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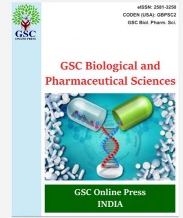

(Research Article)

Hypolipidemic effects of microencapsulated powder fraction from *Hibiscus sabdariffa*. L calyx in hyperlipidic rats

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Abstract

The lipid-lowering activity of microencapsulated and nonmicroencapsulated *Hibiscus sabdariffa* L. powder fractions in rats rendered hyperlipidemic by a hyperlipid diet (HLD) was determined. Dried calyces of *H. sabdariffa* were finely ground and fractionated on a sieve column to retain particle sizes ϕ < 315 µm, and were microencapsulated with maltodextrin (MD). Rats were randomly divided into four groups: normal control group, hyperlipid diet group (negative control groups), hyperlipid diet group supplemented with atorvastatin, non-microencapsulated (NMEPHS) and microencapsulated (MEPHS) powders from H. sabdariffa calyces groups. Rats received atorvastatin, NMEPHS and MEPHS for three weeks. Atorvastatin (10 mg/kg) and individual powders were dissolved in water and administered to rats at a dose of 250 mg/kg body weight for three weeks. Body weight, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate transferase (AST), alanine transferase (ALT), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were measured. Very low-density lipoprotein cholesterol (VLDL-C), hepatosomatic index (HSI), and adiposomatic index (ASI) were calculated. Lipid profil, and MDA were increased in the negative control rat groups compared with the negative control rat group. NMEPHS and MEPHS significantly reduced body weight gain, ASI, HSI, TC, TG, LDL-C, ALT, AST, and MDA and increased HDL-C level significantly. Moreover, SOD and CAT activities were reduced with HLD and significantly increased with HSCP intake. however, the most significant activities were revealed by MEPHS. These results suggest that MEPHS exerts potent lipid-lowering effects, promotes hepatic fat breakdown, and regulates antioxidant enzymes in a more efficient manner.

Keywords: *Hibiscus sabdariffa* calyxes; Powder fraction; Encapsulation; Hypolipidemic activity

1. Introduction

Hyperlipidemia, which refers to an abnormal increase in plasma and tissue lipids, is a potential risk factor leading to atherosclerosis and cardiovascular disease [1]. It is characterized by elevated levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) resulting from increased endothelial production of reactive oxygen species (ROS) such as generations of superoxide ions, nitric oxide, and hydrogen peroxide responsible for lipid peroxidation. Indeed, an excess of ROS towards the antioxidant defense mechanisms lead to oxidative stress. Generally, chemical drugs and surgery are used in the management of hyperlipidemia [2], however, to avoid their side effects patients are increasingly turning to the

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phytotherapeutic treatments that have been reported given excellent results [3, 4]. Indeed, researchers have highlighted the hypolipidemic effects of natural drugs based on plants, among which the *Hibiscus sabdariffa* L. [5].

H. sabdariffa L. is an important medicinal plant that has been widely recognized in tropical and subtropical regions of the world for its medicinal properties. In particular, its calyces which are chemically rich in bioactive components including phenols, flavonoids, tannins, anthocyanins, among others [6, 7]. Previous studies have reported hyperlipidemic, anti-obesity, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, antihypertensive and hepatoprotective properties of the *H. sabdariffa* L extract [5, 8, 9, 10]. Indeed, the heal properties of the *H. sabdariffa* extract has been reported to be significantly correlated to its phytochemical profile [7]. However, as reported by [11], the antiradical profile of extract depends chiefly on the extraction technique. Our research team previously explored an innovative extraction process based on the Alternation of Drying and Grinding and Controlled Differential Sieving process (ADG-CDSp) [7, 12]. It was found that small powder fractions of *H. Sabdariffa* calyces (≤212 µm) concentrated more bioactive ingredients with similar antioxidant properties as that of ethanolic extract powder [7, 12]. Indeed, plant grinding followed by Controlled Differential Sieving process (CDSp) is technically easier to implement for high-volume production compared to the conventional extraction using solvents [7]. While, the solvent used in conventional extraction techniques are specific to the extracted plant components, the CDSp had the advantage of not being specific to a given type of bioactive compounds, thereby concentrating numerous bioactive compounds having several antioxidant properties [7, 12, 13, 14]. Although, CDSp is a reliable technique to obtain an extract rich in biomolecules, the effectiveness health properties of plant extract depend on the preservation of the stability, bioactivity, and bioavailability of its active biomolecules [15]. Indeed, bioactive compounds must be encapsulated after their extraction, as these compounds are very sensitive to environmental exposure, especially to humidity and high temperatures (sensitive to heat, light and oxygen). Even though the encapsulation of *H. sabdariffa* calyxes' extracts have been reported in the literature [16, 17, 18], there are still scarcity of reports on the effect of food processing on the hypolidemic activity of *H. sabdariffa* calyxes' powder (HSCP). Therefore, this study seeks to assess the hypolipidemic effect of encapsulated and unencapsulated *H. sabdariffa* calyxes powder obtained by the ADG-CDSp process.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade from Sigma-Aldrich (Steinheim, Germany). Dried red calyxes of *H. sabdariffa* were purchased at the small market of Ngaoundere located in Adamawa province, Cameroon. Wistar rats (age: 3-4 months; weight: 250–300 g) were purchased from the animal laboratory of the Laboratory of Biophysics, Food Biochemistry and Nutrition (LABBAN) of the National School of Agro-Industrial Sciences, (University of Ngaoundere, Cameroon).

2.2. Hibiscus sabdariffa calyxes Encapsulation

Briefly, *H. sabdariffa* calyces were dried in a ventilated oven (Memmet, UF 55) at 50 °C for 3 hours with 100% opening of the damper. The calyces were then ground with a knife blender during 1 min at a rotor speed of 6,200 g. Thereafter, the powder was sieved in a continuous vibration mode at an amplitude of 0.5 mm for 10 minutes, finally the powder fraction ϕ < 315 µm was encapsulated with MD according to Tchabo *et al* [19] and Choi *et al*. [20] using a spray dryer (SCHULZ INFROPROD, FRA1388/12-078, made in Germany, UE). Concisely, the infeed solution made of MD and HSCP (1:2 w/w) was mixed (3500 rpm for 15 min) with distilled water (to obtain a total solid content of 30 % w/w). The previously prepared mixture was filtered through 315 µm diameter sieves, and spray-dried under the following operating conditions: 100 % suction rate (50 mL/min); inlet temperature of the drying air was 150 ± 1°C, while the pump flow rate was adjusted to maintain an outlet temperature of 70 ± 1°C. Then, the micro-encapsulated HSCP (MEPHS) was packaged into plastic bottles. Besides, HSCP of [212-315 µm] was used as non-encapsulated samples (NMEPHS).

2.3. Experimental animals

The inbreed colonies of *Wistar* rats used for the experiment were raised at the animal house of the Laboratory of Biophysics, Food Biochemistry and Nutrition (LABBAN) of the National School of Agro-Industrial Sciences, University of Ngaoundere, Cameroon. Rats were maintained at room temperature (25 ± 4 °C), 12 h light and 12 h dark and free access to formulated food and water allowed adlibitum [21]

2.4. Hypolipidemic activity evaluation

2.4.1. Induction of hyperlipidemia and experimental protocol

Hyperlipidemic diet (HLD) and normal diet (Table 1) were formulated using the method of Ngatchic et *al* (2013) [21]. The basic constituents of the HLD, were egg yolk and coconut oil respectively used as source of cholesterol and saturated fatty acid. Firstly, the animals were divided in two groups, the first group (n=10) was fed with normal diet ad libitum, while the second group (n = 25) was fed with HLD ad libitum. After 3 weeks, 5 rats of each group were sacrificed, and the lipid profile as well as the oxidative stress parameters was determined in order to verify the establishment of hyperlipidemia and oxidative stress. Then, the remaining rats of the first group (n=5) and the second group (n=20, randomized divided into four groups of 5 rats) were feed with normal died and HLD with ad libitum access to water during 3 weeks, respectively.

Table 1 Composition (%) of experimental diets [21]

Ingredients	Normal diet	High fat diet
Fish powders	20	15
Coconut oil	0	25
Soya oil	5	5
Egg yolk	0	25
Corn starch	59	19
Sugar	5	5
Corn cellulose	5	0
Minerals (bone powder)	5	5
Vitamin (B complex)	1	1

The atorvastatin, the non and encapsulated HSCP were dissolved into distilled water at a volume of 10 mL/kg body weight and administered to the animals by gavage at the dosage of 10 mg/kg (for atorvastatin) and 250 mg/kg (non and microencapsulated HSCP) following the methodology reported by Deli et *al* (2020) [14] and Ngatchic et *al* (2020) [22]. The volume of the HSCP solution or atorvastatin solution administrated to the rats was determined based on the weight of the animals using the equation 1.

$$\text{Volume (ml)} = \frac{\text{Dose}\left(\frac{\text{mg}}{\text{kg}}\right)\text{ X weight of rat (kg)}}{\text{concentration powder solution }\left(\frac{\text{mg}}{\text{m}}\text{L}\right)} \qquad \text{(Equation 1)}$$

The rat groups were splitted as follows: The first group named negative control rats (NeC) which received HLD and distilled water; the second group named positive control rats (PoC) which received HLD and atorvastatin; the third group received HLD and MEPHS; and, the fourth group received HLD and NMEPHS. Finally, the remaining 5 rats in the NoC group were used as normal control rats (NoC) which received the normal diet during treatment. The body weight of rats was recorded at the beginning and the end of the experiments, while the weight of the liver, kidneys, and abdominal fat were recorded after sacrificed.

2.4.2. Preparation of blood plasma and homogenates

Prior sacrifice, the fasted animals (overnight, more than 12h) were anesthetized in a glass jar containing cotton wool that was soak in diethyl ether. The blood was collected from retro-orbital plexus vein of rats. The blood samples were allowed to stand for 4 hours at room temperature, before centrifugation (BIOLAB: Benchtop High Centrifuge, Model N.: BCBH-101) at 3000 rpm for 15 minutes. The supernatant (plasma) was collected in Eppendorf tubes and stored at -4°C prior determination of the lipid profile and oxidative stress markers inside the plasma. Besides, the rats were dissected and the excised livers, kidneys and abdominal fats were washed immediately with saline solution (0,9 %) to remove blood. The organs were weighed in order to determine hepatosomatic and adiposomatic indices, following the equations 2 and 3. Afterwards, liver and kidney homogenates (0.5 g of tissue homogenized in a porcelain mortar with phosphate

buffer of 0.1 M, pH 7.4 having 0.15 M KCl) was centrifuged (3000 rpm for 15 min). The supernatant of the liver and kidney homogenates were collected in Eppendorf tubes and stored at -4°C prior determination of the oxidative stress markers.

$$Hepatosomatic\ index\ = \left(\frac{\text{Liver weight}}{\text{Body weight}}\right) \times 100 \qquad (Equation\ 2)$$

$$Adiposomatic\ index = \left(\frac{\text{Fat weight}}{\text{Body weight}}\right) \times 100 \qquad (Equation\ 3)$$

2.5. Determination of lipid profile

The total cholesterol, the triglycerides (TG), the HDL-cholesterol were assayed using a colorimetric enzymatic method as described by Ngatchic et *al* (2020) [22]. The low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and the atherogenic index (AI) were determined following the equations 4, 5 and 6, respectively.

$$LDL - C\ (mg/dl) = \text{Cholesterol total} - \left(\frac{\text{HDL} - \text{C} + \text{Tryglycérides}}{5}\right) \qquad (Equation\ 4)$$

$$VLDL - C\ (mg/dl) = \text{Cholesterol total} - (\text{LDL} - \text{C} + \text{HDL} - \text{C}) \qquad (Equation\ 5)$$

$$AI\ (mg/dl) = \text{VLDL} - \text{C} \times \left(\frac{\text{LDL} - \text{C}}{\text{HDL} - \text{C}}\right) \qquad (Equation\ 6)$$

2.6. Measurement of transaminases and creatinin activities

The International Federation of Clinical Chemistry (IFCC) method using appropriate assay kits (ENZOPAK SGPT and SGOT) was employed to determine the Alanine transaminase (ALT) and aspartate transaminase (AST) levels as described by Aïssatou et *al* (2017) [2]. Serum creatinine levels were analyzed using an Astra 8 auto-analyzer; Beckman Instruments (Fullerton, CA) following the methodology reported by Aïssatou et *al* (2017) [2].

2.7. Measurement of oxidative stress markers

2.7.1. Lipid peroxidation

The level of lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) as previously described by Deli et *al*. (2020) [14]. In a test tube containing 25 µl of sample, 100 µL of 8.1 % sodium dodecyl sulfate, 800 µL of 20 % acetic acid (pH = 3.5), and 800 µL of 0.8 % thiobarbituric acid (TBA). The mixture was adjusted to 2.125 mL with distilled water and heated in a water bath at 95 °C for 120 min. After cooling at ambient room temperature, 2.5 mL of the n-butanol and pyridine solution (1:1 v/v) was added to the mixture and vigorously stirred. The resultant solution was centrifuged at 3000 rpm for 10 min. Then, the absorbance of the collected supernatant was read at 532 nm against the blank comprising distilled water. The concentration of TBARS was expressed as nmol of malondialdehyde (MDA) per milligram (mg) of protein using the following formula:

$$C = \left(\frac{\text{DO} \times 10^6}{\varepsilon \times \text{L} \times \text{X}}\right) \times DF \qquad (Equation\ 7)$$

Where: C = MDA concentration in nmol/ mg protein, ϵ = MDA molar extinction coefficient, DO = optical density read at 530 nm, L = optical path length (1cm), X = amount of protein in mg/mL, and DF = dilution factor.

2.7.2. Superoxide dismutase activity

The superoxide dismutase (SOD) activity was determined by the method described by Deli et *al* (2020) [14]. Briefly, 0.2 mL aliquot of sample was added to 1.25 mL of carbonate buffer (pH 10.2). The reaction was initiated by adding 0.15 mL of freshly prepared adrenaline to the mixture. After homogenization, the mixture was adjusted to a final volume of 2 mL with distilled water, and the absorbance was read at 480 nm every 30 seconds until 150 seconds to follow the increase in optical density (DO). The reference cuvette contained 1.25 mL of carbonate buffer, 0.15 mL of epinephrine substrate, and 0.2 mL of distilled water. One unit of SOD activity uptake is defined as the amount of SOD required to cause 50 % inhibition of epinephrine oxidation to adrenochrome over 1 min. The SOD activity was expressed in units per milligram of protein (UI/mg of Protein) according to the following formula:

$$\% \, inhibition = \left(\frac{\mathrm{DO_{white}} - \Delta\mathrm{DO_{sample}}}{\mathrm{DO_{white}}}\right) \times 100 \qquad (Equation\ 8)$$

Knowing that 50% inhibition correspond to a unit of activity:

$$A\ (Unit\ of\ SOD\ /\ min\ /\ mg\ Pr) = \left(\frac{\%\ \mathrm{inhibition}}{50}\right) \times \mathrm{DF} \qquad (Equation\ 9)$$

Where: DO = optical density, A = specific activity of SOD, DF = dilution factor

2.7.3. Catalase activity

The Catalase (CAT) activity was assessed by the method described by Deli et *al* (2020) [14]. To 20 µL of sample, 2 mL of phosphate buffer is added, followed by 1 mL of H_2O_2. Then, the absorbance was read at 240 nm against the blank. The catalase activity was expressed as µmol of H_2O_2 hydrolyzed/min/mg protein (IU /mg Pr):

$$Catalase\ activity = \left(\frac{\Delta\mathrm{DO} \times \mathrm{Vmr} \times \mathrm{L}}{\varepsilon \times \mathrm{L} \times \mathrm{Vext} \times \mathrm{X}}\right) \times \mathrm{D}F \qquad (Equation\ 10)$$

Where: ΔDO = Change in optical density per minute; Vmr = Volume of reaction medium (3 mL); L: Optical path length (1 cm); Molar extinction coefficient of catalase (0.036 $mmol^{-1}cm^{-1}$); Vext: Volume of extract introduced into the reaction sample (20 µm); X: Amount of protein in mg /mL and DF: Dilution factor of sample.

2.7.4. Total protein

Photometric colorimetric determination of total proteins in blood plasma and organs supernatant was performed according to the Biuret method using the Human Gesellschemicaft kit [2]. Briefly, 1000 µl of the single dye reagent was added to 20 µL of sample, thereafter the mixture was mixed and incubated for 10 min at 20 °C ± 5. Then, the absorbance was read at 546 nm against the blank. HUMATROL animal serum (STD) was used as a quality control. The protein concentration was calculated according to the following formula:

$$C\ (g/dl) = 8 \times \left(\frac{\Delta\mathrm{DO_{sample}}}{(\Delta\mathrm{DO_{STD}})}\right) \qquad (Equation\ 11)$$

With: C = protein concentration; ΔDO = Change in optical density per minute.

2.8. Statistical analysis

The data obtained were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) followed by Duncan's multiple tests was carried out with Statgraphic software version 16.1.18 (Statgraphics Technologies Inc., 1980, The Plains, Virginia) was used to assess the statistical difference at 5% level. Graphs were plotted using SigmaPlot software version 12.5 (Systat Software Inc, 2013, Bangalore, India).

3. Results and Discussion

3.1. Effect of HSCP obtain by ADG-CDSp on the body and organ weight

Several studies [5, 22, 23], have reported that a high diet saturated in fatty acids and cholesterol induce hyperlipidemia which is associated in an increment of abdominal fat. In our experimental conditions, a hyperlipidic diet (HLD) led to abnormal weight gain in rats, which is well illustrated in table 2.

Indeed, rats fed with HLD showed a significative increase in liver weight and abdominal fat (Afat). This weight gain may be due to the higher energy intake provided by a hyper lipidic diet as reported by Ngatchic et *al* (2013) [21]. However, it was found that HLD combined with HSCP consumption resulted in a significative decrease in fat weight, liver weight, as well as body weight gain (Table 2). In addition, it was noted that NMEPHS performed similarly to atorvastatin, but MEPHS had better impact on fat weight, liver, as well as body weight gain than atorvastatin. Indeed, the similar action of NMEPHS to atorvastatin could be due to the high content of bioactive components, especially phenolic compounds (flavonoid polyphenols, tannins and anthocyanins) present in the powder fraction of *H. sabdariffa* calyx causing the oxidation of fat cells responsible for the increase of the body fat mass. Ngatchic et *al* (2020) [22], also demonstrated

that administration of *Adansonia digitata* pulp powder of particle size class <50 µm to rats fed with a diet high in saturated fatty acids and cholesterol resulted in a significant decrease in weight gain and adiposomatic index.

Table 2 Body weight gain/loss and organ weights rats

Parameters	Weight gain (g)	Liver weight (g)	Abddominal fat (g)	HSI (%)	ASI (%)
Normal control	14.6 ± 3.7^{a}	6.66 ± 0.5^{ab}	5.01 ± 1.1^{a}	3.1 ± 0.5^{a}	2.2 ± 0.2^{a}
Negative control	55.3 ± 6.6^{d}	10.33 ± 1.5^{c}	27.50 ± 0.5^{d}	3.8 ± 0.7^{a}	10.2 ± 1.6^{c}
Positive control	32.3 ± 3.5^{c}	7.66 ± 0.5^{b}	11.66 ± 0.5^{c}	3.1 ± 0.7^{a}	6.5 ± 1.6^{b}
MEPHS	23.6 ± 3.2^{b}	5.66 ± 0.5^{a}	9.01 ± 1.2^{b}	3.3 ± 1.2^{a}	5.2 ± 1.4^{b}
NMEPHS	38.6 ± 1.1^{c}	6.33 ± 0.5^{ab}	13.01± 1.1^{c}	3.3 ± 1.2^{a}	7.1 ± 1.1^{bc}

Values represent means ± standard deviations of 3 measurements. For the same column, numbers with different superscript letters indicate that there is a significant difference ($p < 0.05$). HSI: hepatosomatic index; ASI: adiposomatic index; MEPHS: microencapsulated powder from *H. sabdariffa*; NMEPHS: non-microencapsulated powder from *H. sabdariffa*.

Moreover, the bioactive compounds present in *H. sabdariffa* calyx powder can enhance its ability to reduce fat, liver, as well as body weight gain, however, these bioactive compounds are susceptible to degradation during processing, ingestion, and/or storage by factors such as temperature, oxygen, and light, as well as by conditions in the gastrointestinal tract (pH, presence of enzymes, and interactions with other nutrients) [24, 25]. Lambert et *al* (2007) [26] noted that green tea polyphenols oxidizing easily in beverages and even in the oral cavity leads to substantial levels of hydrogen peroxide. Hence, the positive impact of MEPHS than NMEPHS on weight gain can be attributed to the bioactive components of *H. sabdariffa* powder encapsulated in the maltodextrin matrix which were protected in the gastrointestinal tract, so that they were able to effectively achieve their cites of action and realize the health-promoting hypolipidemic function in these rats. These results corroborate those of Sawale et *al* (2015) [27], who found that encapsulated *Terminalia arjuna* herb added to Vanilla chocolate was effective not only in reducing body weight gain and the relative amount of retroperitoneal fat, but also in significantly reducing lipid droplets in liver tissue when administered to hyperlipidic rats for 60 days. Although no significant difference was found between the different treatment groups and the NoC group when the liver weight of the rats was adjusted to their body weight (hepatosomatic index), the liver size of the rats was significantly increased in the HLD groups compared with the NoC group, due to the accumulation of fat in the liver. MEPHS administration significantly restored the hepatosomatic and adiposomatic indices induced by the hyperlipidic damage compared with those of rats given NMEPHS (Table 2.) This reveals the importance of spray dry encapsulation with maltodextrin in preventing the degradation of bioactive HSCP compounds in the stomach by increasing their bioavailability in the gut where they are generally assimilated.

3.2. Effect of powder of *Hibiscus sabdariffa* (MEPHS) on serum lipid profile

Serum lipid levels of the different groups are presented in Table 3.

After 42-day feeding, a significative increase in serum lipid levels, such as TG, TC, LDL-C, and VLDL-C in HLD-fed rats, whereas HDL-C levels were significatively decreased in NoC rats. The intake of MEPHS showed a significative decrease in serum levels of TG, TC, LDL-C and VLDL-C, whereas there was a significative increase in HDL-C compared with the no-treatment (NeC) rats with a significative decrease in HDL-C. In addition, highest atherogenic index was found in the NeC group and was significantly reduced in the MEPHS group (Table 3). It was found that the HSCP are effective in reducing serum lipids. This is in agreement with previous study [5] that have also demonstrated the effects of different extracts of *H. sabdariffa* calyxes in decreasing serum levels of TG, TC, LDL-C, and VLDL-C, and increasing HDL-C levels. This reduction could be due to the activity of phenolic compounds present in the plant having been identified as potent lipid-lowering agents [23]. The polyphenols present in HSCP contributed effectively to its hypolipid activity. They increased the activity of plasma lipoprotein lipase, lecithin cholesterol acetyl transferase (transporting cholesterol to the liver for excretion) and down-regulated glucose-6- phosphate dehydrogenase, malate dehydrogenase and HMG-CoA [28]. these are all lipogenic enzymes involved in cholesterol metabolism. They also reduced cholesterol levels in the liver, so that the powder could effectively exert its lipid-lowering effect [29]. This action is possible due to lipogenic enzymes involved in cholesterol metabolism such as hepatic HMG-CoA reductase, which is a key cholesterol-limiting enzyme involved in the cholesterol biosynthesis pathway. In addition, it has been reported that consumption of polyphenols leads to a decrease in low-density lipoprotein (LDL-C) levels and an increase in high-density lipoprotein (HDL-C) levels in serum. In our case, the phenolic compounds of HSCP would also have contributed on the one hand to

accelerate the transfer of excess cholesterol from peripheral cells to the liver for catabolism via the reverse cholesterol transport pathway. On the other hand, these compounds would have acted by increasing the inhibition of cholesterol uptake, and the expression of hepatic low-density lipoprotein (LDL) receptors, leading to increased degradation and removal of LDL-C from the blood [1, 23].

Table 3 Effect of non- and microencapsulated powders from *H. sabdariffa* on serum lipid profile

Parameter	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	AI (mg/dl)
Normal control	73.5 ± 10.1^{a}	87.45± 3.1^{c}	22.83± 3.1^{c}	28.01±2.1^{a}	17.49±0.6^{b}	1.23±0.1^{ab}
Negative control	135.2 ± 5.7^{c}	266.2± 9.6^{e}	11.5± 1.4^{a}	62.99±4.5^{c}	53.24±1.9^{d}	5.55±0.9^{d}
Positive control	92.5 ± 3.4^{b}	101.93± 4.3^{d}	17,46± 1.9^{b}	45.68±4.4^{b}	20.38±0.8^{c}	2.65±0.5^{c}
MEPHS	68.7 ± 6.3^{a}	73.4±1.5^{a}	27.26± 1.9^{d}	30.08±5.8^{a}	14.68±0.3^{a}	1.11±0.2^{a}
NMEPHS	98.26±12.6^{b}	78.43± 1.8^{b}	21.43± 2.5^{bc}	45.15±3.3^{b}	15.68±0.3^{ab}	2.11±0.1^{bc}

Values represent means ± standard deviations (n = 3). For the same column, numbers with different superscript letters indicate that there is a significant difference ($p < 0.05$). MEPHS: microencapsulated powder from *H. sabdariffa*; NMEPHS: non-microencapsulated powder from *H. sabdariffa*.

Moreover, the hypolipidic mechanisms of *H. sabdariffa calyx* powder could also be due to the presence of sterols that can interact with intestinal absorption of fats and cholesterols and be excreted in feces [5]. The bioactive compounds present in *H. sabdariffa* calyx powder might enhance its hypolipidic activity. However, the enhanced hypolipidemic action revealed by MEPHS, would be due to the efficient encapsulation of the bioactive components of HSCP in a maltodextrin matrix as described above. These results concur with those of Sawale et *al* (2015) [27] where they noted that the bioactive components present in encapsulated *T. arjuna* not only withstand treatment conditions better, are released effective in the gut but show an effective effect (such as hypolipidemic and antioxidant activities) to treat cardiovascular diseases.

3.3. Effect of microencapsulated powder of *Hibiscus sabdariffa* (MEPHS) on creatinine, ALT and AST levels

The results presented in Table 4 show a significant increase in plasma ALT and AST levels in rats in the HLD group compared to the NoC group.

Table 4 Effect of MEPHS on serum creatinine levels and liver enzyme activities ALT and AST

Paramètres	Creatinin	ALT (UI/L)	AST (UI/L)
Normal control	0.46 ± 0.1^{a}	15.93 ± 3.3^{a}	14.10 ± 2.8^{a}
Negative control	0.53 ± 0.2^{a}	51.40 ± 0.9^{c}	42.43 ± 2.4^{d}
Positive control	0.53 ± 0.1^{a}	23.63 ± 0.8^{b}	22.76 ± 0.9^{c}
MEPHS	0.40 ± 0.1^{a}	13.93 ± 3.4^{a}	15.13 ± 0.8^{a}
NMEPHS	0.33 ± 0.1^{a}	19.4 ± 7.9^{b}	19.00 ± 1.0^{b}

Values represent means ± standard deviations of 3 measurements. For the same column, numbers with different superscript letters indicate that there is a significant difference ($p < 0.05$). MEPHS: microencapsulated powder from *H. sabdariffa*; NMEPHS: non-microencapsulated powder from *H. sabdariffa*.

Elevated levels of these liver marker enzymes are indications of cell membrane damage in hepatocytes that can lead to loss of functional liver integrity [30]. The intake of NMEPHS caused a significant decrease in AST and ALT levels in rats almost to the same extent as atovastatin, while MEPHS supplementation reduced these values to that of the normal control. This result is in agreement with that of Messaoudi et *al* (2019)[4] who showed that the administration of *Citrullus lanatus* seed extract induced a significant decrease in AST and ALT transaminase levels compared to NeC, due to the content of phenolic compounds and flavonoids present in the plant. Similar results were observed in the present study, where the decrease in transaminase levels could be associated with the content of bioactives, mainly that of the high flavonoids present in HSCP [5, 14]. Indeed, the phenolic compounds have the capacity to reinforce the transport function and the permeability of the hepatic cell membrane, their presence would have reduced or even prevented the leakage of enzymes out of the cells and, consequently, the marked reduction of AST and ALT in the bloodstream [31].

The more reducing effect of MEPHS on AST and ALT levels reveals the protective aspect of the encapsulating matrix, phenolic compounds present in the powder against the aggression of gastric juice, as mentioned above. Indeed, maltodextrin as a carbohydrate is fully digested only in the intestine, hence its protective role [32]. Furthermore, administration of HLD and HSCP did not alter serum creatinine levels compared to the NoC group.

3.4. Effect of microencapsulated powder of *Hibiscus sabdariffa* (MEPHS) on lipid peroxidation

As illustrated in Figure 1 (A), the levels of MDA in liver and kidney homogenates and in blood plasma of rats subjected to HLD were significantly elevated compared to rats subjected to the normal diet. This elevation of MDA has been reported to be related to the high intake of polyunsaturated fatty acids (potential targets of free radicals) contained in the diet of these rats [22]. Indeed, singlet oxygen ($^1O_2$) binds directly to the double bonds of the fatty acid to give free radicals such as hydroperoxides [12]. These free radicals will attack the membrane lipid double bonds, inducing a cascade of peroxidation processes leading to the complete disorganization of the membrane, thus altering its exchange, barrier and information functions [33] with the formation of toxic molecules such as malondialdehyde (MDA). MDA levels were noted to be significantly reduced in rats treated with the different *HSCP* and with atorvastatin in liver, kidney and blood plasma.

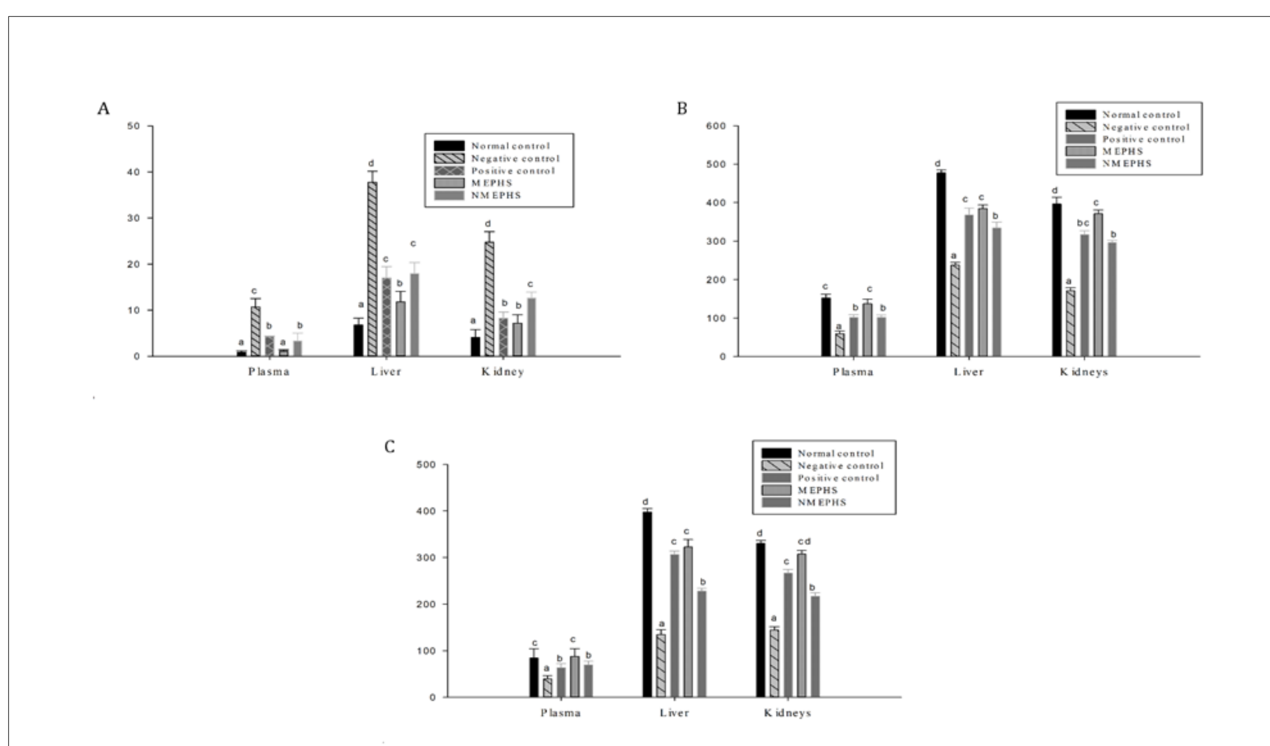

Figure 1 (A) Malondialdehyde (MDA) content. (B) superoxide dismutase (SOD), and (C) catalase (CAT) activities in plasma, liver, and kidney of different groups of rats undergoing the experiment. Error bars represent standard deviation for all samples, bars with different superscript letters differ significatively ($p<0.05$) by Duncan's multiple range test (n=3). MEPHS: microencapsulated powder from *H. sabdariffa*; NMEPHS: non-microencapsulated powder from *H. sabdariffa*.

It is noted that this reduction was more important in the rats that received the MEPHS. Thus, indicating that these treatments are effective in to inhibit the action of free radicals which is the main cause of lipid peroxidation. Similar results were obtained by Deli et *al* (2020) [14] where rats receiving the granulometric fraction < 180 µm of *H. sabdariffa* powders had a lower MDA content both in the liver and in the plasma. Moreover, the inhibitory activity of MDA production induced by MEPHS shows that the phenolic compounds present in this powder were able to protect the membrane cells of the liver, kidney and plasma of the rats. This activity was possible and more effective, due to the protective action of maltodextrin, which allowed the phenolic compounds to reach the majority of their sites of action as previously described. In particular, flavonoids are recognized in the antioxidant mechanism as metal chelators, scavengers of superoxy anions capable of interrupting the propagation of peroxidation by reacting with free radicals such as peroxyls and as hydrogen donors [34].

3.5. Effect of microencapsulated powder of *Hibiscus sabdariffa* (MEPHS) on Superoxide dismutase (SOD) and Catalase (CAT) activities.

As shown in Figure 1 (B and C), the intake of MEPHS showed a significative increase in the levels of SOD and CAT in plasma, liver, and kidney, compared with those of NeC. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are the body's first line of defense against free radicals (e.g. MDA) [35]. In this study, it was found that the levels of SOD and CAT in kidney liver homogenates and blood plasma significantly decreased in HLD-fed rats (Figure 2 and 3). This decrease can be explained by the fact that the activity of these enzymes was insufficient to protect the cells of the organism against oxidative stress due to an overproduction of free radicals caused by the excessive presence of polyunsaturated fatty acids resulting to the HLD received by these rats. Indeed, catalase has as its only substrate hydrogen peroxide which it reduces into water and molecular oxygen to prevent the formation of hydroxyl radicals. It is only localized in peroxisomes. Fortunately, catalase has the capacity to diffuse very easily through membranes and can therefore act at a distance from its production site, wherever hydrogen peroxide is also present [36]. SOD acts synergistically with CAT to defend the body against toxic products of cellular metabolism (MDA) by accelerating the dismutation of superoxide anion to hydrogen peroxide [37]. It was found that the levels of SOD and CAT were elevated in rats fed HLD with the intake of different powders of HSC. The effect was more important in the rats that received the MEPHS. These results further highlight the interest of encapsulation as a means of protection and stabilization of phenolic compounds present in HSCP. Indeed, the maltodextrin matrix used would have controlled the release profile of these compounds for a prolonged or triggered effect in the body, with a more effective antioxidant activity [32, 38].

4. Conclusion

In this work, the effect of encapsulation on the lipid-lowering activity of *H. sabdariffa* calyx powder was studied. A significant decrease in body weight, liver and fat mass, a decrease in plasma lipid levels (TC, LDL-C and VLDL-C), ALT and AST transaminase levels and MDA levels were observed with HSCP intake. This decrease with NMEPHS intake was similar to that observed with the reference drug, atorvastatin, whereas MEPHS intake brought the values close to normal and thus significantly higher than those of the NMEPHS group and the NoC group. In contrast, a significantly greater increase in HDL-C and SOD and CAT activities was observed. The variations of these parameters are closely related to the content of phenolic compounds in the powders, showing that they are mainly responsible for the hypolipidemic activity of H. sabdariffa powders. The best activities obtained by MEPHS show that the phenolic compounds present in this powder were protected by the encapsulation matrix (maltodextrin) during their passage in the stomach and against the handling conditions and external aggressions. This has favored their bioavailability in the intestine where they have improved the lipid-lowering activity of the plant. We can deduce that the protective effect of the encapsulation on the phenolic compounds towards the unfavorable environmental conditions met on the human track can be responsible for their hypolipidemic activity superior to that of the not microencapsulated powder.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

All experimental procedures were approved by the Faculty of Sciences and ethical committee.

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