Journal of Food and Nutrition Sciences
2022; 10(5): 151-164
http://www.sciencepublishinggroup.com/j/jfns
doi: 10.11648/j.jfns.20221005.11
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

Antioxidant and Antiobesogenic Properties of Aqueous Extracts of *Hibiscus sabdariffa*, *Zingiber officinale* and *Mentha spicata* in *Wistar* High-Fat Diet Rats

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To cite this article:

Nkepndep Touohou Suzie Vanissa, Dongho Dongmo Fabrice Fabien, Lienou Lienou Landry, Maffo Tazoho Ghislain, Djeukeu Assongni William, Manz Koule Jules Christophe, Bogning Zangueu Calvin, Mbiatat Hilaire De Goeithe, Ebelle Etame Rebecca Madeleine, Gouado Inocent. Antioxidant and Antiobesogenic Properties of Aqueous Extracts of *Hibiscus sabdariffa, Zingiber officinale and Mentha spicata* in Wistar High-Fat Diet Rats. *Journal of Food and Nutrition Sciences*. Vol. 10, No. 5, 2022, pp. 151-164. doi: 10.11648/j.jfns.20221005.11

Received: August 11, 2022; **Accepted**: September 15, 2022; **Published**: September 28, 2022

Abstract: The world has already undergone several health-related upheavals that have drawn the attention among which obesity. It is an important public health problem as well in developed as in developing countries. In order to sustainably manage this disease and associated damages, medicinal plants could be an alternative solution to synthetic drugs which can present side effects. Thus, the present study aimed at screening *In vitro* and *In vivo* antioxidant and antiobesogenic properties of aqueous extracts of some local plant resources of Cameroon including *Hibiscus sabdariffa* calyces, *Zingiber officinale* rhizomes and *Mentha spicata* leaves. A phytochemical screening was performed on the aqueous extracts of the plants, and their *In vitro* antioxidant activity evaluated. *In vivo* antioxidant and antiobesogenic activities were assessed on male *Wistar* rats. 'Cafeteria' diet was used as obesity-inducer and Orlistat as standard treatment. Through the experiment, the extracts were orally administered at 1 g/kg bw per day for 28 days. *In vivo* antioxidant activity consisted of the evaluation of lipid peroxidation and some enzymes in serum, liver and kidneys. Antiobesogenic properties consisted of determining anthropometric parameters, food consumption pattern, blood lipids profile and glucose content. Hepatic and renal functions were also examined. The screening revealed numerous groups of bioactive compounds in the three tested extracts which also showed reasonable total phenolic content (338.67-1141.12 mgGAE/100g). They revealed excellent antioxidant activity through their good free radical DPPH scavenging potential, reducing power activity, significant decrease of tissue malondialdehyde content and increase of superoxide dismutase and catalase activities. Furthermore, they revealed antiobesogenic effect by their induction of significant reduction ($p < 0.05$) in body mass index, adipose tissue, food intake, blood contents of triglycerides, total cholesterol, LDL-cholesterol, glucose and atherogenic index, while increasing HDL-cholesterol, all compared to untreated obese animals. They exhibited a relatively protective effect on hepatic and renal functions by remedying histopathological damages caused by high-fat diet and by reducing blood transaminases activity, creatinine and urea contents. These effects were comparable or even greater than those observed with Orlistat. The findings of the present research showed that the three plants could be used in the management of obesity and oxidative stress.

Keywords: Hibiscus Sabdariffa, Zingiber Officinale, Mentha Spicata, Obesity, Oxidative Stress

1. Introduction

Obesity or overweight is characterized by an extra amount of adipose tissue to the degree that affects physiological, psychosocial or physical health and wellbeing of an individual [1]. According to WHO, Worldwide global rates of obesity determined by the body mass index (BMI) have almost increased by threefold since 1975 [2-4]. In Cameroon, 11.4% of adults were obese in 2016 against 9.8% in 2012. 11% of children under 5 were overweight in 2019 against 6.5% in 2012 [5]. Obesity is an unintended consequence of 'Western lifestyle' whose economic, social, and technological advances have resulted in urbanization and reduced physical activity. Diets transitioned from natural, organic foods to refined, high fat and high sugar alternatives leading to a nutritional transition parallel to the observed economic growth. These changes led to a rapid increase in the prevalence of obesity [6]. Changes in lifestyle and eating habits, consumption of foods of low micronutrients and bioactive compounds contents associated with a reduction in physical activity as well as genetic, environmental, cultural, and economic factors play a key role in the increasing of obesity prevalence and associated diseases [7-10].

Epidemiological, clinical, and experimental studies have clearly demonstrated that obesity is associated with metabolic complications such as lipids profile impairment and dysfunction of the redox status [11, 12]. In fact, an increase in the amount of accumulated fats contributes to oxidative stress [1]. Oxidative stress is essentially an imbalance between the production of free radicals such as superoxide, hydroxyl, and peroxyl radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants. It leads to many chronic and degenerative diseases such as cancer, atherosclerosis, Parkinson's and Alzheimer's diseases, diabetes, neurodegenerative disorders and aging. Overproduction of these radicals can lead to the aforementioned disorders as a consequence of "oxidative stress/damage" [13].

Obesity is thus considered as a major public health issue which requires immediate control measures. The goal of obesity treatment is to reach and remain at a healthy weight. The treatment consists of improving the overall health and lowering the risk of developing complications related to obesity [14]. Many attempts have been made to correct obesity by designing a number of drugs such as Orlistat, Fibrates, and Sibutramine. However, they have been found to have severe side effects, and are unaffordable [15]. Facing the undesired effects of synthetic drugs and the risks associated with surgery for weight loss, natural products are preferably used because of their effectiveness in the management of overweight, obesity, and many other chronic disorders and their relative safety. Many natural products contain vitamins, minerals, fibres, polyphenols, sterols and alkaloids. These compounds increase the body energy expenditure, decrease calorie intake, act as regulators of fats metabolism and could potentially play a vital role in the prevention and treatment of obesity [16]. The regain of interest in phytomedicine these recent years is due to the richness of plants in certain biomolecules such as flavonoids, anthocyanins, polyphenols and antioxidant compounds. These compounds may be specifically responsible for the beneficial effects in the treatment of numerous pathologies and as alternatives to chemical drugs [17].

Herbal medicines can be defined as crude products or isolated extracts of plants, and they are widely used for the prevention and treatment of many chronic diseases, including obesity [18]; they have fewer side effects than synthetic drugs. It is the case of *Hibiscus sabdariffa* (commonly known as "Bissap" or "Foléré"' in Cameroon), *Zingiber officinale* (known as ginger and used in many countries for thousands of years as a spice), and *Mentha spicata* (commonly known as spearmint). Indeed, it has been shown that aqueous extract of *H. sabdariffa* calyces has many biological properties which are diuretic, diaphoretic, laxative, antihypertensive, vasorelaxant, antioxidant, anti-inflammatory, hepatoprotective, and lipid-lowering activities [19-23]. Rhizomes of *Z. officinale* are widely used in traditional medicine and are prescribed for many ailments including nausea and indigestion [24]. In addition, its pharmacological effects are confirmed, particularly antiemetic [25], anti-inflammatory, antioxidant [26, 27], hypoglycemic, hypolipidemic [28, 29], and anticancer properties [30]. *Mentha spicata* leaves have long been used for medicinal purposes due to its healing, flavoring, antiseptic, and diuretic properties. It accelerates the healing of digestive and urinary disorders, and is a pain reliever [31].

These plants are available in Cameroon where they are commonly consumed as spices, vegetables, drinks or herbal teas. As a result, they could be used to manage obesity and associated disorders. Thus, the objective of the present work was to evaluate *In vitro* and *In vivo* antioxidant and antiobesogenic properties of aqueous extracts of *H. Sabdariffa* calyxes, *Z. officinale* rhizomes and *M. Spicata* leaves. Their effects on liver and kidney functions were also assessed. Male *Wistar* rats were used as *In vivo* model and obesity inducer was "cafeteria" diet.

2. Materials and Methods

2.1. Material

Dried calyxes of *H. sabdariffa* were obtained at Garoua town, North-Cameroon, an important cultivation area while flesh rhizomes of *Z. officinale* and leaves of *M. spicata* were obtained at Douala Sandaga market (Littoral-Cameroon). These plant parts were transported to the Biochemistry Laboratory of the University of Douala.

A total of 45 male *Wistar* rats weighing an average of 200±15 g were used in this study. They were obtained from the Laboratory of Physiology and Animal Biology of the University of Douala.

2.2. Methods

2.2.1. Plants Preparation and Extraction

Once at the laboratory, the plants parts were washed, cleaned, and dried at 45°C in a dry oven (BINDER).

Afterwards, dried materials were finely crushed in a food processor (MOULINEX). The resulting powders were hermetically bagged and stored in a dry place away from any light until extraction. Infusion was used to extract the powders in hot water (100°C) for 10 min using the ratio water to sample 100: 1 (v:w). Filtration was then done using Whatman N°1 filter paper and the filtrate was concentrated under vacuum by rotary evaporation at 40°C. The concentrate was then weighed and stored at 4°C until use. These extracts were subjected to qualitative phytochemical screening in order to seek for the presence of various bioactive compounds.

2.2.2. Phytochemical Screening of Plants Extracts

The crude extracts were subjected to qualitative phytochemical screening to identify presence or absence of selected bioactive compounds particularly saponins, flavonoids, anthocyanins, coumarins, tannins, steroids, glycosides, alkaloids and anthraquinones. This screening was done using standard methods as described by Harborne [31].

2.2.3. Animal Preparation and in Vivo Experiment

All the experiments were conducted in accordance with the internationally accepted guidelines for experimental animals use and the study was approved by University of Douala Institutional Ethics Committee (N° 2714 CEI-Udo/06/2021/M). The animals were housed at the animal handling facility of the Laboratory of Biochemistry, University of Douala. They were kept in cages under standard laboratory conditions and then acclimatized to the environmental conditions for one week before the beginning of the experiment. The rats were randomly divided into nine groups of five animals each and treated as follows: Two control groups with no treatment, one fed with normal diet (NSD) and the other with high-fat diet (HFD). Two experimental groups were constituted for each plant (one fed with NSD and the other with HFD). The animals were orally administered plants extracts at 1 g/kg bw dosage per day while the HFD fed group was administered Orlistat (standard treatment) at the same dosage along the same experimental period. NSD consisted of Standard Laboratory Animals Diet (SLAD) while HFD consisted to '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 [32]. Throughout the experiment which lasted 28 days, food and water were given *ad libitum.* Food intake was recorded daily and animal weights and lengths (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. For this purpose, the rats were euthanized using chloroform to minimize stress and pain during the sacrifice.

The blood was collected by cardiac puncture and some organs were removed. Blood samples were immediately transferred into microvacutainer tubes and centrifuged at 3000 rpm for 10 min to collect clear sera, which were stored in Eppendorf tubes at -20°C until analysis. The collected organs (liver, heart, spleen, kidneys, lungs, and adipose tissue) were rinsed with physiologic water, wrung out and weighed. The liver and kidneys were kept for further analysis. Thus, 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 min and the supernatants were collected, packed in Eppendorf tubes and stored at -20°C until analysis. Other parts of the organs were kept in formalin and stored at room temperature to be used for histology.

2.2.4. Determination of Antioxidant Activity

Antioxidant activities of extracts were determined *In vitro* and *In vivo* through the following protocols.

In vitro antioxidant activity: *In vitro* assays were done from crude plants extracts directly and consisted of evaluating total phenolic content (TPC), free radical scavenging activity and reducing power. TPC was determined by Folin-Ciocalteu's method and expressed as milligrams of gallic acid equivalents per 100 g of extract (mg GAE/100g) as described by Saeed *et al.* [33]. Free radical scavenging activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method described by Bursal and Gulcin [34]. As for the reducing power, it was determined by ferric reducing antioxidant power (FRAP) essay based on Fe(III) to Fe(II) transformation in the presence of the extracts according to Bursal and Gulcin [34]. Ascorbic acid was used as reference.

In vivo antioxidant activity: *In vivo* antioxidant activity was evaluated by determination of tissue markers of oxidative stress. Lipid peroxidation (LPO) and the activities of superoxide dismutase (SOD) and catalase were determined in serum as well as in liver and kidneys homogenates. The LPO consisted at evaluating the level of thiobarbituric acid reactive substance as well as malondialdehyde (MDA) production using the method described by Draper and Hadley [35]. SOD and catalase activities were assayed according to the method of Sun *et al.* [36] and of Atawodi [37] respectively. In parallel, the total protein content was assayed using commercially available total protein kit (www.biolabo.fr, Les Hautes Rives 02160, Maizy, France), employing direct Biuret method [38].

2.2.5. Determination of Anti-Obesogenic Activity

Anti-obesogenic properties of plants extracts was evaluated through *In vivo* experiment described above. It consisted of the determination of animals' anthropometric parameters (body mass index/BMI and organs relative weights), food consumption pattern, blood lipids profile and glucose content.

Anthropometric parameters: Animal weights and lengths, monitored weekly during *In vivo* experiment, were used to determine BMI of rats which is defined as:

$$\text{BMI (g/cm}^2\text{)} = \text{Weight} / (\text{Nose-to-anus length})^2.$$

Relative weights of the organs were evaluated at the end of the experiment using the formula:

$$\text{Relative weight (\%)} = 100 \times [(\text{organ weight}) / (\text{animal weight})]$$

Food consumption pattern: Food consumption behavior of rats was estimated weekly using data from food intake of rats recorded daily during *In vivo* experiment. Practically, each day 250 g of rats chow were given to groups of rats in their cages every morning and food intake was recorded by evaluating the remaining quantity (mass) 24 h after. The masses were calculated nearest to 0.1 g for spillage correction.

Blood lipids profile and glucose: Lipids profile consisted at evaluating of triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL), glucose and atherogenic index. Triglycerides [39], total cholesterol [40], HDL cholesterol [40] and glucose [39] were measured by spectrophotometric methods using test kits from BIOLABO (BIOLABO S. A. S, Paris-France) while LDL cholesterol was calculated using Friedewald's formula [41]: LDL-cholesterol = [Total cholesterol – (HDL-cholesterol + Triglycerides/5)]. The atherogenic index was calculated using the following formula:

Atherogenic index = Total cholesterol/HDL-cholesterol

2.2.6. Effect on Liver and Kidney Functions

Biomarkers of liver functions such as serum Alanine aminotransferase (ALAT) and Aspartate aminotransferase (ASAT) activities [42], and of renal functions such as serum creatinine [42] and urea [43] contents were evaluated by spectrophotometric methods using test kits from BIOLABO.

Moreover, histopathological examination of these tissues was done as described by Mariano and Di Fiore [44]. In fact, 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 (HE). The sections were observed under microscopy for histopathological changes, and their photomicrographs were captured.

2.2.7. Data Management and Statistical Analysis

Raw data were tabulated on MS Excel spreadsheet and organized for statistical analysis, then exported to GRAPHPAD PRISM version 5.9 (GraphPad Software, La Jolla California USA, www.graphpad.com). 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. The data were subjected to descriptive statistics and the results were expressed as mean ± standard error of mean (SEM). The values of $P < 0.05$ were considered significant.

3. Results

3.1. Phytochemical Screening of Plants Extracts

Table 1 shows the phytochemical profile of aqueous extracts of *H. sabdariffa, Z. officinale* and *M. spicata*. There can be noticed the presence of all analyzed compounds in *Z. officinale* while varying in their quantities. *H. sabdariffa* and *M. spicata* presented all the compounds except saponins.

Table 1. *Phytochemical profile of aqueous extracts of plants.*

	Hibiscus sabdariffa	**Zingiber officinale**	**Mentha spicata**
Saponins	-	+	-
Flavonoids	++	++	+++
Anthocyanins	+++	+	+
Coumarins	+++	+	+
Tannins	+++	+++	+++
Steroids	+++	+++	+++
Glycosides	+++	++	++
Alkaloids	++	+	+
Anthraquinones	+++	+	++

(+++): abundantly present, (++): moderately present, (+): fairly present; (-): absent.

3.2. Antioxidant Activity of Plants Extracts

3.2.1. In Vitro Antioxidant Activity

TPC, free DPPH radical activity inhibition and reducing power of aqueous extracts of *H. sabdariffa*, Z. *officinale* and *M. spicata* are presented in table 2. The results show that *M. spicata* presented the highest TPC, free radical inhibition activity and reducing power compared to *H. sabdariffa* and *Z. officinale*. Its free radical inhibition activity and reducing power were significantly higher than for ascorbic acid while those of *H. sabdariffa* and *Z. officinale* were comparable to ascorbic acid.

Table 2. *Polyphenols content and In vitro antioxidant activity of aqueous extracts of plants.*

	Hibiscus sabdariffa	**Zingiber officinale**	**Mentha spicata**	**Ascorbic acid**	**P**
Total phenolic (mgGAE/100g)	410.57 ± 30.78^{a}	380.67 ± 13.97^{a}	1141.12 ± 65.11^{b}	/	0.0013
Free radical inhibition activity DPPH (10^{-5} $(g/mol)^{-1}$)	$4.11 \pm 0.97^{a,c}$	3.35 ± 0.92^{a}	9.41 ± 2.30^{b}	5.93 ± 0.76^{c}	<0.0001
Reducing power FRAP (%)	40.78 ± 5.07^{a}	23.45 ± 6.02^{b}	54.98 ± 5.97^{a}	43.35 ± 8.57^{a}	<0.0001

Values of the same line with different letters are significantly different at $P < 0.05$.

3.2.2. In Vivo Antioxidant Activity of Aqueous Extracts of Plants

Table 3 shows the effect of aqueous extracts of *H. sabdariffa*, Z. *officinale* and *M. spicata* on serum, liver and renal MDA contents of rats with NSD and HFD. It reveals that control groups fed with HFD significantly increased serum MDA content and their liver and renal contents were not affected. The treatment with the three plants significantly decreased MDA blood content in rats under HFD compared to control and reference. Besides, the HFD didn't affect liver and renal MDA contents in animals, compared to NSD as

shown with controls groups. However, the treatment with the three plant extracts didn't affect hepatic MDA content compared to controls while reference significantly decreased it. In kidneys, *H. sabdariffa* and *Z. officinale* significantly decreased MDA content compared to controls while *M. spicata* and reference didn't show significant effect. These effects observed in HFD-fed rats were not reported in NSD-fed ones.

Table 3. *Effect of aqueous extracts of plants on blood, hepatic and renal MDA content of rats with normal and high-fat diets.*

MDA CONTENT	TREATMENT					P[1]
	Controls	Hibiscus sabdariffa	Zingiber officinale	Mentha spicata	Reference	
BLOOD (µmol/L)						
Normal diet	15.79 ± 4.03	14.27 ± 0.91	17.66±4.40	13.60 ± 1.62	-	ns
High-fat diet	19.48 ± 1.69[a]	14.30 ± 1.41[b]	14.34± 0.56[b]	13.84 ± 0.43[b]	17.76 ± 1.89[a]	0.0056
P[2]	0.027	ns	ns	ns	-	
LIVER (µmol/g)						
Normal diet	0.047± 0.002	0.047 ± 0.008	0.051 ± 0.005	0.050 ± 0.003	-	ns
High-fat diet	0.049 ±0.007[a]	0.052± 0.009[a]	0.046 ±0.007[a]	0.055 ±0.004[a]	0.036 ± 0.001[b]	0.0041
P[2]	ns	ns	ns	ns	-	
KIDNEYS (µmol/g)						
Normal diet	0.054± 0.006	0.050 ± 0.006	0.054 ± 0.004	0.055 ± 0.002	-	ns
High-fat diet	0.059 ± 0.004[a]	0.050 ±0.003[b]	0.049 ± 0.004[b]	0.053 ± 0.005[a,b]	0.058 ± 0.002[a]	0.0125
P[2]	ns	ns	ns	ns	-	

P[1]: comparison for a given parameter of values between different treatments (values of the same line with different letters are significantly different); P[2]: comparison for a given parameter and treatment of values between normal diet and high-fat diet; ns: not significant difference at P < 0.05.

The effects of studied extracts on blood, hepatic and renal SOD activities are presented in table 4. Regardless of the considered tissue, it was noticed that this activity was not affected by HFD as referring to controls. In blood, the treatment with plant extracts and reference didn't affect it both with rats under NSD and with those under HFD. However, in the liver, the treatment of HFD-fed rats with the three extracts significantly increased SOD activity as compared to controls while reference did not affect it. With NSD, *H. sabdariffa* and *M. spicata* significantly increased SOD activity and *Z. officinale* didn't affect it. In kidneys of HFD-fed animals, *Z. officinale* and *M. spicata* increased SOD activity while *H. sabdariffa* didn't affect it. However, only *H. sabdariffa* significantly increases SOD activity while *Z. officinale* and *M. spicata* didn't have effect in kidneys from NSD-fed rats.

Table 4. *Effect of aqueous extracts of plants on blood, hepatic and renal SOD activity of rats with normal and high-fat diets.*

SOD activity	TREATMENT					P[1]
	Controls	Hibiscus sabdariffa	Zingiber officinale	Mentha spicata	Reference	
BLOOD (µmol/min/mg proteins)						
Normal diet	23.85± 1.98	23.04 ± 6.28	19.91 ±4.67	21.61 ± 4.08	-	ns
High-fat diet	24.07± 7.73	22.56 ±5.83	30.23 ±8.71	20.95 ± 5.55	27.75 ± 9.19	ns
P[2]	ns	ns	ns	ns	-	
LIVER (µmol/min/mg proteins)						
Normal diet	43.11 ± 10.50[a]	92.44 ± 11.79[b]	53.05 ± 8.46[a]	77.27 ± 4.52[b]	-	<0.0001
High-fat diet	39.38 ± 8.22[a]	56.39 ± 8.61[b]	52.35 ± 9.44[b]	48.06 ± 3.08[b]	34.97± 14.56[a]	0.0004
P[2]	ns	0.0026	ns	0.0076	-	
KIDNEYS (µmol/min/mg proteins)						
Normal diet	34.76± 7.13[a]	56.75± 4.32[b]	36.78± 7.36[a]	30.36 ± 9.11[a]	-	0.0130
High-fat diet	38.46 ±4.75[a]	38.48 ±3.69[a]	50.76± 8.81[b]	54.99 ± 7.63[b]	43.39 ± 3.87[a]	0.0002
P[2]	ns	0.0007	ns	0.0102	-	

P[1]: comparison for a given parameter of values between different treatments (values of the same line with different letters are significantly different); P[2]: comparison for a given parameter and treatment of values between normal diet and high-fat diet; ns: not significant difference at P < 0.05.

Table 5, which presents the effect of plant extracts on blood, hepatic and renal activities of catalase, shows that HFD significantly decreased the activity in blood but increased it in liver and kidneys. In blood, the treatment led to significant increase in the activity compared to control group and regardless to diet; this effect was similar with reference. In the liver of HFD-fed rats, *Z. officinale* and *M. spicata* significantly decreased catalase activity as compared to controls while *H. sabdariffa* and reference didn't affect the parameter. In NSD-fed animals, *H. sabdariffa* increased the enzyme activity while *Z. officinale* and *M. spicata* didn't have significant effect on it. In kidneys, the treatment with plant extracts didn't affect these activities as compared to the controls groups and regardless of the diet.

Table 5. *Effect of aqueous extracts of plants on blood, hepatic and renal catalase activity of rats with normal and high-fat diets.*

CATALASE activity	TREATMENT					P[1]
	Controls	Hibiscus sabdariffa	Zingiber officinale	Mentha spicata	Reference	
BLOOD ($nmolH_2O_2$/min/mg proteins)						
Normal diet	54.47± 6.88[a]	125.74 ± 21.49[b]	155.58 ± 24.38[b]	146.97± 21.94[b]	-	<0.0001
High-fat diet	32.31 ± 2.19[a]	77.40 ± 8.93[b]	130.74 ± 34.20[c]	107.92 ± 36.98[b,c]	106.95 ±12.74[c]	<0.0001
P[2]	0.0009	<0.0001	ns	ns	-	
LIVER ($nmolH_2O_2$/min/mg proteins)						
Normal diet	53.88 ± 12.75[a]	101.36 ± 24.83[b]	40.74 ± 3.26[a]	41.65 ± 5.49[a]	-	0.0018
High-fat diet	99.02 ± 26.91[a]	138.37± 39.59[a]	47.34 ± 12.24[b]	75.40 ± 17.16[c]	98.71±14.16[a]	<0.0001
P[2]	0.0230	ns	0.00218	0.0095	-	
KIDNEYS ($nmolH_2O_2$/min/mg proteins)						
Normal diet	23.38±5.67	31.20± 2.17	31.90± 7.93	21.01 ±8.91	-	ns
High-fat diet	64.01 ± 8.52	60.15 ± 1.07	70.46 ± 6.36	54.17 ± 12.19	68.06 ± 10.75	ns
P[2]	0.0002	ns	0.0003	0.0046	-	

P[1]: comparison for a given parameter of values between different treatments (values of the same line with different letters are significantly different); P[2]: comparison for a given parameter and treatment of values between normal diet and high-fat diet; ns: not significant difference at $P < 0.05$.

3.3. Antiobesogenic Activity of Aqueous Extracts of Plants

3.3.1. Anthropometric Parameters

Table 6 presents the effect of plant extracts on animals BMI. It shows, regarding to controls groups, that HFD significantly affected the BMI. In fact, BMI of the control NSD group didn't significantly change during the 4 experiment weeks while the parameter significantly increased in HFD-fed group, starting from the 2nd week. This confirms that cafeteria diet leads to an important weight gain. Otherwise, the treatment with the three extracts prevented this weight gain since any significant variation was noted in HFD-fed groups treated with extracts regardless of the plant. This effect is similar to that noted with the reference group as well as with NSD-fed groups treated with the extracts.

Table 6. *Effect of aqueous extracts of plants on body mass index of rats with normal and high-fat diets.*

BMI (g/cm²)	Week					P
	0	1	2	3	4	
Control + normal diet	0.50 ± 0.08	0.51±0.10	0.51 ± 0.05	0.51 ± 0.14	0.52 ± 0.03	ns
Control + high-fat diet	0.52± 0.08[a]	0.56± 0.05[a]	0.65± 0.04[b]	0.65 ± 0.05[b]	0.65± 0.06[b]	0.031
Hibiscus sabdariffa + normal diet	0.48 ± 0.04	0.48 ± 0.06	0.47 ± 0.07	0.47 ± 0.11	0.47± 0.10	ns
Hibiscus sabdariffa + high-fat diet	0.47 ± 0.03	0.48 ±0.06	0.49± 0.06	0.52 ± 0.06	0.53± 0.06	ns
Zingiber officinale + normal diet	0.48 ±0.06	0.47± 0.03	0.48 ± 0.03	0.49 ± 0.02	0.50± 0.04	ns
Zingiber officinale + high-fat diet	0.46 ± 0.04	0.47 ±0.04	0.48± 0.03	0.49 ± 0.02	0.50± 0.04	ns
Mentha spicata + normal diet	0.50± 0.07	0.46± 0.07	0.44 ± 0.12	0.47 ± 0.14	0.46 ± 0.12	ns
Mentha spicata + high-fat diet	0.49 ± 0.02	0.46 ± 0.02	0.53 ± 0.05	0.55 ± 0.05	0.55 ± 0.05	ns
Reference + high-fat diet	0.51 ±0.04	0.53 ±0.02	0.53 ± 0.03	0.54 ± 0.03	0.54 ± 0.03	ns

The effect of extracts on organs relative weights is presented in table 7. It shows that apart from adipose tissue for which the relative weight significantly varied with diet and treatment, no significant change was noted with other organs (liver, heart, spleen, kidneys and lungs) regardless of the diet and treatment. In fact, HFD-fed control animals presented a significant increase in adipose relative weight compared to NSD-fed control animals. Moreover, a significant decrease was experienced in adipose relative weight HFD-fed groups treated with the three extracts compared to HFD-fed controls; this effect was also noted with the reference group. The result of the treatment with extracts is not the same with NSD where a slight increase was noticed in adipose tissue relative weight in treated groups when compared to the control group.

Table 7. *Effect of aqueous extracts of plants on organs relative weight of rats with normal and high-fat diets.*

RELATIVE WEIGHT (%)	TREATMENT					P[1]
	Control	Hibiscus sabdariffa	Zingiber officinale	Mentha spicata	Reference	
LIVER						
Normal diet	1.93 ±0.78	2.32 ± 1.10	2.56 ±0.48	2.78 ± 0.78	-	ns
High-fat diet	2.35 ±0.23	2.49 ± 0.17	2.14 ±0.32	2.31 ±0.05	2.41 ± 0.10	ns
P[2]	ns	Ns	ns	ns	-	
HEART						
Normal diet	0.25 ± 0.02	0.28 ±0.13	0.27 ±± 0.04	0.30 ± 0.07	-	ns
High-fat diet	0.24 ± 0.02	0.24 ±0.01	0.26 ±0.03	0.26± 0.03	0.26 ± 0.01	ns
P[2]	ns	ns	ns	ns	-	
SPLEEN						
Normal diet	0.30 ± 0.07	0.32 ±0.18	0.35 ±0.09	0.36 ± 0.09	-	ns
High-fat diet	0.32 ± 0.07	0.35 ±0.04	0.38 ±0.09	0.35± 0.03	0.31 ± 0.02	ns
P[2]	ns	ns	ns	ns		

RELATIVE WEIGHT (%)	TREATMENT					P[1]
	Control	Hibiscus sabdariffa	Zingiber officinale	Mentha spicata	Reference	
KIDNEYS						
Normal diet	0.56±0.06	0.52 ±0.24	0.46 ±0.13	0.53 ±0.13	-	ns
High-fat diet	0.47 ± 0.06	0.46 ±0.05	0.48 ±0.07	0.48 ±0.05	0.51 ± 0.04	ns
P[2]	ns	ns	ns	ns	-	
LUNGS						
Normal diet	0.70±0.20	0.61 ±0.17	0.78 ±0.26	0.74 ±0.23	-	ns
High-fat diet	0.48 ±0.21	0.61 ±0.14	0.62 ±0.18	0.53 ±0.13	0.65 ± 0.15	ns
P[2]	ns	ns	ns	ns	-	
ADIPOSE TISSUE						
Normal diet	0.90 ±0.09[a]	1.28 ±0.63[b]	1.12 ±0.80[b]	0.96 ±0.28[a]	-	0.0344
High-fat diet	4.77 ± 0.69[a]	2.81 ±0.47[b]	3.00 ±1.06[b]	2.63 ±0.47[b]	1.25 ± 0.83[c]	<0.0001
P[2]	<0.0001	0.008	0.0299	0.0009	-	

P[1]: comparison for a given parameter of values between different treatments (values of the same line with different letters are significantly different); P[2]: comparison for a given parameter and treatment of values between normal diet and high-fat diet; ns: not significant difference at P < 0.05.

3.3.2. Food Consumption Pattern

Figure 1 presents the effect of aqueous extracts of *H. sabdariffa*, *Z. officinale* and *M. spicata* on food consumption patterns of animals. It was noticed that weekly food intake was relatively the same between all the groups along the first couple of weeks of the experiment regardless of the treatment and the diet (30-37 g/rat the 1st week and 48-59 g/rat the 2nd week) applied. From the 3rd week, the food intake was higher in animals under NSD (79-90 g/rat the 3rd week and 90-101 g the 4th week) compared to HFD animals (57-71 g/rat the 3rd week and 58-79 g the 4th week). Likewise, the food intake varied with the treatment from the 3rd week of experiment. In fact, for control groups it increased from 65-81 g/rat the 3rd week to 85-100 g/rat the 4th week and from 70 to 78 g/rat for the reference group. On the contrary, the food intake was relatively constant from the 3rd to the 4th week for groups treated with plant extracts independently of the applied diet (from 57-90 g/rat the 3rd week to 58-90 g/rat the 4th week).

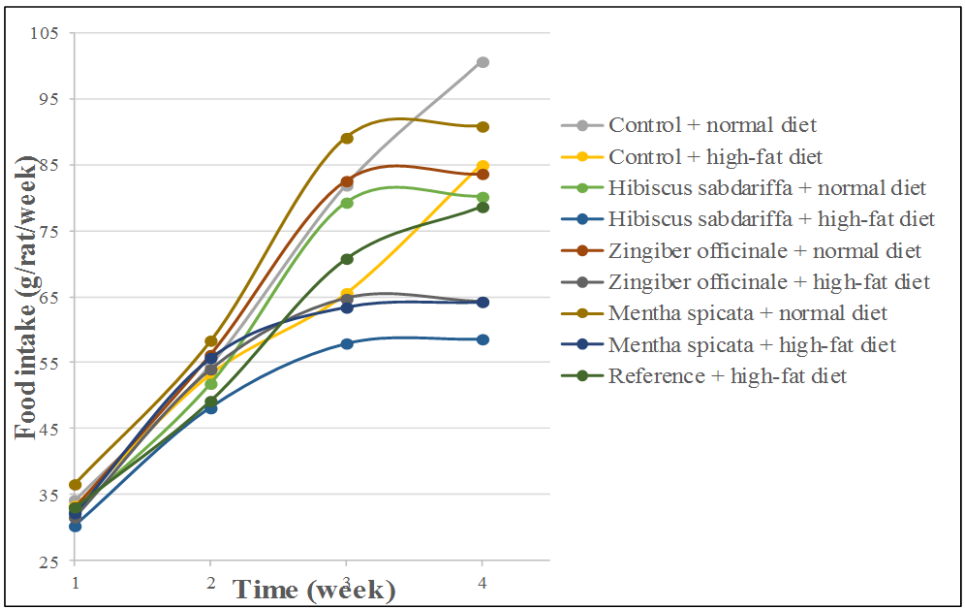


Figure 1. *Effect of aqueous extracts of plants on food pattern consumption of rats with normal and high-fat diets.*

3.3.3. Blood Lipids Profile and Glucose

The effect of aqueous extracts of *H. sabdariffa*, *Z. officinale* and *M. spicata* on serum lipids profile and glucose content is shown in table 8. As compared to the control and NSD-fed animals, HFD-fed presented significantly high serum contents in triglycerides, total cholesterol and LDL-cholesterol. This observation was correlated with the results obtained above with BMI and relative weight of adipose tissue. Meanwhile, HDL-cholesterol, glucose contents and atherogenic index were not significantly affected with HFD. The treatment with the three plant extracts allowed to significant reduction in triglycerides, total cholesterol, LDL-cholesterol, glucose content and in atherogenic index in HFD-fed animals compared to control HFD group. Otherwise, the treatment led to significant increase in HDL-cholesterol. These effects with plant extracts were similar, even sometimes slightly higher when compared to the reference group. The observations with HFD-fed animals were the same as in NSD-fed ones for all the parameters except triglycerides and HDL-cholesterol for which no significant variation between treated and control groups were noted.

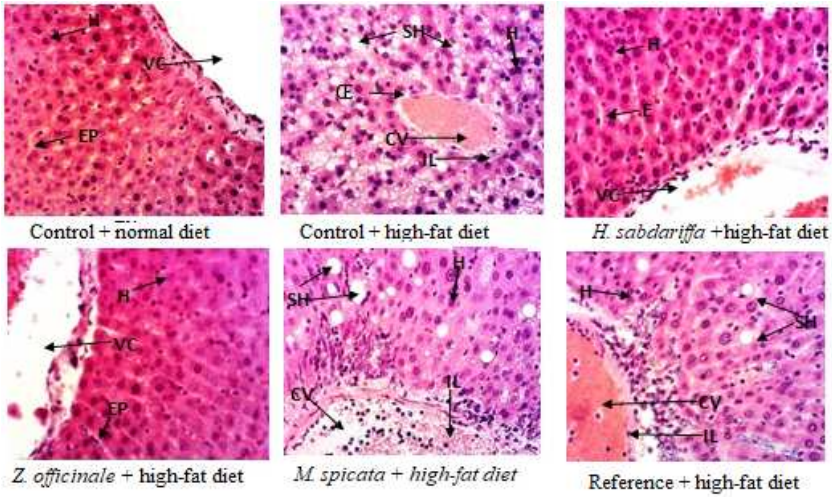
Table 8. *Effect of aqueous extracts of plants on blood lipid profile and glucose of rats with normal and high-fat diets.*

	TREATMENT					P[1]
	Control	Hibiscus sabdariffa	Zingiber officinale	Mentha spicata	Reference	
TRIGLYCERIDES (g/L)						
Normal diet	1.38 ± 0.20	1.01± 0.01	1.16 ±0.46	1.04 ±0.21	-	ns
High-fat diet	2.06 ± 0.16[a]	0.88±0.19[b]	0.78 ±0.09[b]	0.94 ±0.08[b]	1.21 ±0.63[b]	0.0142
P[2]	0.0018	ns	ns	ns	-	
TOTAL CHOLESTEROL (g/L)						
Normal diet	1.26 ± 0.03[a]	0.49 ± 0.08[b]	0.48 ±0.07[b]	0.64 ±0.010[c]	-	<0.0001
High-fat diet	1.51 ± 0.12[a]	0.71 ± 0.07[b]	0.57 ±0.11[c]	0.92 ±0.08[d]	1.01 ±0.19[d]	<0.0001
P[2]	0.0071	0.0031	ns	0.0073	-	
HDL-CHOLESTEROL (g/L)						
Normal diet	0.23± 0.04	0.19± 0.01	0.19 ±0.02	0.19 ±0.04	-	ns
High-fat diet	0.21 ± 0.06[a]	0.20 ±0.09[a]	0.21 ± 0.02[a]	0.35 ±0.10[a]	0.12 ±0.03[b]	0.0034
P[2]	ns	ns	ns	0.0245	-	
LDL-CHOLESTEROL (g/L)						
Normal diet	0.76 ± 0.07[a]	0.11 ±0.03[b]	0.20 ± 0.01[c]	0.30 ±0.02[d]	-	<0.0001
High-fat diet	1.07 ± 0.11[a]	0.32 ±0.05[c]	0.27 ± 0.01[c]	0.64 ±0.02[d]	0.85 ±0.05[b]	<0.0001
P[2]	0.0023	0.004	<0.0001	<0.0001	-	
ATHEROGENIC INDEX						
Normal diet	5.72 ± 1.19[a]	2.53 ±0.43[b]	2.52 ± 0.18[b]	3.52 ±0.65[c]	-	0.0051
High-fat diet	7.60 ± 2.24[a]	4.07 ±1.50[b]	2.69 ± 0.50[c]	2.95 ±1.33[c]	8.86 ±3.19[a]	0.00136
P[2]	ns	ns	ns	ns	-	
GLUCOSE (g/L)						
Normal diet	0.77 ± 0.13[a]	0.61 ±0.01[b]	0.61 ± 0.03[b]	0.59 ±0.04[b]	-	0.0051
High-fat diet	0.70 ±0.08[a]	0.64 ±0.03[b]	0.61 ±0.04[b]	0.68 ±0.06[a,b]	0.72 ±0.04[a]	0.00136
P[2]	ns	ns	ns	0.0468	-	

P[1]: comparison for a given parameter of values between different treatments (values of the same line with different letters are significantly different); P2: comparison for a given parameter and treatment of values between normal diet and high-fat diet; ns: not significant difference at $P < 0.05$.

3.4. Effect of Aqueous Extracts of Plants on Renal and Hepatic Functions

The macroscopic observation of animals' organs particularly liver, heart, spleen, kidneys and lungs didn't show any particular sign of inflammation in NSD-fed rats, as well in the control group as in extracts-treated animals. Contrarily, HFD-fed control and reference groups presented slight lung inflammations characterized by swelling and redness; it was not the case with the rats receiving plant extracts. Heart and spleen showed uniform structures in all groups regardless of the diet and the treatment. Moreover, in HFD-fed control some fat deposits were observed on certain organs including the liver and kidney.



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

Figure 2. *Micrograph of rats' liver after experiment.*

Concerning the effect of extracts on hepatic and renal functions, table 9 shows ALAT and ASAT activities as well as urea and creatinine contents in blood. For hepatic functions, ALAT and ASAT activities of HFD-fed groups were not

significantly different from control values. However, plants extracts treatments led to a slight decrease in the activities of the enzymes whatever the treatment and diet, except for ASAT activity in NSD-fed groups where the extracts significantly increased it compared to control. The results of histopathological examination of liver in control NSD-fed rats and all HFD-fed groups are presented in figure 2. It shows that in comparison with NSD control group, there is a vascular congestion (thickening of vascular membrane accompanied with a relative presence of blood cells in the vessels) in favor to a slight inflammation of hepatic cells on one hand, and a fatty liver (steatosis) progressing to steatosis-hepatitis in HFD control group on the other hand. The same observation was made with reference groups and the group treated with *M. spicata*. The groups receiving *H. sabdariffa* and *Z. officinale* exhibited a normal aspect.

Concerning renal functions, table 9 shows a significant increase in HFD-fed animals' blood contents in creatinine and urea as compared to control groups. These contents were significantly lower in groups under HFD, treated with plant extracts as well as with reference. In NSD groups, extracts didn't significantly affect creatinine and urea contents compared to the control.

Table 9. *Effect of aqueous extracts of plants on blood ALAT, ASAT, urea and creatinine of rats with normal and high-fat diets.*

	TREATMENT					P[1]
	Controls	Hibiscus sabdariffa	Zingiber officinale	Mentha spicata	Reference	
ALAT (UI/L)						
Normal diet	21.75± 5.04[a]	11.50 ±3.78[b]	11.74 ± 4.95[b]	11.09 ± 3.04[b]	-	0.0070
High-fat diet	17.04 ±1.76	11.64 ±7.76	10.04 ±7.03	14.30 ± 7.64	14.45 ±2.82	ns
P[2]	ns	ns	ns	ns	-	
ASAT (UI/L)						
Normal diet	18.71 ±6.72	22.21 ±11.50	27.74 ±15.26	18.60 ± 12.67	-	ns
High-fat diet	39.06 ±15.99	27.76 ±± 13.67	16.49 ±11.92	37.77 ±16.51	25.34 ±17.51	ns
P[2]	ns	ns	ns	ns	-	
CREATININE (mg/L)						
Normal diet	2.91 ±0.74	2.43 ±0.92	2.68 ±0.28	3.10 ±0.45	-	ns
High-fat diet	20.99 ±3.27[a]	16.36 ±3.93[a,b]	14.81 ± 6.79[a,b]	14.26 ±1.01[b]	14.17 ±5.13[a,b]	<0.0001
P[2]	0.0001	ns	0.0218	0.0095	-	
UREA (mg/dL)						
Normal diet	46.67 ±17.32	34.20 ±6.49	31.82 ± 6.60	33.64 ±4.78	-	ns
High-fat diet	73.96 ±5.71[a]	61.37 ±7.75[a,b]	52.80 ± 9.22[b]	51.46 ±11.24[b]	43.03 ±17.88[b]	0.0247
P[2]	0.0242	0.0017	0.0101	0.0207	-	

P[1]: comparison for a given parameter of values between different treatments (values of the same line with different letters are significantly different); P[2]: comparison for a given parameter and treatment of values between normal diet and high-fat diet; ns: not significant difference at P < 0.05.

These observations are confirmed by the results of histopathological examination of kidneys of NSD control and of all HFD groups as shown in figure 3. In fact, in comparison with the NSD control group, there is a mesangial expansion characterized by a glomerulus presenting an enlarged mesangium, and consequently a narrowed urinary space in the HFD control group. The same observation was made with the reference group; the groups which received plants extracts exhibited normal kidneys aspect.

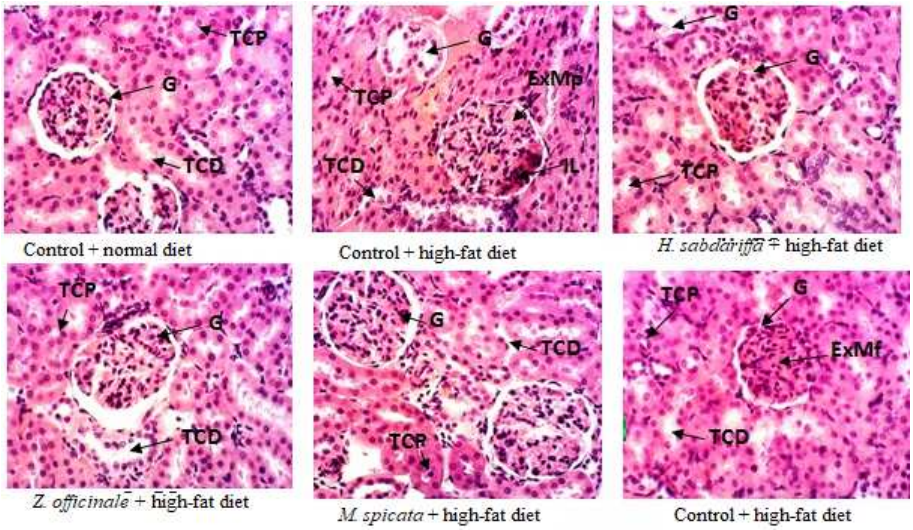


Figure 3. *Micrograph of rats' kidneys after experiment.*

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

4. Discussion

In order to contribute to the management of obesity through the enhancement of natural resources, this study has been performed with the objective of evaluating the *In vitro and In vivo antioxidant and* antiobesogenic properties of aqueous extracts of *H. Sabdariffa* calyxes, *Z. officinale* rhizomes and *M. Spicata* leaves and their effect on liver and kidney functions on high-fat induced obese *Wistar* rats. The phytochemical screening showed the presence of several groups of bioactive compounds in the three plants extracts in particular anthocyanins, tannins, steroids, anthraquinones, glycosides, alkaloids, flavonoids and steroids. This result corroborates with those of Maffo *et al.* [45], Bashige *et al.* (2020) [46] and Hamid *et al.* [47] who confirmed the presence of these compounds in *H. Sabdariffa* calyxes, *Z. officinale* rhizomes and *M. Spicata* leaves respectively. Most of these compounds have been shown to have health beneficial effects including protection against DNA cleavage, stimulation of cytokine production, anti-inflammatory and antimicrobial activities, protection against oxidative stress, and inhibition of weight gain, decrease in adipose tissue and regulation of the lipid profile [48]. Indeed, flavonoids are considered to be excellent antioxidants [49] and have anti-inflammatory, hypotensive, hypoglycemic, cardioprotective and anticancer effects [50]. The presence of tannins suggests the capacity of these plants to be used as anti-diarrheal and anti-hemorrhagic agents [51]. Alkaloids possess analgesic and antispasmodic [52], detoxifying, anti-hyperlipidemic, hypotensive and antihypertensive effects [53]. Anthocyanins reduce oxidative damage in living systems and act as anti-inflammatory and anti-hepatotoxic molecules [54].

Regarding antioxidant activity, the extracts have expressed an acceptable total phenolic compounds (TPC) value. Phenolic compounds are widely distributed in plant resources with redox properties that allow them to act as reducing agents (hydrogen donors), singlet oxygen scavengers or metal chelators [55]. They also have a diuretic activity by acting on the retention of potassium [56]. These extracts also have good free radical inhibition and reducing power activities. Maffo et al. [45] Jing et al. [57] and Anour et al. [58] showed that the H. Sabdariffa calyxes, Z. officinale rhizomes and M. Spicata leaves contain vitamins (group B, A, C, D and E), minerals and macronutrients which are necessary for the organism as they play a protective role against lipid peroxidation induced by fatty diet in hepatic and renal tissues. Likewise, the presence of minerals in plants extracts helps to restore the catalytic activities of various antioxidant enzymes acting as cofactors in enzymatic reactions in order to maintain their catalytic activity. These elements constitute essential transition metals in the defense against the metabolic disorder caused by the fatty diet. As for, In vivo antioxidant activity, SOD and catalase are considered to be primary enzymes involved in the direct elimination of reactive oxygen species (ROS). SOD plays an important role in protecting cells against the toxic effect of O_2•- by catalyzing its disproportionation reactions. The enzymes require copper and zinc for its activity. SOD maintains the concentration of superoxide radicals at low levels and therefore plays an important role in the defense against oxidative stress. Its biological role is to allow the disproportionation of $O_2 \cdot$-, H_2O_2 produced in this reaction is eliminated by catalase, one of the most active enzymes in humans. It is a hemoprotein which protects tissues against damages caused by ROS. MDA is one of the end products formed during the breakdown of polyunsaturated fatty acids mediated by free radicals. In fact, following oxidative stress, lipoperoxides are formed, including MDA which results from the fragmentation of polyunsaturated fatty acids [59]. The high amount of toxic metabolites in the body leads to an increase in free radicals. These initiate lipid peroxidation through increasing the concentration of MDA which is a biomarker of oxidative stress and one of the most studied consequences of ROS on lipid membranes. Thus, studied plants extracts would ensure the regulation of the serum, the liver and the renal biological markers of oxidative stress through an increase in SOD and catalase activities and inhibition in MDA production. Such effects reflect the antioxidant capacity of plants, which is generally attributed to their richness in bioactive and phenolic compounds. A large number of studies previously confirmed this hypothesis which suggests that it is the polyphenols content of the plant which is responsible for its high antioxidant activities. These molecules can trap and neutralize free radicals, inhibit the enzymes responsible for their formation and be chelators of certain metal ions [60].

Regarding antiobesogenic activity, this study showed that the treatment of obese rats with extracts induced a decrease in body weight gain, hence in BMI and food intake. The decrease in weight could be explained by several mechanisms:

Increased mandatory energy expenditure and adaptive thermogenesis: according to Kajimura *et al.* [61], most natural anti-obesity products regulate body weight through increased mandatory energy expenditure. They regulate the expression of thermogenin, a key protein in thermogenesis in brown adipose tissue, which converts energy from food into heat [62].

Suppression of appetite: the biological mechanisms of appetite and satiety are regulated by a complex interaction of neurological and hormonal signals. Many studies have shown that certain food ingredients (minerals, phytocompounds, etc.) could provide effects favorable to satiety and be beneficial for weight control. The mechanism underlying improved satiety includes an increase in norepinephrine level and subsequent activation of sympathetic nervous system activity resulting in increased satiety and energy expenditure, suppression of hunger and increased lipids oxidation [63].

Composition of plants: Many studies revealed that *H. sabdariffa*, *M. spicata* and *Z. officinale* are rich in Mg^{2+} [46, 64]. Gehan [64], showed that Mg^{2+} has a specific ability to increase lipid excretion by forming complexes between insoluble salts and fatty acids and preventing their intestinal absorption.

In addition, the results of the present research showed evidence that consumption of HFD causes a significant increase in adipose tissue while other organs (liver, spleen, lungs, kidneys and heart) were not affected in experimental groups compared to control groups. Indeed, the HFD induced body weight increase, associated with an accumulation of adipose tissue [65]. However, a decrease was noticed in adipose tissue of HFD-fed rats treated with plant extracts compared to control; the same effect was observed with the reference. These observations show the extracts have an effect on adipose tissue, therefore, they contain bioactive compounds which are likely to have an effect on satiety (and weight control), resulting in fat reserves loss [66].

This work confirms that the HFD induces deregulation of the lipid profile with an increase in blood triglycerides and cholesterol contents. It has been noted that plant extracts have hypocholesterolemic, hypertriglyceridemic and even hypoglycemic effects. Their administration induced a significant decrease in serum total cholesterol, LDL-cholesterol, triglycerides and atherogenic index compared to the control. No significant effect was noted on HDL-cholesterol levels in treated groups while the reference expressed a reduction in HDL cholesterol. These results suggest a regulatory effect of the extracts on lipids profiles. The studies of Maffo *et al.* [45], Alizadeh-Navaei *et al.* [67]. and Anour *et al.* [58] showed that extracts of *H. Sabdariffa* calyxes, *Z. officinale* rhizomes and *M. Spicata* leaves contain bioactive compounds capable of inducing a significant decrease in blood triglycerides, total cholesterol, LDL-cholesterol as well as an increase in HDL-cholesterol in rats under HFD. One possible explanation for these results is the presence of flavonoids in the extracts. These secondary metabolites possess anti-hyperlipidemic, anti-hypercholesterolemic [68] and anti-obesity [69] properties. The anti-hypercholesterolemic effect of flavonoids may be due to inhibition of acyl-CoA cholesterol acyltransferase activity [70] and cholesterol absorption [71]. This is also true for the alkaloids present in our extracts. Studies showed that these metabolites induce the decrease in the level of cholesterol, triglycerides by increasing the expression of hepatic receptor of low density lipoproteins, and inhibit synthesis of lipids in human hepatocytes by activation of adenosine monophosphate kinase [72]. Our results are in accordance with those of Ben *et al.* [73] and Zhan *et al.* [74] which demonstrated the cholesterol lowering effect of alkaloids. Regarding their hypoglycemic effect, studies on considered plants extracts have shown a stimulation of insulin release from β pancreatic islets cells [56, 75]. Extracts from hibiscus calyxes and ginger rhizome have been shown to reduce serum glucose, cholesterol and triglyceride levels, possibly due to the elevation of the activated form of protein kinase through AMP in the liver, resulting in a significant decrease in lipid accumulation and an improvement in insulin sensitivity [76].

Concerning hepatic and renal functions, a relative protecting effect of the plant extracts would have kept the organs safe. In fact, the results showed a significant decrease in the blood transaminases content of extracts-administered rats compared to the controls. Agreeing with the study of Mahesh *et al.* [77], this means that bioactive compounds from plants inhibit liver damage caused by the HFD. This effect can be explained by the presence of flavonoids, steroids, tannins and alkaloids in the extracts, as the hepatoprotective property of these metabolites have already been shown [78, 79]. Indeed, Rudenskaya *et al.* [80] showed that alkaloids decrease ASAT and ALAT activities as well as triglycerides concentration, confirming their hepatoprotective effect. Moreover, HFD significantly increased blood creatinine and urea contents compared to NSD. However, the administration of plant extracts resulted in a considerable reduction of these concentrations. This is an indication that extracts regulated renal function, and this may be due to the metabolites present in extracts. Indeed, it has been found that all the bioactive compounds present in studied extracts can repair liver and kidneys damages caused by toxic agents, regenerate damaged hepatocytes and reduce inflammation [45, 58, 81]. To confirm the biochemical data, histological examination of the liver and kidneys of HFD rats was performed. The results showed that this diet caused a slight infiltration of hepatic cells, hepatic steatosis in the liver and glomerular hypertrophy in the kidneys; these disruptions were not experienced in NSD group. However, the administration of extracts showed a protective and curative effect as they corrected the damaged tissues.

5. Conclusion

We demonstrated in this study that the aqueous extracts of *H. Sabdariffa* calyxes, *Z. officinale* rhizomes and *M. Spicata* leaves possess antioxidant and antiobesogenic activities revealed by their richness in vital phytochemical agents. In addition, these plant extracts seem not disrupting hepatic and renal functions, but would rather eradicate damages caused to these organs by a HFD. The study showed that *H. Sabdariffa* calyxes, *Z. officinale* rhizomes and *M. Spicata* leaves could be used in prevention and management of obesity and associated damages as oxidative stress.

We recommend, for prevention or treatment of obesity, the regular consumption of *H. Sabdariffa* calyxes, *Z. officinale* rhizomes and *M. Spicata* leaves, may be as herbal tea. It would be not trivial to consider the combiner use of the three plants in order to optimize their properties.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Availability

The data used in this study are available from the corresponding author upon request.

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