, glucose, lower atherogenic index and higher level of HDL-cholesterol when compared to the control groups. These results suggest a regulatory action of FX on blood lipids and the glucose level. This could be due to the presence of bioactive compounds capable of inducing a significant decrease in blood triglycerides, total cholesterol, LDL-cholesterol as well as an increase in HDL-cholesterol in case of HFD-fed. For example, it is known that flavonoids possess anti-hypertriglyceridemic and anti-hypercholesterolemic properties through inhibition of acyl-CoA cholesterol acyltransferase and cholesterol absorption [26]. Also, alkaloids are able to induce a decrease in the levels of cholesterol and triglycerides by increasing the expression of hepatic receptor of LDL and to inhibit the synthesis of lipids in human hepatocytes by activation of adenosine monophosphate kinase [27]. Regarding hypoglycemic effect, bioactive compounds of FX extract were able to raise the amount of the activated form of protein kinase through AMP in the liver, resulting in a significant decrease in lipid accumulation and improvement of sensitivity to insulin [26-27]. The

effects noted with FX were comparable to those observed with the reference group except for the HDL-cholesterol, glucose content and atherogenic index which did not present any significant difference with control. Likewise, these effects were generally superior to those obtained with the plants administered individually as showed by Nkepndep et al. [6].

Effect of aqueous extract of FX on hepatic and renal functions

Macroscopic observation revealed some fat deposits on the liver and kidneys of HFD-fed controls which was not observed in FX-treated rats. Blood ASAT and ALAT activities, creatinine and urea rates (Table 2) and histopathological examination of these organs (Fig 4-Appendix 2) in controls groups showed that HFD affects hepatic and renal functions. Indeed, HFD-fed animals presented higher blood enzymes activity, creatinine and urea rates than NSD-fed rats. Moreover, they presented a vascular congestion in favor to a slight inflammation of hepatic cells, and a fatty liver (steatosis) progressing to steatosis-hepatitis (Fig 4A-Appendix 2). Additionally, a mesangial expansion and consequently a narrowed urinary (Fig 4B- Appendix 2) were also observed. FX alleviated these abnormalities caused by HFD feeding. Indeed, among the animals fed with HFD, those receiving FX presented a lower blood ALAT and ASAT activities as well as a lower blood contents in creatinine and urea. Their liver and kidneys exhibited normal features comparable to NSD-fed control group. This effect can be explained by the presence of flavonoids, steroids, tannins and alkaloids in the extract, which are able to decrease ASAT and ALAT blood activities. It has been found that bioactive compounds present in FX extract can repair liver and kidneys damages caused by toxic agents, regenerate damaged hepatocytes and reduce inflammation [21-23]. These results obtained from subjects treated with FX were comparable to those from with the reference group, or even higher than the results obtained with individual plants [6], there by indicating a synergistic effect between compounds from different plants.

Conclusion

This study revealed that the best tea blend sample is composed of 80% of *H. sabdariffa*, 10% of M*. spicata* and 10% of *Z. officinale.* It showed good antiobesogenic properties with a significant reduction in the food intake, body mass index, adipose tissue, blood triglycerides, total cholesterol, LDL-cholesterol, glucose, and atherogenic index, while increasing blood content in HDL-cholesterol when compared to the control groups. The results were comparable to those from the reference groups treated with a standard medicine, Orlistat. In addition, the developed tea blend formulation appears to have a corrective effect and reverse damages caused to the kidney and liver of rat subjects by HFD-fed. The study showed that this developed herbal tea blend could be safely used in the prevention and management of obesity and associated oxidative stress damages.

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Conflicts of Interest

Authors declare no conflict of interest.

Ethics Approval

This study was approved by the University of Douala Institutional Ethics Committee (N°2714CEI-Udo/06/2021/M) and All animal experiments were complied with the ARRIVE guidelines and carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Consent to Participate

Informed consent was obtained from each participant for sensory attributes evaluation

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Appendix 1

Table 1: Sensory Attributes, TPC and Antioxidant Activity of Different Formulated Herbal Tea Blends

Herbal tea code	Composition (%)			Sensory attributes					TPC (mgGAE/g)	Antioxidant activity	
	Hibiscus sabdariffa	*Mentha spicata*	*Zingiber officinale*	Color	Aroma	Flavor	Aftertaste	Overall acceptability		DPPH free radical scavenging activity (10^{-5} mol/g)	FRAP essay IC50 (ppm)
F1	80.00	10.00	10.00	7.13 ± 0.83^{c}	6.75 ± 0.78^{b}	6.25 ± 1.39^{c}	7.25 ± 0.92^{c}	7.25 ± 1.28^{c}	$5.28 \pm 0.95^{a,b}$	18.40 ± 4.01^{c}	2017.89 ± 211.68^{a}
F2	56.70	33.30	10.00	6.88 ± 1.03^{b}	6.56 ± 0.98^{b}	6.50 ± 1.00^{c}	$6.82 \pm 1.01^{b,c}$	6.38 ± 0.94^{b}	$4.03 \pm 0.70^{a,b}$	6.11 ± 1.87^{b}	5587.56 ± 1558.08^{b}
F3	56.70	10.00	33.30	6.88 ± 0.80^{b}	6.56 ± 0.74^{b}	6.25 ± 0.81^{c}	7.06 ± 0.70^{b}	6.44 ± 0.88^{b}	4.13 ± 0.81^{a}	$5.81 \pm 1.35^{a,b}$	6187.56 ± 1058.51^{b}
F4	10.00	56.70	33.30	5.86 ± 1.42^{a}	$6.00 \pm 1.59^{a,b}$	$5.06 \pm 2.13^{a,b}$	6.13 ± 1.14^{a}	5.56 ± 1.88^{a}	6.02 ± 0.89^{b}	$5.77 \pm 1.45^{a,b}$	10359.98 ± 1997.39^{c}
F5	33.30	56.70	10.00	6.88 ± 0.91^{b}	$6.25 \pm 1.00^{a,b}$	6.38 ± 1.13^{c}	7.06 ± 0.94^{b}	6.50 ± 1.20^{b}	3.68 ± 0.63^{a}	$4.30 \pm 0.69^{a,b}$	$9980.11 \pm 1297.39^{b,c}$
F6	33.30	10.00	56.70	6.06 ± 1.44^{a}	5.81 ± 1.36^{a}	4.63 ± 1.83^{a}	6.63 ± 1.22^{b}	5.63 ± 1.42^{a}	$4.22 \pm 1.01^{a,b}$	3.85 ± 1.01^{a}	$8799.27 \pm 1025.37^{b,c}$
F7	10.00	80.00	10.00	6.81 ± 0.86^{b}	6.69 ± 0.96^{b}	6.50 ± 0.94^{c}	6.75 ± 0.86^{b}	6.75 ± 0.94^{b}	7.35 ± 0.87^{c}	$6.60 \pm 0.1.67^{b}$	11075.29 ± 1011.15^{c}
F8	10.00	33.30	56.70	5.44 ± 1.63^{a}	6.25 ± 1.15^{b}	5.44 ± 1.54^{b}	5.89 ± 1.51^{a}	5.88 ± 1.42^{a}	$5.24 \pm 0.97^{a,b}$	5.00 ± 0.78^{a}	9786.17 ± 1781.28^{c}
F9	10.00	10.00	80.00	5.75 ± 1.07^{a}	6.50 ± 1.07^{b}	5.94 ± 1.14^{c}	6.00 ± 1.09^{a}	$5.81 \pm 0.99^{a,b}$	$4.28 \pm 0.39^{a,b}$	3.16 ± 0.39^{a}	11785.58 ± 2112.56^{c}
F10	33.33	33.33	33.33	6.25 ± 0.95^{b}	6.25 ± 0.83^{b}	6.06 ± 0.76^{c}	6.38 ± 1.07^{b}	$6.06 \pm 0.89^{a,b}$	3.22 ± 0.37^{a}	3.77 ± 0.69^{a}	$8258.57 \pm 1144.17^{b,c}$
P				<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TPC: total phenolic compounds
Values of the same column with different letters are significantly different

Appendix 2

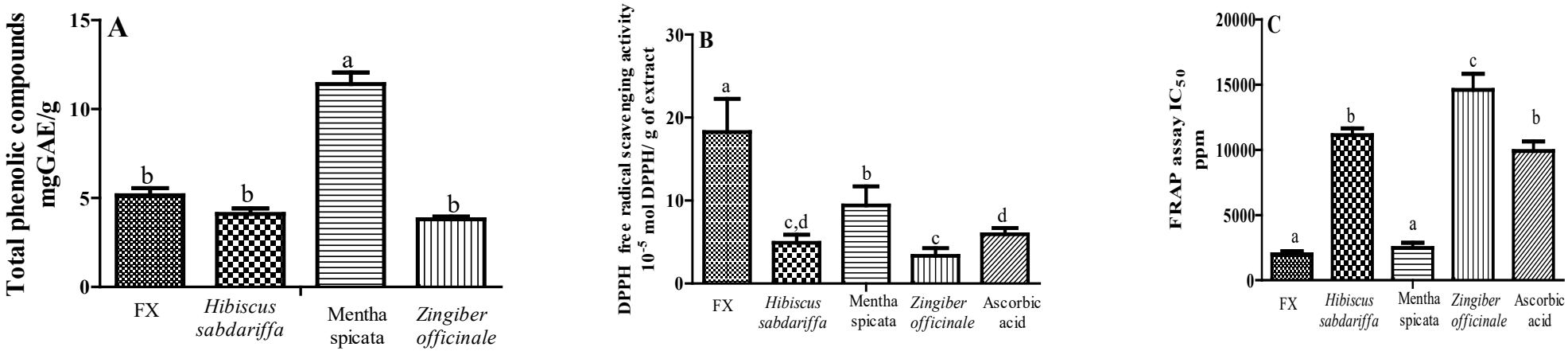


Figure 2: Total Phenolic Compounds (A), DPPH Free Radical Scavenging Activity (B) and Ferric Reducing Antioxidant Power (FRAP) Assay of FX, Individual Plants and Ascorbic Acid Used as Reference

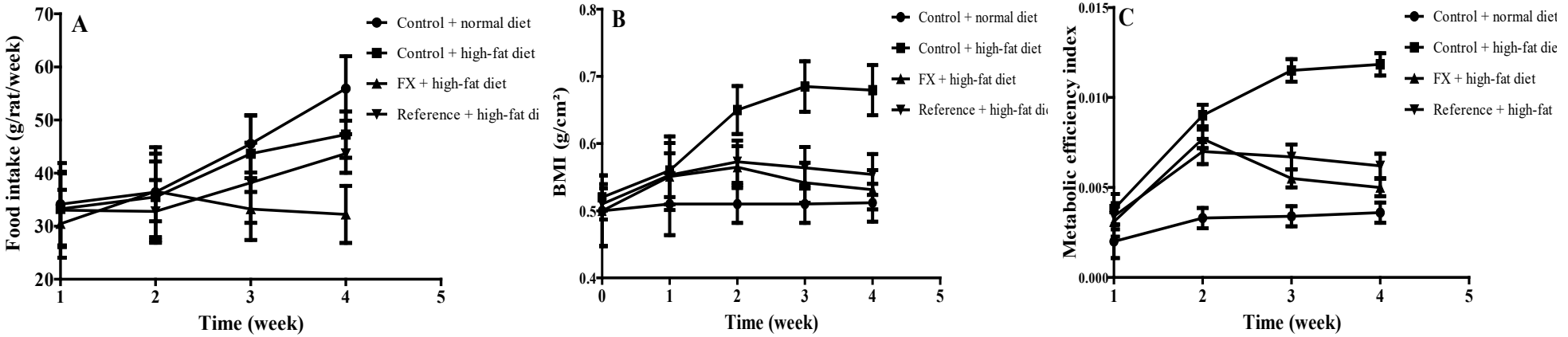
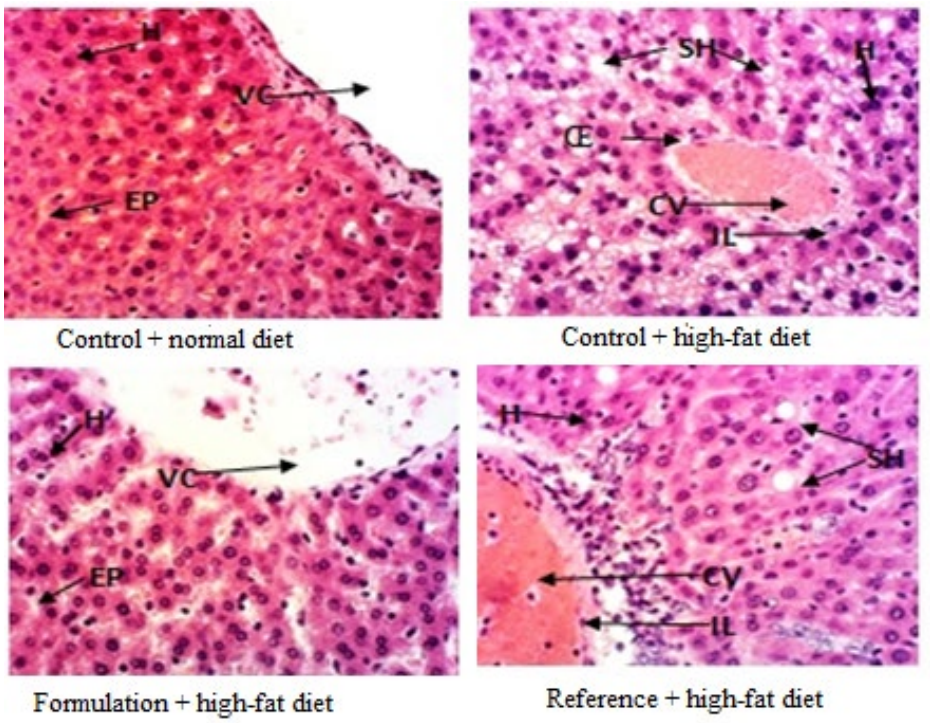
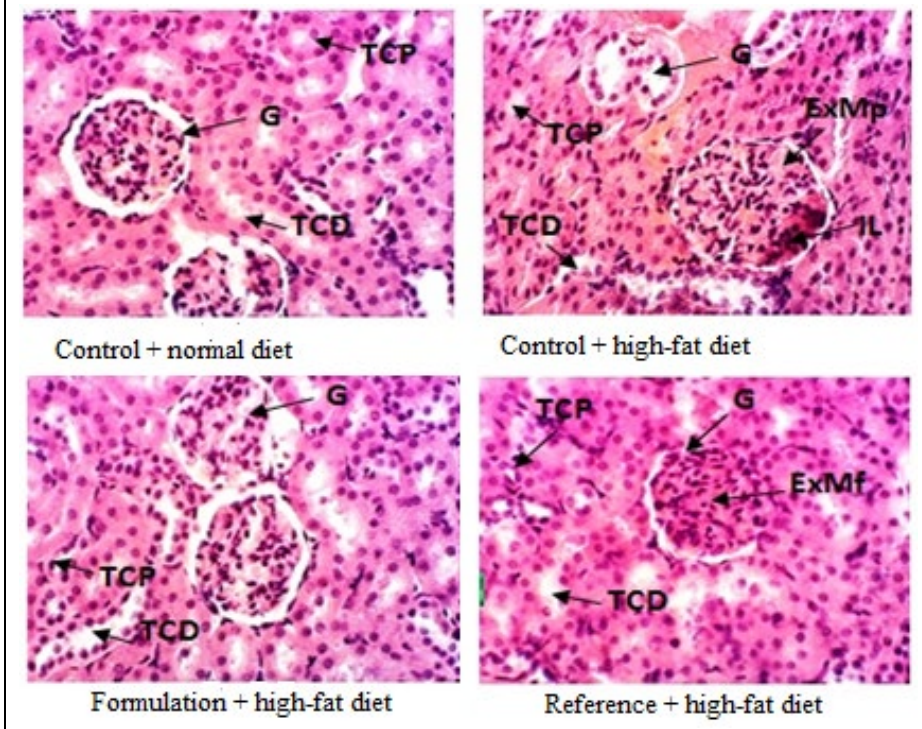


Figure 3: Effect of the Aqueous Extract of FX on Food Pattern Consumption (A), BMI (B) and Metabolic Efficiency Index (C) of Rats





H: Hepatocyte, EP: Portal space, Œ: Edema, CV: Vascular congestion, SH: Hepatic steatosis, IL: Leukocyte infiltration, VC: Centrilobular vein. Magnification 200x.

G: Glomerulus; TCD: Distal convoluted tube; TCP: Proximal bypass tube; ExMp: Pronounced mesangial expansion; ExMf: Non-pronounced mesangial expansion; IL: Leukocyte infiltration. Magnification 200x.

Figure 4. Micrograph of Rats' Liver (A) and Kidneys (B) After the Experiment