Original Article

Sonchus maritimus Extract-Loaded Niosomes Bioconjugated by Linoleic Acid in Hepatic Encephalopathy Induced by High-Fructose Diet in Albino Wistar Rats

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ABSTRACT

One of the major roles of nanotechnology in the pharmaceutical field is to provide a facility to improve drug delivery systems and design smart nanocarriers with the potential to deliver specific biomolecules to the target site for treatment. This study evaluated *Sonchus maritimus*-loaded niosomes (SmE-N) in hepatic encephalopathy induced by a high-fructose diet (HFD) in rats. High-performance liquid chromatography (HPLC) analysis of *Sonchus maritimus* extracts (SmE), the synthesis of niosomes, and their characterization were performed. For the *in vivo* study, 24 male rats were haphazardly divided into 4 groups (n=6): control, HFD (35%), HFD+SmE-N (50 mg/kg/day), and HFD+metformin (50 mg/kg/day). Clinical behaviors and biological markers were assessed for all groups. The *in vitro* results of the chromatographic analysis revealed that *Sonchus maritimus* contains important phenolic acids, including gallic acid, vanillic acid, chlorogenic acid, and caffeic acid, as well as diverse flavonoids, including quercetin, rutin, and naringin bioactive compounds. The niosome formulation, characterized by the encapsulation efficiency of SmE, reached up to 61.40%. The *in vivo* results of the HFD showed a significant change in behavior parameters, liver glycogen, transaminase enzymes, brain protein, and acetylcholine esterase levels. In addition, there was a significant increase in malondialdehyde levels and a decrease in glutathione, superoxide dismutase, and glutathione peroxidase activities in the HFD group compared to the control group. Furthermore, the histopathological observation recorded a profound modification in the liver and brain tissues of the HFD group. In contrast, the treatment with SmE-N and metformin assured a partial amelioration in the noticed parameters compared to the HFD group, but SmE-N led to a better improvement than metformin compared to the control group. In conclusion, the use of SmE-N bioconjugated by linoleic acid seems powerful in treating the complications of fructose-induced metabolic disorders due to its hepato-neuroprotective abilities.

Keywords: Hepatic encephalopathy, Metabolic disorder, Niosome, Oxidative stress, *Sonchus maritimus*

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1. Introduction

Hepatic encephalopathy is a neurological condition caused by the complication of chronic liver disease through exposure to several hepatotoxins, such as viral hepatitis and drug toxicity, leading to brain inflammation and resulting in cerebral edema, behavioral changes, and mental confusion in advanced cases (1). Among the most prevalent etiologies of chronic liver disease is non-alcoholic fatty liver disease, which is associated with mild cerebral dysfunction and cognitive decline, according to a recent study by Hadjihambi *et al.* (2). The nutritional structure of our lifestyle is responsible for the increasing rate of metabolic disorders due to the excessive consumption of fructose, which is widely used in the food industry in the form of high-fructose corn syrup products (3). There has been a lot of interest in the use of natural substances as medicines, including polyunsaturated fatty acids such as linoleic acid, which serves as functional food "bioactive lipids", targeting the liver to regulate energy metabolism and maintain metabolic homeostasis (4). Due to the pathomechanism complications of metabolic disorders, recent studies have focused on the properties of polyphenols, such as chlorogenic acid and caffeic acid, to discuss their therapeutic implications in metabolic disorders and apply them in future directions. *Sonchus maritimus* belongs to the *Asteraceae* family, which is one of the largest and most economically important families possessing medicinal impact (5). The genus *Sonchus* is a class of eatable wild plants that are widely scattered in Africa, Asia, and Europe. The *Sonchus* species contains various bioactive compounds, such as flavonoids, steroids, tocopherols, fatty acids, saccharides, and coumarins (6). Nowadays, research in nanotechnology has spread to almost all scientific fields to create new options and solve various complications (7). Nanotechnology has grown dramatically to create new forms of nanomaterials in different fields, including diagnosis and medical treatment, structural materials, energy production, and molecular calculations (8). One of the major roles of nanotechnology in pharmaceutical fields is to provide a facility to improve drug delivery systems (9) and design smart nanocarriers that not only have the potential to deliver specific biomolecules to the target site for treatment but also have the advantages of enhancing medication solubility, protecting medicinal components from damaging enzymes, and controlling medication in the circulatory system (10). This study aimed to evaluate the therapeutic effect of *Sonchus maritimus* extract-loaded niosomes (SmE-N) conjugated by linoleic acid on hepatic encephalopathy induced by a high-fructose diet in albino Wistar rats.

2. Materials and Methods

2.1. Collection of Plant Samples

Sonchus maritimus samples were collected from Djamaa village in El-Oued state, Algeria, in November, and their taxonomy was verified by a botanist (Pr. Halis Youcef) in the Center of Scientific and Technical Research on Arid Regions (Touggourt, Algeria). The leaves were washed with distilled water and allowed to totally dry out at room temperature. Before being used, they were ground into powder and stored at room temperature.

2.2. Preparation of the Leaf Extracts

To prepare the aqueous extract, 10 g of *Sonchus maritimus* dry powder was added to 100 ml of distilled water. The mixture was then macerated at room temperature for 24 h and dried in a stove at 50°C after being filtered by filter paper.

2.3. Method of Chromatographic Analysis by HPLC

Before injection, the aqueous extracts of *Sonchus maritimus* were filtered. The experimental conditions were as follows: 1) temperature: 30°C; 2) injection volume: 20 µL; 3) the column with stationary phase C18 (150 mm of length, 4.6 mm of diameter); 4) mobile phase: A: acetonitrile and B: 2% glacial acetic acid solution (pH=2.6); 5) debit: 0.5 ml/min; and 6) gradient: 0-5 min: 5% A, 25-30 min: 35% A, and 35-45 min: 70% A. The HPLC system linked with a detector read at λ=280 nm for polyphenols and at 360 nm for flavonoids.

2.4. Preparation of Niosomes

The niosome was prepared according to the following method: 100 µg of non-ionic surfactants, including tween 80, 50 µg of linoleic acid to bioconjugate as a stabilizer, and 30 mg of cholesterol were dissolved in 100 mL ethanol and chloroform with a 2:1 ratio in a round-bottom flask. The organic solvents were eliminated under a vacuum in a rotary evaporator (BUCHI R-210 Rotavapor®, Switzerland) to form a thin layer on the wall of the flask. The residual solvents were evaporated in a vacuum oven for 12 h at 30°C. Afterward, the layer was dispersed with 10 mL of aqueous extract solution and sonicated in an ultrasonic bath (Digital Ultrasonic Cleaner UC-230D, Spain) at 50°C for 60 min to produce an aqueous niosomal suspension containing SmE. The phytoniosome suspension was left to mature overnight at

room temperature and then stored in a refrigerator for further studies.

2.5. Optical Microscopy

The prepared niosomes were examined under an optical microscope (Optika B-293, Italy) equipped with a camera (Optika C-B5, Italy) with a magnification of ×400. A thin layer of the diluted vesicular mixture was spread on a glass slide and covered with lamella for structural evaluations, such as lamellarity and uniformity of shape and size.

2.6. Encapsulation Efficiency of Niosomes

To determine the encapsulation efficiency of the encapsulated drug in niosomes, the SmE in niosomes was separated from the nonencapsulated SmE by centrifugation of the prepared formulation at 15700 g for 30 min at 4°C. The supernatant was separated, and the pellets were disrupted by isopropyl alcohol. The amounts of encapsulated bioactive compounds and free compounds present in the supernatant were measured. The analysis was performed and repeated three times. The encapsulation efficiency (EE%) was calculated as follows:

EE%=100×(the amount of encapsulated compound/initial amount of compound)

2.7. Animals

Twenty-four male albino Wistar rats were obtained from the Institute Pasteur of Algiers (7-8 weeks old and weighing 173.08±3.48 g). They were housed in plastic cages at the animal house of the Faculty of Natural Sciences and Life, University of El-Oued, Algeria. The animals were kept under standard conditions (temperature 25±2°C, 12/12 h dark/light cycle). Throughout the experiments, they were acclimatized to this condition with standard food and free access to water provided ad libitum. All experimental procedures were in accordance with the international guidelines provided by the local Ethics Committee (06 EC/DCMB/FNSL/EU2021) of the Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El-Oued, Algeria.

2.8. Experimental Design

After two weeks of acclimatization, the 24 rats were randomly divided into four groups of six as follows: Group 01 (Control): the control group receiving water, Group 02 (HFD): high-fructose diet group, Group 03 (HFD+SmE-N): high-fructose diet group treated by SmE-loaded noisome, and Group 04 (HFD+Met): high-fructose diet group treated by metformin. Groups 2, 3, and 4 rats were given a diet containing 35% fructose for 13 weeks. The rats were then treated orally with metformin (50 mg/kg b.w/day) and SmE-N (50 mg/kg b.w/day) through intraperitoneal injection during the last four weeks. The rats were weekly weighed, their food and water intakes were measured, and their behavioral (clinical) signs were evaluated. The clinical grading scores of behaviors were measured as follows (Table 1).

Table 1: Clinical grading scores of rat's behaviors.

Clinical grade	Behavior
0	Normal behavior
1	Mild lethargy
2	Decreased motor activity
3	Sever ataxia, no spontaneous righting reflex
4	No reaction to pain stimuli

2.9. Sacrifice, Blood Sampling, and Tissue Collection

The animals were sacrificed at the end of the treatment period and after 12 h of fasting while being lightly anesthetized with chloroform (94%) administered via inhalation. During the decapitation, blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes, which were numbered for each rat, the plasma was separated by centrifugation at 1500 rpm for 10 min, the plasma samples were stored at -20°C until the assessment of the lipid profile and biochemical parameters, and the fasting blood glucose of each rat was measured by a glucometer (Vital Chek®, China). The liver and brain were carefully removed and washed in 0.9% sodium chloride (NaCl). The organs were weighed and then stored in a freezer at -20°C until the preparation of homogenates to determine glycogen, protein, acetylcholinesterase activity, lipid peroxidation, and oxidative stress.

2.10. Plasma biochemical parameters

Glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), and the lipid profile, including triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C), were determined in the plasma using (Mindray BS-200, China), and glycosylated hemoglobin was measured using (Medcoon, Germany) and commercial reagent kits (Bio Lab, France, and Spin, Spain). Very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were calculated according to the following equations:

VLDL-C=Triglyceride/5

LDL-C=TC–VLDL-C–HDL-C

2.11. Preparation of Homogenate and Tissue Biochemical Parameters

A part of the brain or liver tissue of all experimental groups was homogenized in a cold Tris-bufferred saline solution (TBS, pH=7.4). The obtained homogenate was then centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was used for the assessment of acetylcholine esterase (AChE) activity, lipid peroxidation, and oxidative stress. The protein content in both homogenates was measured according to the Bradford method using bovine serum albumin as the standard (11). Liver glycogen was estimated using the Anthrone reagent according to the method described by Duvhåteau and Florkin (12). The AChE activity in the brain was evaluated according to the method described by Ellman *et al.* (13).

2.12. Oxidative Stress Parameters

Malondialdehyde (MDA) and glutathione (GSH) levels in the homogenates of the liver and brain were obtained using the methods described by Yagi (14) and Weckbecker and Cory (15), respectively. The superoxide dismutase (SOD; EC 1.15.1.1) and glutathione peroxidase (GPx; EC 1.11.1.9) activities in both homogenates were determined by the methods described by Beauchamp and Fridovich (16) and Flohe and Gunzler (17), respectively.

2.13. Histological Analysis

The brain and liver of each rat were removed after the sacrifice and immersed in a fixative solution (formaldehyde 4% and phosphate buffer with pH=7.6) for 48 h, dehydrated in an ascending grade of ethanol, cleaned by toluene, and immersed into paraffin blocks. Using a rotator microtome, the immersed specimens were sliced into sections of 5 µm thickness and then colored with hematoxylin-eosin. The histopathological evaluation was performed using an optical microscope (Optika B-293, Italy) equipped with a camera (Optika C-B5, Italy). The analysis of photomicrographs was carried out using the image processing software, Optika.

2.14. Statistical Analysis

The obtained results are expressed as Mean±SEM (Mean±Standard Error of Mean), and the Student's t-test was applied to perform the analysis of the data, which is based on the comparison between two means. The Minitab software (Version 19) and Excel (Version 2019) were used to perform the tests and the curves.

3. Results

3.1. HPLC Analysis of *Sonchus maritimus* Leaf Aqueous Extract

The results of the chromatographic analysis revealed the richness of the leaf aqueous extract of *Sonchus maritimus* in different polyphenols through the appearance of peaks in the chromatogram of different phenolic acids, including gallic acid, vanillic acid, chlorogenic acid, and caffeic acid, with diverse flavonoids, including quercetin, rutin, and naringin, as illustrated in figure 1 and table 2.

3.2. Morphological Characterization and Encapsulation Efficiency of SmE-N

Figure 2 presents a micrograph image of the formulated niosomes at a magnification of ×400 prepared by the thin film hydration method. The microscopic observation gave an idea of the shape of the formulated vesicles. Our preparation demonstrated that niosomes were spherical in shape and had small vesicles and some aggregations, as noticed in the micrographs. The *Sonchus maritimus* extract was loaded into niosomes without affecting their structure or morphology. Our formulation showed a good encapsulation efficiency of up to 61.4%, as presented in Table 3.

3.3. Growth Parameters

The initial body weight of the rats was statistically similar. The results of their final body weight, food intake, and water intake demonstrated a highly significant decrease (P<0.001) in all experimental groups compared to the control group. However, there was a significant increase in metformin and SmE-N groups compared to the HFD, with the exception of water intake in SmE-N groups.

Table 2 Chemical structure of identified compounds by HPLC analysis in leaves aqueous extract of *S.maritimus*

N^{br}	Retention Time (minutes)	Area	Height	Area %	Height %	Name	Formula
1	5.296	22785	1632	0.360	0.616	Gallic acid	$C_7H_6O_5$
2	13.289	30467	1435	0.482	0.541	Chlorogenic Acid	$C_{16}H_{18}O_9$
3	15.558	11524	933	0.182	0.352	Vanillic Acid	$C_8H_8O_4$
4	16.129	53432	1346	0.845	0.508	Caffeic Acid	$C_9H_8O_4$
5	28.499	9261	424	0.146	0.160	Rutin	$C_{27}H_{30}O_{16}$
6	34.840	10463	369	0.165	0.139	Naringin	$C_{27}H_{32}O_{14}$
7	44.838	738821	6678	11.678	2.519	Quercetin	$C_{15}H_{10}O_7$

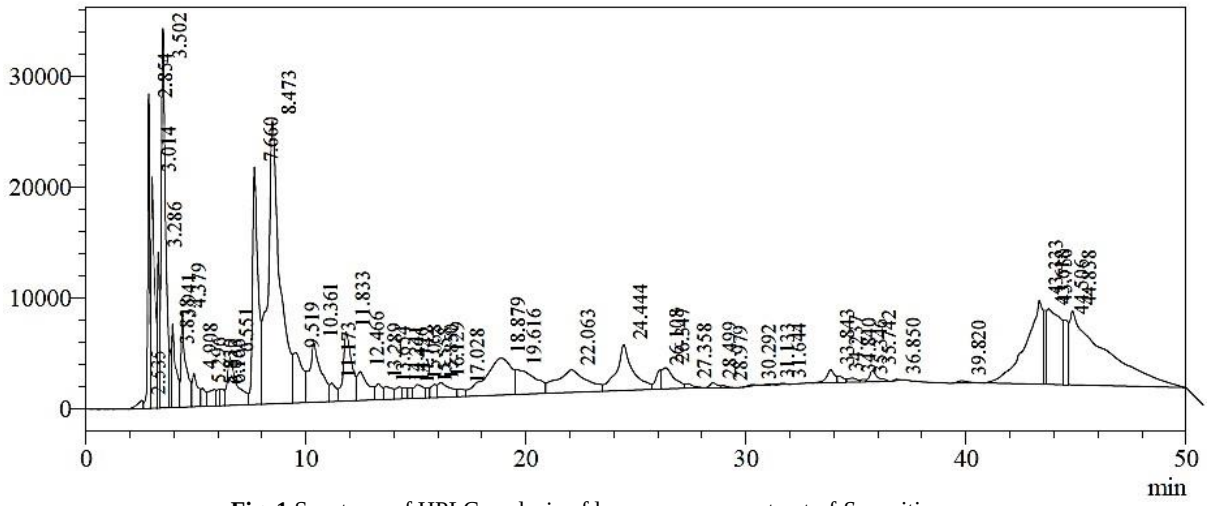

Fig. 1 Spectrum of HPLC analysis of leaves aqueous extract of *S.maritimus*

The relative liver weight results showed a highly significant increase (P<0.001) in the experimental rats compared to the control, while there was a significant decrease (P<0.001) in the metformin and SmE-N groups compared to the HFD group (Table 4).

3.4. Biochemical Parameters

Table 5 shows a significant change in biochemical results, indicating a highly significant increase (P<0.05) of blood glucose, glycated hemoglobin, total cholesterol, triglyceride, VLDL-C, and LDL-C levels, transaminase activities (GPT and GOT activities) as well as liver and protein levels, and a significant decrease in HDL-C, liver glycogen levels, and AChE activity in the HFD group compared to the control. In comparison with the HFD group, both groups treated with SmE-N and metformin showed a significant decrease (P<0.01) in all parameters mentioned above, with a significant increase (P<0.01) in HDL-C level and AChE activity, but no significant change in triglyceride and VLDL-C levels. Therefore, the results demonstrated the important effect of SmE-N in improving the biochemical marker in rats compared to metformin against biochemical disorders induced by a high-fructose diet.

3.5. Brain Markers and Clinical Score

Our results demonstrated a significant decrease (P<0.001) in AChE activity and a significant increase (P<0.001) in brain protein levels and clinical scores in the HFD group compared to the control group.

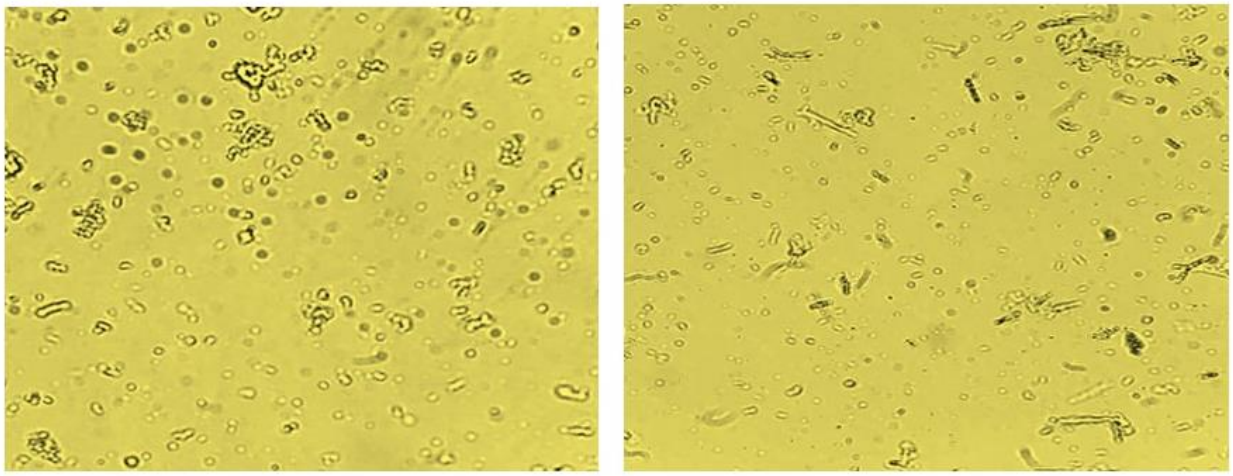

Fig. 2 Optical microscopic images of SmE-N at magnification ×400.

Table 3 Total phenol contents (TPC) and encapsulation efficiency of niosomes.

	TPC (mg)	Percentage of TPC (%)
100 mg of SmE	**3.0817±0.0241**	**100**
Niosomes	**1.8926±0.0374**	**61.41±1.21**
Supernatant	**1.189±0.0241**	**38.584± 0.831**
Encapsulation Efficiency (%)	**61.409 ± 0.924**	

Table 4 Growth parameters of control, HFD and treated groups.

Parameters	Control (n=6)	HFD (n=6)	HFD+SmE-N (n=6)	HFD+Met (n=6)
Initial body weight (g)	**179.17±4.72**	**173.33±6.38**	**171.67±7.24**	**172.00±7.18**
Final Body Weight (g)	**217.50±4.91**	**127.77±5.41*****	**153.50±3.67**^{***c}	**168.75±3.64**^{***c}
Relative liver Weight (g/100g b.w)	**2.1776±0.0178**	**3.200±0.133*****	**2.6362±0.0330**^{***c}	**2.8853±0.0378**^{***c}
Food intake (g/rat/day)	**9.500±0.0990**	**5.160±0.0317*****	**6.160±0.0317**^{***c}	**5.500±0.0990**^{***b}
Water intake (ml/rat/day)	**20.815±0.004**	**8.250±0.205*****	**8.373±0.304*****	**9.305±0.377**^{***a}

Values are expressed on (mean ± SEM): * P<0.05, **P<0.01, ***P<0.001: comparison with control group; a P<0.05, b P<0.01, c P<0.001: comparison with HFD group.

Table 5 biochemical markers of control, HFD and treated groups.

Parameters	Control (n=6)	HFD (n=6)	HFD+SmE-N (n=6)	HFD+Met (n=6)
Blood Glucose (mg/dL)	113.50±1.16	153.67±3.69***	126.32±5.24^{b}	114.33±1.32^{c}
Glycosylated Hemoglobin (%)	6.9±0.0516	7.5±0.0258***	4.9±0.620^{*b}	1.9±0.155^{***c}
Liver glycogen (mg/g of tissue)	4.868±0.290	1.845±0.164***	4.495±0.432^{c}	3.495±0.332^{***c}
Triglyceride (g/L)	1.6067±0.0429	2.04±0.0459***	2.05±0.0317***	1.88±0.0989*
Total cholesterol (g/L)	0.6667±0.0166	0.9825±0.0210***	1.1025±0.0187^{***c}	0.7867±0.0166^{***c}
HDL-Cholesterol (g/L)	0.216±0.0015	0.1867±0.0107*	0.2850±0.0166^{**b}	0.3100±0.00447^{***c}
LDL-Cholesterol (g/L)	0.1785±0.0119	0.4473±0.0400***	0.374±0.00346^{***c}	0.186±0.0192^{c}
VLDL-Cholesterol (g/L)	0.321±0.008	0.408±0.00918***	0.410±0.00635***	0.376±0.0198*
GOT activity (IU/L)	116.56±3.62	193.2±21.5*	122.10±5.75^{c}	135.19±3.16^{**c}
GPT activity (IU/L)	46.05±1.98	66.58±3.19***	37.97±1.92^{**c}	48.50±2.16^{c}
Protein of liver (mg/g of tissue)	4.130±0.133	6.725±0.150***	6.161±0.350***	4.614±0.164^{*c}

Values are expressed on (mean ± SEM): * P<0.05, **P<0.01, ***P<0.001: comparison with control group; a P<0.05, b P<0.01, c P<0.001: comparison with HFD group.

However, the niosomal therapeutic system improved these parameters through a significant elevation in AChE activity and a significant decrease in protein levels and clinical scores compared to the HFD rats. Metformin also improved brain parameters, but less than SmE-N, as indicated by clinical scores (Table 6).

3.6. Oxidative Stress Parameters

Our data showed a highly significant increase (P<0.001) in the liver MDA levels in the HFD group and a significant decrease (P<0.001) in the brain MDA levels in the group treated with metformin compared to the control group. Furthermore, the comparison to the control group demonstrated that a high-fructose diet led to a highly significant decrease (P<0.001) in enzymatic and non-enzymatic antioxidants and GPx/GSH reports in the liver and brain homogenates of the HFD rats and a significant decline in the HFD+Met and HFD+SmE-N groups. Both metformin and SmE-N treatments improved and significantly increased the GSH levels, the SOD and GPx activities, and the GPx/GSH report. In parallel, they significantly decreased MDA levels in the liver and brain, while the GPx/GSH reported no statistically significant increase in the liver in the SmE-N group (Table 7).

3.7. Histopathological Study

The histopathological examination of the liver tissue section of the control group revealed a normal hepatocyte structure, while the HFD group showed huge alterations, including necrosis, hemorrhage, infiltration of inflammatory cells, and vacuolization of the cytoplasm with the appearance of intracytoplasmic vacuoles. However, the HFD+SmE-N group showed a better liver structure than that presented in the HFD+Met group for hepatocyte damage. The HFD+Met group was characterized by a large number of distinguished intracytoplasmic vacuoles (lipid droplet vacuoles) compared to the HFD group. The microscopic analysis of the brain section of the control group presented a normal histological structure, while the section of the HFD group demonstrated a big change from the degeneration of cells, inflammation, and hemorrhage, but SmE-N improved these alterations and showed a normal histological structure better than that in the Met group (Figure 3 and Table 8).

Table 6 Brain markers and clinical score of control, HFD and treated groups.

Parameters	Control (n=6)	HFD (n=6)	HFD+SmE (n=6)	HFD+Met (n=6)
Clinical score	0	$6.00\pm1.43^{***}$	$3.00\pm0.793^{**b}$	$3.6\pm0.855^{***a}$
Protein of brain (mg/g of tissue)	10.481±0.007	$13.959\pm0.243^{***}$	$11.384\pm0.094^{***c}$	$12.052\pm0.147^{***c}$
AChE activity (µmol/min/mg of prot)	11.65±1.00	$4.768\pm0.497^{***}$	$9.150\pm0.442^{***c}$	$8.128\pm0.584^{***c}$

Values are expressed on (mean ± SEM): * P<0.05, ** P<0.01, *** P<0.001: comparison with control group; a P<0.05, b P<0.01, c P<0.001: comparison with HFD group.

Table 7 - lipid peroxidation and antioxidant markers of liver and brain of control, HFD and treated

Parameters		Control (n=6)	HFD (n=6)	HFD+SmE-N (n=6)	HFD+Met (n=6)
MDA (nmol/mg of prot)	Liver	6.006±0.620	$11.258\pm0.338^{***}$	4.453 ± 0.815^{c}	6.08 ± 1.10^{b}
	Brain	2.6338±0.0295	2.702±0.137	2.5422 ± 0.0440^{b}	$1.856\pm0.113^{***c}$
GSH (nmol/mg of prot)	Liver	1.9879±0.0193	$1.1164\pm0.0548^{***}$	$1.579\pm0.114^{*b}$	$1.3693\pm0.0457^{***b}$
	Brain	0.4717±0.0153	$0.3772\pm0.0163^{***}$	$0.4152\pm0.0187^{*}$	0.4648 ± 0.0070^{c}
SOD activity (mUI/mg of prot)	Liver	2.09±0. 37	$0.76\pm0.04^{***}$	$1.46\pm0.20^{*b}$	2.36 ± 0.28^{b}
	Brain	1.26±0.07	$0.85\pm0.07^{**}$	1.22 ± 0.02^{c}	$1.07\pm0.05^{*b}$
GPx activity (µmol/mg of prot)	Liver	42.249±0.769	$26.161\pm0.496^{***}$	$29.24\pm1.08^{**b}$	$33.77\pm0.794^{***c}$
	Brain	10.628±0.061	$9.097\pm0.100^{***}$	$10.033\pm0.177^{**c}$	$9.741\pm0.210^{***a}$
(GPx /GSH) $x10^{3}$	Liver	23.223±0.178	$18.518\pm0.054^{***}$	$18.501\pm0.241^{***}$	22.380 ± 0.630^{c}
	Brain	27.130±2.02	$17.182\pm0.630^{***}$	$25.109\pm0.647^{**c}$	$24.615\pm0.462^{***c}$

Values are expressed on (mean ± SEM): * P<0.05, ** P<0.01, *** P<0.001: comparison with control group; a P<0.05, b P<0.01, c P<0.001: comparison with HFD group.

Tabale 8 Grading of histological changes in liver and brain sections of control, HFD and treated groups.

Parameters		Control	HFD	HFD+SmE-N	HFD+Met
Inflammation	Liver	-	+++	+	++
	Brain	-	+++	-	+
Necrosis	Liver	-	+++	+	++
	Brain	-	+++	-	+
Hemorrhage	Liver	-	+++	+	++
	Brain	-	+++	-	+
Cytoplasmic vacuolization	Liver	-	+++	+	++
	Brain	-	-	-	-
Intracytoplasmic vacuoles	Liver	-	++	+	+++
	Brain	-	-	-	-

none (-) ; moderate (+) ; severe (++) ; very severe (+++)

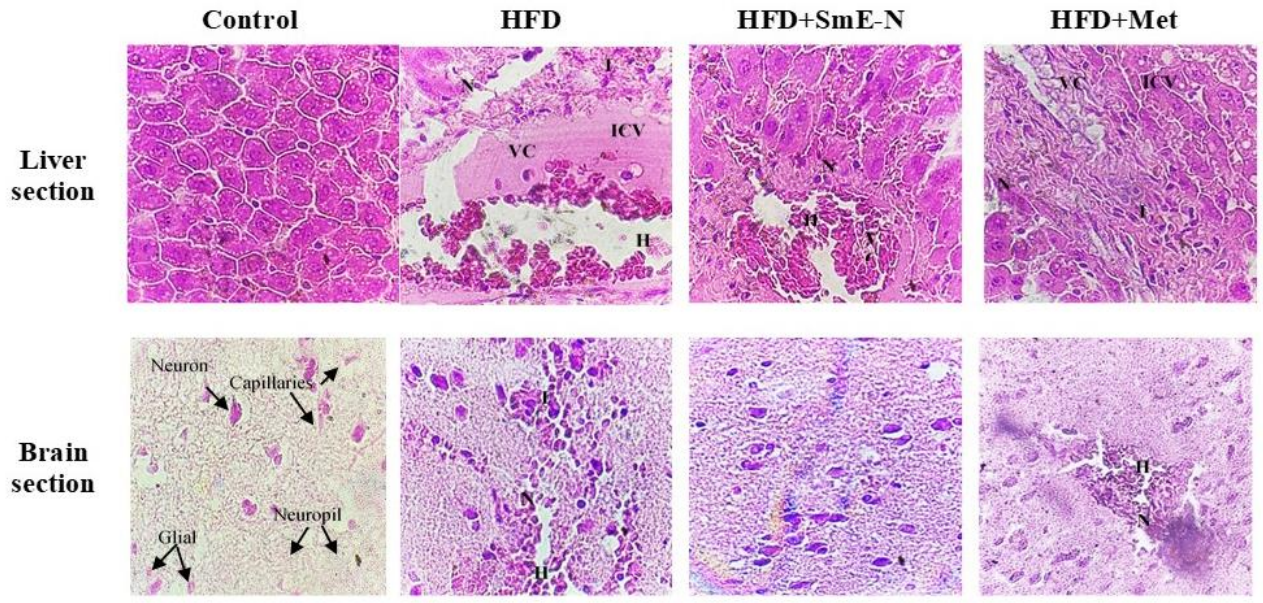

Fig. 3 Histological photomicrographs of liver and brain section of control, HFD and treated groups with hematoxylin and eosin (H&E), magnification ×400, I: inflammation; N: necrosis; VC: vacuolar cytoplasm; H: hemorrhage; ICV: intracytoplasmic vacuole.

4. Discussion

Our obtained HPLC results are confirmed by previous studies that identified some of these phyto-compounds in *Sonchus* plants (6). This reflects its importance as an antioxidant and its power against pathogenic bacteria and different metabolic diseases. The size and morphological surface appearance of the niosome were determined by an optical microscope. Our formulation was consistent with the results of Yeo *et al.* (18). They observed a round cinnarizine noisome under an optic microscope. Niosomes were used for encapsulating bioactive compounds. The encapsulation efficiency reached up to 61.4%, indicating an important amount of entrapped bioactive molecules in niosomes, which reflects evidence of its formulation. The increase in metabolic needs opposes the maintenance of a constant body weight, and the maintenance of a lowered or elevated body weight is connected with variations in cellular energy consumption, while the elevation of relative liver weight may be due to necrosis or the histological section (19). The SeM-N improved all these parameters, especially the body weight, followed by raising food intake and water intake due to phyto-compounds contained in the aqueous extract.

Previous investigations revealed that the aqueous extract of the plant may recover the total body weight (5) and linoleic acid in SmE-N, which are considered polyunsaturated fatty acids ameliorating body composition and increasing muscle mass, clarifying its wide use in body configuration (4). The administration of a high-fructose diet in the current study induced a significant change in metabolic parameters, including hyperglycemia and hyperlipidemia. Insulin resistance is responsible for maintaining a high level of blood glucose, which leads to glycosylation of hemoglobin. The decrease in liver glycogen level is also related to glucose tolerance and resistance to insulin, which may discuss the inability of fructose to stimulate insulin production from β-cells in the pancreas due to the low density of fructose transporter and GLUT 5 in the mentioned cells (20). Fructose intake increases TG by increasing the production of free fatty acids and acetyl-CoA through activating the carbohydrate response element-binding protein and stimulating the activity of all three lipogenesis enzymes (adenosine triphosphate citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase); as a result, there will be an increase in TG synthesis and esterification and a decrease in export and oxidation of fat (21). Kumar *et al.* revealed that the consumption of 20% fructose in drinking water for 12 weeks increased the expression and activity of HMG-CoA reductase, which leads to excessive synthesis of endogenous cholesterol (21). Excessive fructose intake promotes the accumulation of fat in the liver, which can induce non-alcoholic fatty liver disease that causes liver injuries and oxidative stress with the inflammation of hepatocytes leading to the increase of GOT and GPT activities, as well as the carbonylation of proteins that assemble into large aggregates resistant to degradation pathways (20). The hypoglycemic effect of SmE-N was consistent with the findings of Khorasgani and Khani's study in diabetic rats (22). Previous studies have shown that some *Sonchus* plants enhance liver glycogen synthesis by activating phosphoenolpyruvate carboxykinase, which is the key enzyme to regulate hepatic gluconeogenesis (6). Niosomes ameliorated the lipid profile due to their polyphenol compound of the entrapped *Sonchus maritimus* extract, as reported in previous studies that the hydro-alcoholic extract of rosemary had beneficial effects in lipid metabolism by diminishing circulating triglyceride levels and their accumulation in hepatocytes and LDL-C, enhancing HDL-C levels, and suppressing the HMG-CoA reductase activity (21) (23). The presence of these phyto-compounds may also explain the effect of SmE-loaded niosomes on reducing transaminase enzyme levels in the blood. The complication of chronic liver disease leads to brain inflammation, causing hepatic encephalopathy progression and resulting in neuropathological conditions including cerebral edema, behavioral changes, and mental confusion in advanced cases (1). This has appeared in our investigation by increasing the clinical score and brain protein levels and decreasing the AChE activity in the brain of the HFD group. Behavioral and neurological dysfunction can result from neuroinflammation due to the metabolic dysfunction induced by excessive fructose consumption (24). The upregulation of beta-secretase-1 and the accumulation of amyloid beta (Aβ) plaques, in addition to pro-inflammatory mediators, such as TNF-α and IL-6, as indicated in previous studies, could be the causes of elevated protein in the brain. A high-fructose diet subsequently accumulates acetylcholine, which leads to the hyperstimulation of nicotinic and muscarinic receptors and disrupts the neurotransmission in the cholinergic synapse (1). The clinical score improved by the treatment with SmE-N is linked to improvements in brain markers, which are associated with its beneficial effect on the liver. The encapsulated SmE content of flavonoids, including naringin, rutin, and quercetin, as well as bioconjugated linoleic acid, reduces the abnormal accumulation of Aβ and downregulates pro-inflammatory cytokines (25). The AChE activity was restored upon treatment with SmE-N, and based on a previous study, chlorogenic acid included in SmE upregulates the activity of AChE and shows a neuroprotective effect on the mouse brain. The excessive consumption of fructose elevates the generation of free radicals and causes an imbalance in the antioxidant defense system. The significant decrease of brain antioxidant markers in the HFD group is in agreement with other studies by Ekici *et al*. revealing that long-term fructose consumption leads to oxidative stress, reduces the level of glutathione, and suppresses cerebral enzymatic antioxidants, including the activities of SOD and GPx (26). SmE-N ameliorated the oxidative stress induced by the high-fructose diet in the liver and brain by suppressing lipid peroxidation and enhancing the GSH level, as well as SOD and GPx activities, due to the ability of flavonoids and phenolic compounds in SmE to effectively inhibit lipid peroxidation, scavenge peroxyl radicals, and neutralize oxidizing free radicals, such as hydroxyl radicals and superoxide (27). Several previous findings demonstrate the hepatoprotective and neuroprotective effects of quercetin and rutin as a result of their strong antioxidant power by reducing oxidative stress damage in tissues (27). Furthermore, the presence of metallic elements, such as Mn, in *Sonchus maritimus* can enhance the activity of antioxidant enzymes, which serve as co-factors (5). Mirzaei *et al.* confirmed the histopathological alteration of the liver and brain by a high

intake of fructose, which helps to stimulate the production of free radicals in the tissues and leads to their inflammation and the appearance of vacuolar degeneration of cytoplasm in the liver tissue (28). The spread of intracytoplasmic vacuoles in hepatocytes is caused by droplets of lipid in the cytosol, which accumulate due to lipid dysfunction caused by fructose, as described above. These histological changes in the structure of the liver cells have been associated with a higher status of steatosis and damage, which relates to the upregulation of macrophage marker expression and inflammatory cell infiltration (29). Because a high lipid influx into the liver increases mitochondrial oxidative activity, which progresses the ROS formation that causes the up-production of inflammatory cytokines, it leads to the necrosis of cells (27). The cerebral inflammation is due to the contribution of fructose to upregulate the extracellular accumulation of Aβ, which alters the antioxidant defense system of the brain (26). Treatment by SmE-N gave an important histological amelioration in the brain and liver sections, which suggests that the components of the plant extract included in niosomes are responsible for these reparations. Numerous findings have been widely exhibited in several *in vivo* studies about the neuroprotective effects of phenolic acids and flavonoids through the anti-inflammatory cytokines by suppressing proinflammatory cytokine secretion and the antioxidant effect by enhancing the antioxidant defense system in the brain, including naringin, quercetin, and rutin (30). *Sonchus maritimus* extract-loaded niosomes showed their therapeutic power against the complications of metabolic disorders induced by a high-fructose diet due to a high EE%, which means an important amount of bioactive molecules of SmE entrapped in niosomes, reflecting evidence of their formulation and ensuring their delivery of bio-compounds to the target affected organs, through the observed beneficial effect in treating hepatic encephalopathy through the hypolipidemic, hypoglycaemic, and antioxidant effects shown in behavioral amelioration. These activities are due to the richness of *Sonchus maritimus* leaf aqueous extract in phenolic acids. In addition, the presence of linoleic acid in the nanocarrier of niosomes helps direct the biomolecules to target the affected liver for treatment.

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Authors' Contribution

Conceptualization and supervision: S.D.

Writing original draft: S.C.

Preparing analysis of the laboratory parameters: SC., I.B. Writing review and editing S.C., S.D., Y.R.

All authors have read and agreed to publish version of the manuscript.

Ethics

The Ethics Committee of the Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El Oued, was consulted and approved (reference 28/BMC/FSNV/2022)

Conflict of Interest

The authors declare no competing interests.

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