Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Hypocholesterolemic effects of a polysaccharide isolated from Balangu (*Lallemantia royleana*) seeds in Wistar rat fed on a cholesterol-containing diet

Mohammed Abdulameer Farhan[1], Amal Feki[2], Intissar Kammoun[2], Malek Aroui[2], Manel Naifar[3], Rim Kallel[4], Fatma Ayadi-Makni[3], Tahia Boudawara[4], Choumous Kallel[5], Adil Abaed Hassoni[6], Ibtissem Ben Amara[2*]

[1] Ministry of Labor and Social Affairs/National Center for Occupational Health and Safety, ALfurat ALawsat Technical University, Department of Biological Control, Laboratory of postgraduate Studies, Babylon city, Iraq
[2] Laboratory of Medicinal and Environment Chemistry, University of Sfax, Higher Institute of Biotechnology, BP 261, 3000 Sfax, Tunisia
[3] Laboratory of Biochemistry, CHU Habib Bourguiba,University of Sfax, Sfax, Tunisia
[4] Laboratory of Anatomopathology, CHU Habib Bourguiba, University of Sfax, Sfax, Tunisia
[5] Hematology Laboratory, CHU Habib Bourguiba 3029. Sfax University. Tunisia
[6] Department of Biology. AL_ Musayyib Technical College. Alfurat Alawsat Technical University, Iraq

ARTICLE INFO

Original paper

Article history:
Received: July 16, 2022
Accepted: August 31, 2022
Published: September 30, 2022

Keywords:

Polysaccharides, Lallemantia royleana, Antioxidant activity, Hypocholesterolemic

ABSTRACT

The present study aimed to evaluate the antioxidant effects of polysaccharide (PS) isolated from Balangu Shirazi (*Lallemantia royleana)* seeds *in vitro* as well as on the hypercholesterolemic diet-induced liver and kidney injury in adult rats. PS was structurally characterized by Fourier-transformed infrared, which confirmed the presence of bands characteristic of polysaccharides. Functional properties of PS were investigated based on water solubility index, holding and emulsifying capacities. The antioxidant activities were confirmed by DPPH radical scavenging assays, reducing power and the chelating effect assay. The administration of PS to a hypercholesterolemic diet for 30 days in Wistar rats significantly improved the liver and kidney levels in malondialdehyde, advanced oxidation protein products, glutathione, superoxide dismutase, glutathione peroxidase and in vitamin C. The oxidative stress profile was confirmed by hematological and plasma biochemical parameters. In addition, histological alterations were significantly ameliorated in liver and kidney tissues. The study strengthens the hypothesis that the herbal polysaccharide can be used as a novel antioxidant and hypocholesterolemic compound against hyperlipidemia-induced atherosclerosis.

Doi: http://dx.doi.org/10.14715/cmb/2022.68.10.6

Introduction

Hyperlipidemia is not only a health problem, but a social one as well. The number of people suffering from this disease is increasing rapidly worldwide and has become the center of public attention (1). Hyperlipidemia, induce to long-term complications of cholesterolemia serious diseases by generating reactive oxygen species (ROS) (2). Oxidative stress induced by oxygen radicals has been implicated in the pathogenesis of various diseases. Finding natural, safe, as well as effective treatments for dyslipidemia and hyperlipidemia is one of the major quests of medical scientists.

Medicinal, functional, and nutraceutical herbs have been used as food and for medicinal purposes for centuries. Research interests have focused on various herbs that possess hypolipidemic and hypocholesterolemic properties that may be useful for reducing the risk of cardiovascular diseases (3).

Lallemantia royleana, popularly known as Balangu Shirazi, is one of the Iranian medicinal plants that belong to the *Lamiaceae* family. It is one of the five species of Iranian *Lallemantia*, which grows wild in several areas. It is extensively represented in different regions of Asia, Europe and Middle East countries especially various regions of Iran (4,5). *L royleana* has been used in Iranian traditional and folklore medicine as a diuretic, tonic, aphrodisiac, and antitussive remedy and in the treatment of various nervous, hepatic, and renal disorders (6). Seeds powders have been used in some southern parts of Iran as an atonic medication and a remedy for psychotic diseases (7). *Lallemantia royleana* contains similar biologically active compounds such as polysaccharides (8). These polysaccharides are an important class of biological polymers in plants and typically play either structure-or capacity-related parts (9). Polysaccharides have attracted the attention of researchers due to their great bioactivities, such as antioxidant, anti-inflammation, antibacterial, antidepressant, antitumor, and anti-atherosclerosis activities (10).

In this paper, the polysaccharide extracted from *L royleana* (PS) was characterized for its structural and functional properties based on water solubility index, holding and emulsifying capacities. The PS antioxidant activity was determined by DPPH radical scavenging assays, reducing power and the chelating effect. PS was also, tested for its ability to prevent oxidative stress related to hypercholesterolemia on liver and kidney tissues the in of rat model.

* Corresponding author. Email: ibtissem.benamara@isbs.usf.tn

Materials and Methods

Extraction and purification of water-soluble polysaccharide

The polysaccharide from *Lallemantia royleana* (PS) was extracted referring to Liu et al (11). In brief, the PS was mixed with 95% (v/v) ethanol at 4 °C for 24 h. The water phase was dialyzed at 4 °C against distilled water for 2 days, then concentrated by rotary evaporation under reduced pressure (Rotary evaporator, Heidolph, Germany) and freeze-dried using freeze dryer (Bioblock Scientific Christ ALPHA 1–2, IllKrich-Cedex, France) to obtain polysaccharide.

FT-IR spectrometric analysis

The polysaccharide FT-IR range was obtained using a Perkin-Elmer Spectrum infrared spectrometer. Films were analyzed using 10 scans per minute at a resolution of 4 cm^{-1} in the wavenumber region between 400 cm^{-1} and 4000 cm^{-1}.

Functional properties

Water solubility index (WSI)

Water solubility was determined through the method described by Ben Slima et al (12) and it was calculated according to the following formula:

$$WSI\ (\%) = \frac{\text{weight of the dissolved solid in the supernatant}}{\text{weight of the dry solid}} \times 100$$

Water holding capacity (WHC) and Oil Holding Capacity (OHC) of PS

The WHC and the OHC were recorded referring to the method of Ktari et al (13). The WHC was calculated referring to the following formula:

$$WHC\ (\%) = \frac{\text{weight of the tube content after drainingt}}{\text{weight of the dry solid}} \times 100$$

The emulsifying capacity of PS

Emulsifying capacity (EC) and its stability were undertaken by referring to the method of Freitas et al (14). After 1, 24 and 168 h, emulsification indices E1, E24 and E168, were computed as follows:

$$EC\ (\%) = \frac{He}{Ht} \times 100$$

Where, He (mm) is the emulsion layer height and Ht (mm) is the mixture overall height after t hours.

Antioxidant properties of PS

Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the samples (polysaccharide or the antioxidant reference) was assessed by referring to the method of Blois (15). The absorbance was measured spectrophotometrically at 517 nm using model JENWAY 6300 spectrophotometer. All determinations were performed in triplicate.

Iron (Fe^{2+}) chelating activity

The chelating ability of the polysaccharide extract is determined by following the inhibition of the formation of the Fe (II) -Ferrozine after incubating the samples with the divalent iron according to the method of Carter (16). Furthermore, the negative control contains all reagents except the test sample, which is replaced by an equal volume of distilled water. EDTA has been used as a reference chelator.

Reducing antioxidant power assay.

The reducing power of PS was performed by referring to the Oyaizu method (17). The absorbance of the samples was estimated against blank at 700 nm. BHT was deployed as standard.

In vivo effects of polysaccharide extracted from *Lallemantia royleana*

Animals and experimental design

Ethics statement

The experimental, as well as animal procedures, were conducted referring to the Natural Institute of Health Guidelines for Animal Care and approved by the "Institute Ethical Committee Guidelines" for the care and use of laboratory animals.

Treatment

Wistar male rats weighing about 180 ± 10g, obtained from the Central Pharmacy (SIPHAT, Tunisia), were randomly, divided into four groups of 8 animals each:

- Group I: Normal control rats were fed a standard diet.
- Group II: Rats were fed a high-fat diet (normal diet supplemented with 8% of fats and 1% of cholic acid/kg diet), to induce hyperlipidemia for 4 weeks.
- Group III: Rats received a high-fat diet and were treated with fluvastatin (10 mg/kg, body weight/daily) for 4 weeks (18).
- Group IV: Rats received a high-fat diet and were treated with PS extracted from *Lallemantia royleana* by gastric gavages route (200 mg/kg of body weight/daily) for 4 weeks (19).

During the experimental period (30 days), food and water intake of the animals were monitored daily. At the end of the experimental period, the animals of different groups were killed by cervical decapitation to avoid stress.

Blood samples were collected in heparin tubes, some others were collected in EDTA. Heparin tubes were centrifuged at 2200 ×g for 15 min. Plasma samples were next removed and served for the determination of biochemical parameters. Blood samples collected with EDTA were used for the determination of red blood cells (RBCs), white blood cells (WBCs), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet number, and MCH concentration (MCHC) by electronic automate Coulter MAXM (Beckman Coulter, Inc, Fullerton, CA).

Liver and kidney tissues were quickly removed. All the samples were homogenized (10% w/v) in an appropriate ice-cold buffer (100 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) with an Ultra Turrax homogenizer, and centrifuged at 10.000×g for 15 min at 4°C. The resulting supernatants were deployed for various biochemical assays. Other samples were immediately fixed in 10% formalin solution for histological investigation.

Liver and kidney parameters of oxidative stress

The malondialdehyde (MDA) levels were determined spectrophotometrically according to Draper and Hadley (20) methods and expressed as nmoles of MDA/mg tissue.

According to the method performed by Witko et al (21), advanced oxidation protein products (AOPP) levels of each homogenate were calculated using the extinction coefficient of 261 cm^{-1} mM^{-1} and the results were expressed as µmoles/mg protein.

Superoxide dismutase (SOD) activity was estimated according to the method of Beauchamp and Fridovich (22) and it was expressed as units/mg of protein.

Glutathione peroxidase (GPx) activity was determined according to Flohé and Günzler (23). The GPx enzyme activity was expressed as nmoles of GSH oxidized/min/mg protein.

Both liver and kidney glutathione (GSH) contents were measured at 412 nm using the method of Ellman et al (24) and expressed as µg/g tissue.

Acid ascorbic content was determined spectrophotometrically by the dinitrophenyl-hydrazine method described by Jacques-Silva et al (25). The absorbance was estimated at 540 nm as well as results were expressed as microgram/milligram protein.

Biochemical markers in plasma

Plasma levels of creatinine, uric acid, urea, bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by colorimetric methods using commercial reagent kits (References: 20151, 20091, 20143, 20102, 20094, and 20012, respectively) purchased from Biomaghreb (Ariana. Tunis. Tunisia).

Histological studies

For histological studies, some portions of kidney and liver tissues were immediately fixed in 10% of formalin solution and processed in a series of graded ethanol solutions. Organs were next embedded in paraffin, serially sectioned (3 µm) and stained with hematoxylin_eosin (H-E). Six slides were prepared from each organ. All sections of liver and kidney organs were estimated for the degree of injury.

Statistical analysis

Values of each parameter are expressed as the mean± standard deviation (x±SD). Duncan's multiple range tests provided mean comparisons with the level of statistical significance set at $p<0.05$. Statistical analyses were performed using SPSS Software using Duncan's test performed after variance analysis (ANOVA).

Results

Infrared Spectrum Analysis

FT-IR spectroscopy is displayed to characterize the structure of polymers as well as to determine their organic functional groups for the qualitative measurements of organic functional groups (Fig 1). The infrared spectrum of PS ranged from 4000 to 500 cm^{-1}. As presented in Fig. 1, PS displayed typical peaks of polysaccharides at 3284.79, 2928.51, 2166.51, 1595.44, 1411.83, 1320.78, 1030.92 and 893.61cm^{-1}. The broad peak at 3284.79 cm^{-1} was due to the stretching vibrations of terminations formed by hydroxyl groups, a weak band at 2928.51and 2166.51 cm−1 are assigned to a C-H stretching vibration. The peak at 1595.44 cm^{-1} was attributed to the carboxylate anion group (C=O). The most important peaks were observed at 1411.86 and 1320.78 $cm^{-1,}$ generally attributed to the stretching vibration of the ester sulfate groups (S=O). The most important peaks observed at 1411.86 and 1320.78 cm−1, were generally attributed to the stretching vibration of the ester sulfate groups (S=O). The peak assigned to 1320.78 cm^{-1}, might be related to the stretching vibrations of S–O of sulfate. Furthermore, the band at 1030 cm^{-1} was attributed to C–O–C glycosidic bond vibrations and ring vibrations, which overlapped with C–O–H link bond.

Functional properties of PS

Different functional proprieties of PS extracted from Lallemantia royleana were studied as illustrated in Table 1. PS was characterized by high water solubility and emulsion capacities; this former is maintained elevated and stable during 168 h. In addition, the PS displayed an interesting WHC, highlighting the potential of PS from *Lallemantia royleana* as a strong candidate for medical uses, while the OHC was weak.

Antioxidant activities of PS

Radical-scavenging activity (DPPH)

Data revealed a potent antioxidant activity of PS as described by DPPH activity (Fig 2A). In fact, the scavenging capacity increases with increasing concentration. At the final concentration (2.5 mg/ml), the antioxidant activity reached about 70%, but still lower than the reference (DPPH).

Ferrous ions chelating activity

The Ferrous ions chelating activity of PS and EDTA used as reference were determined at different concentrations (0-10 mg/ml). As illustrated in Fig 2B, PS presented an increase in chelating activity, which increased with the concentration of the sample. At the highest concentration (10 mg/ml), S1 reached about 78%, lower than EDTA with about 100% at the same concentration.

Reducing antioxidant power

The reducing antioxidant power of the polysaccharide is shown in Fig 2C. The result suggested that PS had a significant reducing power, which enhanced when concentra-

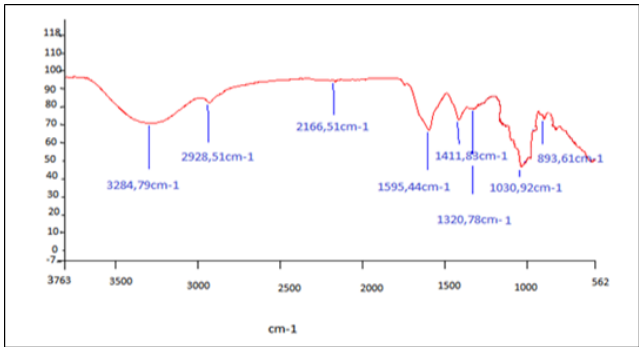

Figure 1. FT-IR spectrum of *Lallemantia royleana* polysaccharides.

Table 1. Functional properties of PS.

WSI	WHC	OHC	EC1	EC24	EC168
67.31±2.36%	10.22±4.44%	22.98±0.23%	89.5±0.02%	83.2±0.23%	71.44±1.81%

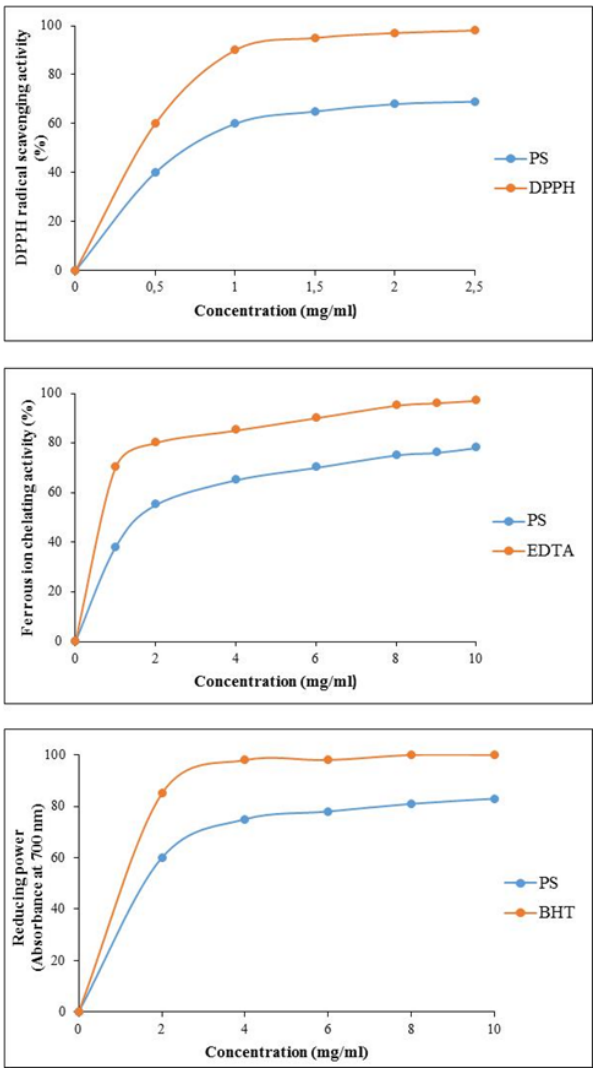

Figure 2. DPPH radical scavenging activity **(A)**, chelating effect **(B)** and reducing power **(C)** of polysaccharide extracted from *Lallemantia royleana* in comparison with DPPH, EDTA and BHT, respectively.

tions increased. BHT, used as a standard reference, showed similar reducing power of PS.

In vivo effects of PS

Effect of PS on morphological parameters

The weight of the animals was monitored daily for 30 days. The results showed that the hypercholesterolemic group induced a significant increase in body weight when compared to the initial body weight and to the control group ($p<0.05$). Meanwhile, the administration of Fluvastatin or PS significantly reduced ($P<0.05$) their body weights by 11.82% and 11.13%, respectively, compared to the control group (Table 2).

Additionally, in the hypercholesterolemic group, daily food intake and relative liver and kidney weight significantly increased, when compared to the control group ($p<0.05$). Co-administration of PS caused a normal liver and kidney weight.

Effect of PS on hematological parameters

Compared with the control group, an increase in the number of RBC, WBC and platelet were observed. However, no significant differences were observed in Hb, HT, MCV, MCH and MCHC in hypercholesterolemic rats (Table 3). Treatment with PS improved all parameters, which reached control values.

Assessment of oxidative stress markers

Estimation of MDA and AOPP levels

Table 4 represents the MDA and AOPP levels in the liver and kidney of control and treated rats for 30 days. Results showed a significant difference between the hypercholesterolemic and control groups. Our results revealed an increase in lipid peroxidation and protein oxidation in the liver and kidney of the rats treated with PS, as evidenced by the increase in the MDA and AOPP levels ($P<0.001$; $P<0.05$) when compared to the cholesterol-treated group (Table 4).

Enzymatic and non-enzymatic antioxidant status

Our *in vivo* study was revealed to test the capacity of PS to ameliorate the antioxidant defense against hypercholesterolemia.

Data showed a significant decrease in GPx, GSH, SOD and Vitamin C activities in the liver and in the kidney homogenates in the hypercholesterolemic treated group, when compared to the control rats (Table 4). However, the administration of PS to hypercholesteroemic rats ameliorated significantly these parameters in both tissues.

Biochemical assays

Effect of PS on blood lipid profile in hypercholesterolemic-rats

A significant increase in CT, TG and LDL and a decrease in HDL levels in the plasma of hypercholesterolemic-rats, when compared to the control group. However, PS administered to hypercholesterolemic-rats regulates lipid profile,

Table 2. Initial and final body weight, food intake and relative liver-kidney weights of controls and hypercholesterolemic rats co-treated with the Fluvastatin or the polysaccharide extracted from *Lallemantia royleana* for 30 days.

Parameters	Groups			
	Control	Cholesterol	Fluvastatin	Cholesterol +polysaccharide
Initial Body weight (g)	196.87±2.39^{a}	195.75±1.71^{a}	197±2.45^{a}	198.75±2.22^{a}
Final Body weight (g)	301.87±1.44^{a}	342.37±2.86^{c}	314±2.58^{b}	304.25±2.98^{a}
Daily food intake (g/day)	14.39±1.98^{a}	22.05±1.74^{d}	18.18±1.33^{b}	16.06±1.64^{c}
Relative organ weight (g/100 g body weight)				
Liver	4.02±0.06^{a}	6.05±0.05^{d}	4.91±0.08^{c}	4.18±0.03^{b}
Kidney	1.31±0.01^{a}	1.37±0.004^{b}	1.42±0.03^{c}	1.45±0.01^{d}

Results are expressed as mean of three experiments ± SD. The number of determinations was n =8.

$^{a.b.c.d}$In the same line indicate significant differences between different groups of rats ($p < 0.05$).

Table 3. Hematological parameters of controls and hypercholesterolemic rats co-treated with the Fluvastatin or the polysaccharide extracted from *Lallemantia royleana* for 30 days.

	Groups			
Parameters	**Control**	**Cholesterol**	**Fluvastatin**	**Cholesterol+ polysaccharide**
RBC count (10^6/ml)	6.07±0.74[a]	7.41±0.34[b]	7.03±0.43[b]	7.06±0.54[ab]
Hb (g/dl)	11.63±0.84[a]	12.62±0.32[a]	12.38±0.71[a]	11.97±0.75[a]
HT (%)	36.7±0.92[a]	40.33±0.74[b]	39.96±1.18[b]	40.07±0.87[b]
MCV (mm^3/RBC)	57.37±1.29[a]	56.45±0.45[a]	55.36±1.05[a]	55.3±0.75[a]
MCH (pg/RBC)	19.6±1.15[a]	18.05±0.71[a]	18.4±0.57[a]	18.41±0.79[a]
MCHC (g/dl)	35.8±1.64[b]	34.8±0.75[b]	31.8±0.27[a]	32.13±0.15[a]
WBC (10^3/ml)	12.96±0.4[a]	13.9±0.84[b]	12.26±1.02[a]	12.38±0.38[a]
Plt (10^3/ml)	537.67±4.51[a]	938.25±14.84[c]	718.2±29.25[b]	686.67±17.47[b]

Results are expressed as mean of three experiments ± SD. The number of determinations was n =8.
[a.b.c.]In the same line indicate significant differences between different groups of rats ($p < 0.05$).

Table 4. Levels of MDA, GSH, AOPP, Vitamin C and GPx activities in the liver and kidney of controls and hypercholesterolemic rats co-treated with the Fluvastatin or the polysaccharide extracted from *Lallemantia royleana* for 30 days.

	Groups			
Parameters	**Control**	**Cholesterol**	**Fluvastatin**	**Cholesterol+ polysaccharide**
Liver				
MDA (nmoles MDA/g tissue)	58.32±2.94[a]	85.10±3.51[c]	62.63±1.41[b]	52.66±7.82[a]
GSH (mg/g tissue)	182.55±14.27[a]	82.80±14.53[d]	163.59±15.43[b]	184.22±22.39[a]
AOPP (mmoles/mg proteins)	0.039±0.014[a]	0.099±0.02[b]	0.061±0.0018[b]	0.034±0.02[a]
Vitamin C (µg/mg protein)	125.57±10.81[a]	76.71±11.59[d]	101.59±11.64[b]	118.04±11.18[a]
GPx (nmoles of GSH/min/mg protein)	5.65±0.24[a]	2.66±0.60[b]	4.59±0.31[a]	5.15±1.36[a]
SOD (U/mg of protein)	79.13±13.87[a]	52.28±3.44[b]	84.41±6.18[a]	81.67±15.89[a]
Kidney				
MDA (nmoles MDA/g tissue)	62.5±6.94[a]	93.72±3.32[b]	69.14±3.48[a]	69.67±2.48[a]
GSH (mg/g tissue)	342.61±32.97[a]	205.36±17.87[b]	363.42±34.71[b]	370.63±31.64[b]
AOPP (mmoles/mg of proteins)	0.02±0.006[a]	0.084±0.015[c]	0.038±0.004[b]	0.033±0.01[a]
Vitamin C (µg/mg of protein)	135.61±12.25[a]	78.76±11.18[c]	118.94±13.23[b]	129.68±11.99[a]
GPx (nmoles of GSH/min/mg protein)	1.61±0.69[a]	0.51±0.05[b]	1.34±0.25[a]	1.85±0.61[a]
SOD (U/mg protein)	38.63±6.77[a]	26.39±9.85[b]	38.64±3.89[a]	38.84±8.98[a]

Results are expressed as mean of three experiments ± SD. The number of determinations was n =8.
[a.b.c.d]In the same line indicate significant differences between different groups of rats ($p < 0.05$).

Table 5. Effects of the Fluvastatin and the polysaccharide extracted from Lallemantia royleana on lipid profile of hypercholesterolemic treated rats.

	Groups			
Parameters	**Control**	**Cholesterol**	**Fluvastatin**	**Cholesterol+ polysaccharide**
CT (mmol/l)	1.54±0.03[a]	1.68±0.03[b]	1.57±0.01[a]	1.55±0.01[a]
TG (mmol/l)	1.17±001[a]	2.05±0.04[d]	1.40±0.04[c]	1.25±0.03[b]
HDL (mmol/l)	1.03±0.001[a]	1.028±0.05[a]	1.029±0.0008[a]	1.03±0.001[a]
LDL (mmol/l)	0.01±0.001[a]	0.046±0.04[b]	0.025±0.003[a]	0.017±0.001[a]

Results are expressed as mean of three experiments ± SD. The number of determinations was n =8.
[a.b.c.]In the same line indicate significant differences between different groups of rats ($p < 0.05$).

as shown by the significant decrease in plasma CT, TG and LDL levels by 7.73%, 39.02% and 63.04%, respectively and the increase in HDL level by 0.19% (Table 5).

Effect of PS on liver-kidney functions in hypercholesterolemic-rats

Table 6 revealed that hypercholesterolemic rats undertook an increase in terms of AST, Gamma GT, urea and uric acid rats by 32% and 43%, respectively; while the ALT, CK and creatinine levels decreased by 22%, 45% and 10%, respectively in the hypercholesterolemic group, when compared to the control rats. Interestingly, a polysaccharide extracted from *Lallemantia royleana* restored these parameters to normal values as compared to controls.

In our experimental conditions, obese animals showed a significant increase in plasma renal dysfunction levels

Table 6. Effect of Fluvastatin and polysaccharides extracted from Lallemantia royleana on plasma biochemical parameters of hypercholesterolemic treated rats.

Parameters	Groups			
	Control	Cholesterol	Fluvastatin	Cholesterol+ polysaccharide
Liver				
AST (Ul/L)	140.82±1.40[a]	189.12±1.49[d]	150.06±1.23[c]	146±0.71[b]
ALT (Ul/L)	42.04±1.30[a]	62.44±1.56[d]	50.76±1.71[c]	44.57±1.09[b]
CK (Ul/L)	3100.51±1.51[a]	4899.28±1.47[d]	3695.84±1.82[c]	3328.57±1.64[b]
Gamma GT (Ul/l)	2.39±0.23[a]	7.57±0.84[c]	3.5±0.35[b]	2.53±0.36[a]
ALP (UI/L)	260.8±1.64[a]	329±1.58[d]	288.8±1.30[c]	269±1.58[b]
Kidney				
Urea (mmol/L)	3.37±0.22[a]	4.12±0.31[b]	3.58±0.24[a]	3.39±0.04[a]
Creatinine (mmol/L)	36.9±0.74[a]	47.72±0.94[c]	38.9±0.93[b]	36.8±0.83[a]
Uric acid (mg/L)	99.8±1.48[a]	216.6±1.51[c]	104.16±1.60[b]	101.3±1.71[a]

Results are expressed as mean of three experiments ± SD. The number of determinations was n =8.
[a.b.c.] In the same line indicate significant differences between different groups of rats ($p < 0.05$).

of albumin, urea and creatinine by 43.34%,216.84% and 112.29%, respectively, compared to control rats ($P < 0.05$). However, the administration of PS to obese rats improved all renal toxicity indices (Table 6).

Histopathological studies

In the hypercholesterolemic rats, liver and kidney histological data showed numerous abnormalities (Fig 3 and Fig 4). Light microscopic examination indicated a normal liver structure in controls, and polysaccharides administered rats, showing a normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein. While with the hypercholesterolemic rats, severe histopathological changes were observed. Hypercholesterolemia caused marked leucocyte infiltration, vacuole formation and steatosis (Fig 3 C-D).

Upon kidney histological examination, renal tissue architecture showed normal glomeruli and tubules (Fig 4A-B). In obese rats, the kidney revealed a broaden Bowman's space, tubular obstruction, vacuole formation and infiltration of leucocytes (Fig 4B-C). The severe kidney damage, shown in hypercholesterolemic rats significantly reduced, when PS and fluvastatin were administered to hyperlipidemic rats for 30 days. In fact, in these groups, the kidney histological aspect was similar to that of controls (Fig 4 E-F-G-H).

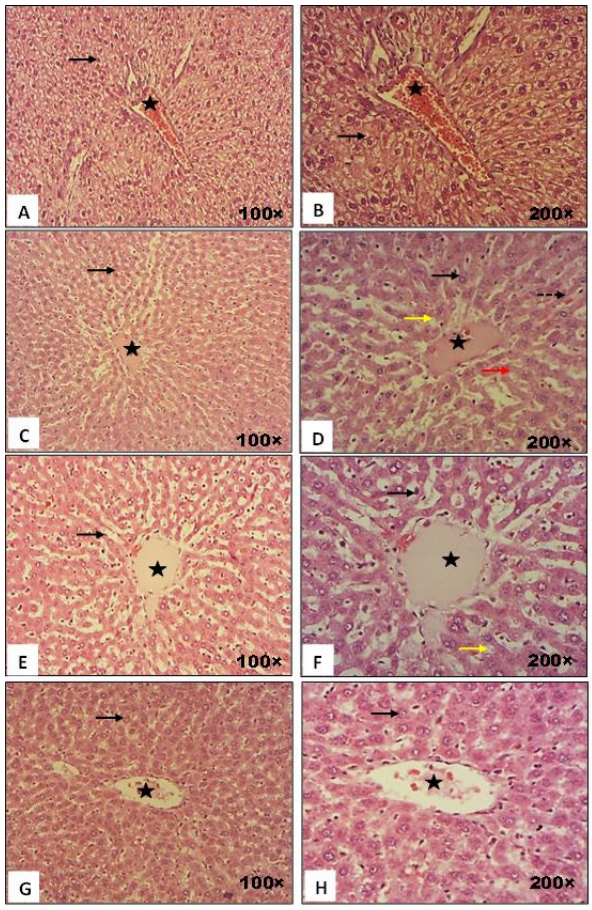

Figure 3. Histological sections of liver of experimental rats. **(A-B)** normal rats; **(C-D)** hypercholesterolemic rats; **(E-F)** rats treated with Fluvastatin; **(G-H)** hypercholesterolemic rats treated with polysaccharide extracted from *Lallemantia royleana*. Arrows indicate: Hepatocytes : ;Central vein: vc ; Necrosis : ; Apoptosis : ; Mitosis : Vacuolated hepatocytes : ; Leukocyte infiltration : ;Vascular congestion:

Discussion

Hypercholesterolemia has significant cardiac consequences, kidney failure and liver damage (26). Thus, the present study was designed to evaluate the antioxidant activities and the chemical composition of PS extracted from *Lallemantia royleana* and the possible beneficial effect of its administration on hematological and plasma lipid parameters, liver-kidney functions in adult rats fed with a cholesterol-rich diet.

Interestingly, Herbal medicines are plentiful sources of bioactive compounds; especially PS can be used as antioxidants for the prevention of oxidative damage in living organisms. The PS is a complex and heterogeneous macromolecule and their biological and pharmacological activities normally result from a complex interaction of several structural features, including glycosidic bonds between monosaccharides, the molecular weight, and sugar residue composition.

Antioxidants have a positive effect on human health as they can protect the human body against damage induced

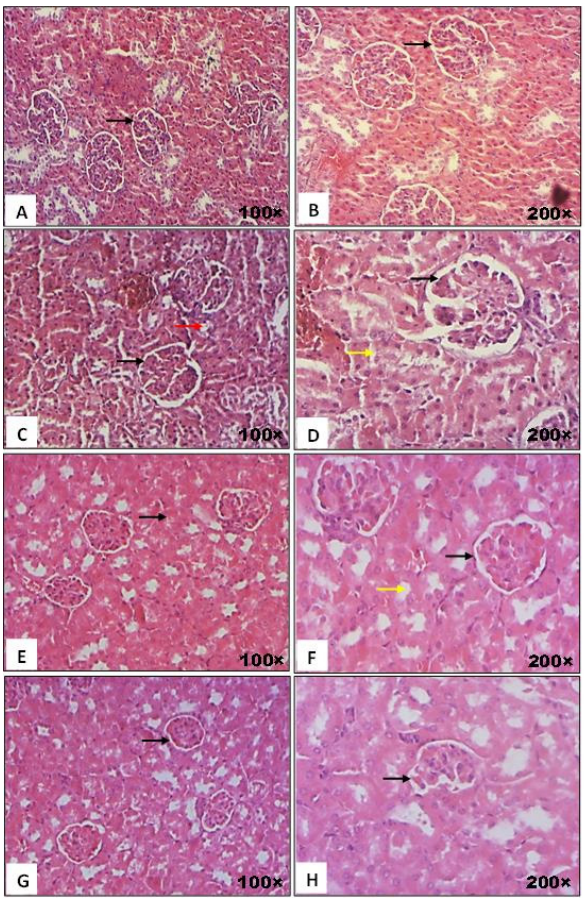

Figure 4. Histological sections of Kidney of experimental rats. **(A-B)** normal rats; **(C-D)** hypercholesterolemic rats; **(E-F)** rats treated with Fluvastatine; **(G-H)** hypercholesterolemic rats treated with polysaccharide extracted from *Lallemantia royleana*.**T:** tubule; **G:** glomeruli Tubular light : , Glomerular space : , Apostosis : ,Leukocyte infiltration: , Necrosis :

by reactive oxygen species (ROS), which can attack macromolecules such as membrane lipids and lead to health disorders and inflammatory diseases with severe tissue injuries (27). Our *in vivo* experimental study revealed that hypercholesterolemia induced damage in the liver and kidney of treated rats.

In fact, it has been reported that oxidative stress, and especially ROS, may affect the protein expression profile, as shown by the elevation in MDA and AOPP levels in the liver and kidney tissues, when compared to the control group. These data are similar to those reported by Anila (28). Interestingly, the abnormal rise in lipid peroxidation was reduced upon polysaccharides administration generally related to the antioxidant properties of polysaccharides extracted from *Lallemantia royleana*, as shown in our *in vitro* study by DPPH-scavenging, the ferric reducing power assay and chelating effect tests (29). Oxidative stress occurs when the antioxidant defense system is overwhelmed by ROS production. Antioxidants and other cell redox state-modulating enzyme systems act as the first line of defense against ROS in all cellular and extracellular compartments (30). In fact, the activity of SOD and GPx are significantly decreased in the liver and kidney in hypercholesterolemic rats. The decrease, which occurred in the liver and kidney, indicated that free radical production exceeded the capacity of detoxification mechanisms in both tissues. The disruption in antioxidant enzymatic activities indicated oxidative stress damage caused by cholesterol (26). However, a significant improvement in GPx and SOD activities through the supplementation of PS extracted from *Lallemantia royleana*, confirmed the protective effect and the antioxidant capacity of this polymer.

In addition, non-enzymatic antioxidants such as glutathione and vitamin C play a vital role in protecting cells from oxidative damage. Data revealed that GSH and vitamin C levels in hypercholesterolemic rats decreased significantly when compared to the normal ones. Accordingly, the increase in GSH and Vitamin C contents in the PS-supplemented group may be attributed to the ability of this polymer to improve the kidney and liver defense against free radicals (3).

It's known that the development of oxidative stress-induced cell damage, tissue injuries and functional impairment as evidenced by hepatic function tests, such as elevated plasma lipase profile, demonstrated the severity of the hepatic damage. In fact, the hypercholesterolemic effect may be due to the increased dietary cholesterol intake and subsequent increased rate of intestinal cholesterol absorption (31). In the present work, results showed an elevation in cholesterol, LDL and triglyceride levels in hypercholesterolemic rats, with a slight reduction in HDL level. It has been reported that complex alimentary lipids are converted into triglycerides and free fatty acids by intestinal lipase and that lipase inhibitors reduce postprandial plasma lipid level *via* retarding the liberation of TG and free fatty acids and their absorption (19). In fact, our *in vivo* study report that PS extracted from *Lallemantia royleana*is inhibits the key enzyme related to hypercholesterolemia as lipase activity with a decrease in the level of TG, cholesterol, LDL-cholesterol with a normal rate in HDL-cholesterol. This could be explained by the hypolipidemic effect of this polymer.

The previous data were confirmed by histological sections of the livers of treated rats for 30 days. Light microscopic examination revealed the appearance of lipid droplets, which correspond to a deposit of fat in the hepatocytes. The liver abnormality was associated with lipid metabolism, which is manifested by the appearance of lipid vacuolization in the liver of rats subjected to a hyperlipidemic diet. While the rats exposed to PS and fluvastatin have a protective effect against hypercholesterolemia, as shown by a marked decrease in lipid vacuolization and steatosis. Our results are similar to those of Suanarunsawat et al (32), who showed that changes in hepatic tissue results in the fat deposition formed steatosis and appears in the histological sections as empty vacuoles. However, in hypercholesterolemic rats treated with PS, significant protection in liver function was observed biochemically by lowering hepatic cytolysis markers regression of liver fat, confirming the beneficial effect of this compound against hyperlipidemia. Interestingly, in PS-treated rats, the histopathological changes were completely alleviated, indicating the effectiveness of this polymer in mediating cholesterol-induced hepatotoxicity.

Additionally, in obese rats treated with PS, significant protection of liver function was observed biochemically by a lowering of elevated liver enzyme markers. In fact, a significant decrease in AST, ALT and ALP activities was noted. Similarly, the therapeutic effect and potential of PS in antihyperlipidemic actions prevent kidney dysfunctions and showed a decrease in the plasma biomarkers indicating renal perturbation such as urea, creatinine and uric acid.

These findings are in agreement with several reports. For instance, Kammoun et al (3) reported the positive effect of sulfated polysaccharides extracted from the green alga *Ulva lactuca* in antihyperlipidemia actions to prevent liver-kidney dysfunctions. In fact, an increase in the density of microvessels in the lower and middle cortex of the kidneys and morphological damage was noted. This alteration affects the distribution and regulation of intra-renal blood (33). In addition, hyperlipidemia induces renal disorders leading to renal insufficiency, which may be the result of damage to tubules or glomeruli (34). These modifications could be due to the accumulation of free radicals resulting from an enhancement in lipid peroxidation in the renal tissues (35). In view of all these results, it can be concluded that the administration of PS to hypercholesterolemic rats resulted in a partial restoration of the renal disorders, as shown in the histological aspect. This is in agreement with Sathivel et al (36), which showed the anti-hyperlipidemic nature of the sulfated polysaccharide of *Ulva lactuca.*

Furthermore, this study showed abnormalities in some blood cell parameters in hypercholesterolemic rats, objectified by a significant increase in the number of RBC, WBC and platelet, which indicate the activation of defense mechanism due to the enhancement in the LDL and triglyceride levels and thereby the installation of hypercholesterolemia. Nevertheless, treatment with PS improved all parameters, which reached control values.

Conclusion

The present study showed that polysaccharide extracted from *Lallemantia royleana* was efficient in the protection against hyperlipidemia by reducing plasma TC, TG, LDL and lipid peroxidation in animals fed by a high-cholesterol diet. Therefore, this polymer could be considered as a promising functional agent to prevent atherosclerotic disease. However, as the accurate mechanism is not yet clear, further studies are needed in order to check the potential therapeutic use of this polysaccharide.

Acknowledgments

The present work was funded by the Ministry of Higher Education and Scientific Research, Tunisia.

Conflict of interest

We confirm that there are no known conflicts of interest associated with this publication.

Author's contribution

Mohammed Abdulameer Farhan: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization.

Amal Feki, Intissar Kammoun and Malek Aroui: Investigation.

Manel Naifar, Rim Kallel, Fatma Makni Ayadi, Tahia Boudawara, Choumous Kallel and Adil Abaed Hassoni: Supervision.

Ibtissem Ben Amara: Resources, Supervision, Writing - review & editing.

References

1. Ktari N, Belguith-Hadriche O, Ben Amara I, Ben Hadj A, Turki M, Makni-Ayedi F, et al. Cholesterol regulatory effects and antioxidant activities of protein hydrolysates from zebra blenny (Salaria basilisca) in cholesterol-fed rats. Food Funct 2015;6:2273–82. https://doi.org/10.1039/C5FO00492F.
2. Kaliora AC, Dedoussis GVZ, Schmidt H. Dietary antioxidants in preventing atherogenesis. Atherosclerosis 2006;187:1–17. https://doi.org/10.1016/j.atherosclerosis.2005.11.001.
3. Kammoun I, Ben Salah H, Ben Saad H, Cherif B, Droguet M, Magné C, et al. Hypolipidemic and cardioprotective effects of *Ulva lactuca* ethanolic extract in hypercholesterolemic mice. Arch Physiol Biochem 2018;124:313–25. https://doi.org/10.1080/13813455.2017.1401641.
4. Behbahani BA, Imani Fooladi AA. Shirazi balangu (Lallemantia royleana) seed mucilage: Chemical composition, molecular weight, biological activity and its evaluation as edible coating on beefs. Int J Biol Macromol 2018;114:882–9. https://doi.org/10.1016/j.ijbiomac.2018.03.177.
5. Razavi SMA, Cui SW, Ding H. Structural and physicochemical characteristics of a novel water-soluble gum from Lallemantia royleana seed. Int J Biol Macromol 2016;83:142–51. https://doi.org/10.1016/j.ijbiomac.2015.11.076.
6. Ghannadi A, Zolfaghari B. Compositional analysis of the essential oil ofLallemantia royleana(Benth. in Wall.) Benth. from Iran. Flavour Fragr J 2003;18:237–9. https://doi.org/10.1002/ffj.1215.
7. Safa O, Soltanipoor MA, Rastegar S, Kazemi M, Nourbakhsh Dehkordi K, Ghannadi A. An ethnobotanical survey on hormozgan province, Iran. Avicenna J Phytomedicine 2013;3:64–81.
8. Farhadi N. Structural elucidation of a water-soluble polysaccharide isolated from Balangu shirazi (Lallemantia royleana) seeds. Food Hydrocoll 2017;72:263–70. https://doi.org/10.1016/j.foodhyd.2017.05.028.
9. Denny TP. Involvement of Bacterial Polysaccharides in Plant Pathogenesis. Annu Rev Phytopathol 1995;33:173–97. https://doi.org/10.1146/annurev.py.33.090195.001133.
10. Feki A, Amara IB, Bardaa S, Hajji S, Chabchoub N, Kallel R, et al. Preparation and characterization of polysaccharide based films and evaluation of their healing effects on dermal laser burns in rats. Eur Polym J 2019;115:147–56. https://doi.org/10.1016/j.eurpolymj.2019.02.043.
11. Liu G, Xu S, Chen L. Chemical composition and bioactivities of a water-soluble polysaccharide from the endodermis of shaddock. Int J Biol Macromol 2012;51:763–6. https://doi.org/10.1016/j.ijbiomac.2012.07.012.
12. Ben Slima S, Ktari N, Trabelsi I, Moussa H, Makni I, Ben Salah R. Purification, characterization and antioxidant properties of a novel polysaccharide extracted from Sorghum bicolor (L.) seeds in sausage. Int J Biol Macromol 2018;106:168–78. https://doi.org/10.1016/j.ijbiomac.2017.08.010.
13. Ktari N, Feki A, Trabelsi I, Triki M, Maalej H, Slima SB, et al. Structure, functional and antioxidant properties in Tunisian beef sausage of a novel polysaccharide from Trigonella foenum-graecum seeds. Int J Biol Macromol 2017;98:169–81. https://doi.org/10.1016/j.ijbiomac.2017.01.113.
14.] Freitas F, Alves VD, Carvalheira M, Costa N, Oliveira R, Reis MAM. Emulsifying behaviour and rheological properties of the extracellular polysaccharide produced by Pseudomonas oleovorans grown on glycerol byproduct. Carbohydr Polym 2009;78:549–56. https://doi.org/10.1016/j.carbpol.2009.05.016.
15. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature 1958;181:1199–200. https://doi.org/10.1038/1811199a0.
16. Carter P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal Biochem 1971;40:450–8. https://doi.org/10.1016/0003-2697(71)90405-2.
17. Oyaizu M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared

from glucosamine. Jpn J Nutr Diet 1986;44:307–15. https://doi.org/10.5264/eiyogakuzashi.44.307.

18. Mnafgui K, Hamden K, Ben Salah H, Kchaou M, Nasri M, Slama S, et al. Inhibitory Activities of *Zygophyllum album* : A Natural Weight-Lowering Plant on Key Enzymes in High-Fat Diet-Fed Rats. Evid Based Complement Alternat Med 2012;2012:1–9. https://doi.org/10.1155/2012/620384.
19. Ben Gara A, Ben Abdallah Kolsi R, Chaaben R, Hammami N, Kammoun M, Paolo Patti F, et al. Inhibition of key digestive enzymes related to hyperlipidemia and protection of liver-kidney functions by Cystoseira crinita sulphated polysaccharide in high-fat diet-fed rats. Biomed Pharmacother 2017;85:517–26. https://doi.org/10.1016/j.biopha.2016.11.059.
20. Draper HH, Hadley M. [43] Malondialdehyde determination as index of lipid Peroxidation. Methods Enzymol., vol. 186, Elsevier; 1990, p. 421–31. https://doi.org/10.1016/0076-6879(90)86135-I.
21. Witko V, Nguyen AT, Descamps-Latscha B. Microtiter plate assay for phagocyte-derived Taurine-chloramines. J Clin Lab Anal 1992;6:47–53. https://doi.org/10.1002/jcla.1860060110.
22. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971;44:276–87. https://doi.org/10.1016/0003-2697(71)90370-8.
23. Flohé L, Günzler WA. [12] Assays of glutathione peroxidase. Methods Enzymol., vol. 105, Elsevier; 1984, p. 114–20. https://doi.org/10.1016/S0076-6879(84)05015-1.
24. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95. https://doi.org/10.1016/0006-2952(61)90145-9.
25. Jacques-Silva MC, Nogueira CW, Broch LC, Flores ÉMM, Rocha JBT. Diphenyl Diselenide and Ascorbic Acid Changes Deposition of Selenium and Ascorbic Acid in Liver and Brain of Mice: DEPOSITION OF SELENIUM AND ASCORBIC ACID IN LIVER AND BRAIN OF MICE. Pharmacol Toxicol 2001;88:119–25. https://doi.org/10.1034/j.1600-0773.2001.d01-92.x.
26. Kolsi RBA, Jardak N, Hajkacem F, Chaaben R, jribi I, Feki AE, et al. Anti-obesity effect and protection of liver-kidney functions by Codium fragile sulphated polysaccharide on high fat diet induced obese rats. Int J Biol Macromol 2017;102:119–29. https://doi.org/10.1016/j.ijbiomac.2017.04.017.
27. Chen L, Long R, Huang G, Huang H. Extraction and antioxidant activities in vivo of pumpkin polysaccharide. Ind Crops Prod 2020;146:112199. https://doi.org/10.1016/j.indcrop.2020.112199.
28. Anila L. Antioxidant action of flavonoids from Mangifera indica and Emblica officinalis in hypercholesterolemic rats. Food Chem 2003;83:569–74. https://doi.org/10.1016/S0308-8146(03)00155-9.
29. Sardarodiyan M, Arianfar A, Sani AM, Naji-Tabasi S. Antioxidant and antimicrobial activities of water-soluble polysaccharide isolated from Balangu seed (Lallemantia royleana) gum. J Anal Sci Technol 2019;10:17. https://doi.org/10.1186/s40543-019-0174-4.
30. Garoui E, Amara IB, Driss D, Elwej A, Chaabouni SE, Boudawara T, et al. Effects of Cobalt on Membrane ATPases, Oxidant, and Antioxidant Values in the Cerebrum and Cerebellum of Suckling Rats. Biol Trace Elem Res 2013;154:387–95. https://doi.org/10.1007/s12011-013-9746-0.
31. Zulet MA, Barber A, Garcin H, Higueret P, Martínez JA. Alterations in Carbohydrate and Lipid Metabolism Induced by a Diet Rich in Coconut Oil and Cholesterol in a Rat Model. J Am Coll Nutr 1999;18:36–42. https://doi.org/10.1080/07315724.1999.10718825.
32. Suanarunsawat T, Devakul Na Ayutthaya W, Songsak T, Thirawarapan S, Poungshompoo S. Lipid-Lowering and Antioxidative Activities of Aqueous Extracts of *Ocimum sanctum* L. Leaves in Rats Fed with a High-Cholesterol Diet. Oxid Med Cell Longev 2011;2011:1–9. https://doi.org/10.1155/2011/962025.
33. Bentley MD, Rodriguez-Porcel M, Lerman A, Sarafov MH, Romero JC, Pelaez LI, et al. Enhanced renal cortical vascularization in experimental hypercholesterolemia. Kidney Int 2002;61:1056–63. https://doi.org/10.1046/j.1523-1755.2002.00211.x.
34. Gonca S, Ceylan S, Yardimoglu M, Dalçık H, Yumbul Z, Köktürk S, et al. Protective Effects of Vitamin E and Selenium on the Renal Morphology in Rats Fed High-Cholesterol Diets. Pathobiology 2000;68:258–63. https://doi.org/10.1159/000055935.
35. Ben Amara I, Troudi A, Garoui E, Hakim A, Boudawara T, Zeghal KM, et al. Protective effects of selenium on methimazole nephrotoxicity in adult rats and their offspring. Exp Toxicol Pathol 2011;63:553–61. https://doi.org/10.1016/j.etp.2010.04.007.
36. Sathivel A, Raghavendran HRB, Srinivasan P, Devaki T. Anti-peroxidative and anti-hyperlipidemic nature of Ulva lactuca crude polysaccharide on d-Galactosamine induced hepatitis in rats. Food Chem Toxicol 2008;46:3262–7. https://doi.org/10.1016/j.fct.2008.07.016.