Inhibition of key digestive enzymes related to diabetes and protection of β-cell and liver- kidney functions by Hypnea spinella sulfated polysaccharide in diabetic rats

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Abstract

The present study investigates the protective effects of sulfated polysaccharide isolated from *Hypnea spinella* alga (HSSP) on alloxan-induced stress oxidant, hepatic dysfunction and histological changes in male rats liver, pancreas and kidney. The chemical characterization of HSSP using various assays such as FIR, XRD and GC-MS spectroscopy. Our results showed that HSSP reduced the activity of α-amylase in serum, pancreas and intestine, as well as a reduction of blood glucose level. In addition, HSSP enhanced superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), reduced lipid peroxidation in the hepatic, pancreatic and renal antioxidant enzymes and improved the liver-kidney dysfunction parameters by decreasing of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), creatinine, albumin and urea rates in plasma. Moreover, HSSP treatment in diabetic rats protects against alloxan induced pancreatic β-cells and hepatic cells damages.

Introduction

Nowadays, the popularity of seaweed has become a more versatile form of a food ingredient that can be incorporated directly or indirectly in the preparation of foods and beverages. Due to its functionality seaweed and its products are of particular importance in the food industry, as components in fertilizers, animal feed supplements and additives for functional foods [1].

Polysaccharides, a kind of biological macromolecules existed in almost all organisms, have attracted worldwide interest of researchers owing to their non-toxic characteristics, diversified structures, multiple biological activities and functional properties.

Polysaccharides are described as an energy reserve and structural compounds of all organisms including marine and higher plants. In seaweed, the polysaccharides are the most important macro-molecules which hold more than 80% of their weights. These resistant polysaccharides are termed as dietary fibers, which are not digested in the body but due to enzymatic action of micro-organisms in the gut can be fermented to varying degrees [2].

Sulfated polysaccharides have attracted increasing attention due to their diversified biological and pharmacological activities, such as anti-viral, immune enhancement, anti-tumor, antidiabetic and antioxidant effects [29]. These activities are extensively attributed to the interaction between polysaccharide and intestinal microorganisms to prove a functional and medicinal property of sulfated polysaccharides.

Many new drugs have evolved for diabetes in recent years including oral hypoglycemic agent such as metformin, acarbose, sitagliptin, etc., and insulin and its analogues have been clinically applied for the treatment of diabetes. However, most of them have been proven to have some toxicity and side effects [12]. Therefore, there is an urgent demand for seeking safer and more effective intervention strategies that may breakthrough these restrictions. Bioactive compounds from seaweed are found safe and effective against type-2-diabetes, which reverse carbohydrate metabolism enzymes [30].

Previous studies have also shown that polysaccharides possess significant antidiabetic activities through multiple ways, primarily including preventing gastric emptying, inhibition of amylase and glycosidase activities, boosting insulin secretion, improving insulin function and remodeling intestinal microflora [18]. Therefore, algae polysaccharides may be the potent therapeutics for diabetes and its complications.

As novel bioactive molecules isolated from marine macro-algae have anti-diabetic, antihypertensive and other bioactive properties, investigation of bioactive molecules from marine algae has been an attractive field to search potent drug candidates. This is the first report that investigates anti-diabetic effects of sulfated polysaccharides derived from red algae, *Hypnea spinella*.

Mediterranean Tunisian coast marine flora, with its large contains of seaweeds, most of them have not yet been investigated for anti-diabetic, anti-obesity and anti-hypertensive potencies. Therefore, the objective of this research sought to investigate, for the first time, the inhibitory potential of a sulfated polysaccharide extracted from *Hypnea spinella* (HSSP) alone on key enzymes related to diabetes *in vitro* and in diabetic rats. This is the first study for this red algae, focusing on the composition, chemical characterization, functional properties and beneficial effect of the sulfated polysaccharide on serum lipid, hepatic and renal parameters, considering its antioxidant properties and histopathological changes in induced diabetic rat.

Methods And Materials

Algal materials

Hypnea spinella, a red algae, (Phylum Rhodophyta, Class Florideophyceae, Order Gigartinales, Family Hypneaceae) was collected from the Mediterranean in various areas of the coastal region of Golf Gabes (Tunisia), in June 2020, at a depth between 1 and 3 m.

The plant was identified at the Stazione Zoologica ‘A. Dohrn’, Functional and Evolutionary Ecology Laboratory, Punta S. Pietro, Ischia, Italy. The sample was washed three times with tap water to remove the salt, epiphytes and sand attached to the surface, then carefully rinsed with fresh water and distilled water. Dried alga sample was ground and sifted through a 50-mesh standard testing sieve and maintained in bottles till ready for extraction.

Method of extraction of HSSP

The polysaccharide extract from *H. spinella* (HSSP) was prepared as described by Ananthi et al. [5] with minor modifications. Briefly, 300 g of the dried algae powder was depigmented with acetone stirring overnight at room temperature and with ethanol twice under reflux, followed by hot water extraction at 90–95 °C for 3–4 h 3 times. The syrup was then filtered through a Whatmann No°3 filter paper, concentrated to 1/4 of the original volume, cooled and precipitated with three volumes of ethanol overnight at 4°C. The precipitate was collected by centrifugation and washed 3 times with 75% ethanol,

dehydrated and lyophilized to get a dried crude sulfated polysaccharide. The polysaccharide extraction yield was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Dry weight of alcohol precipitate}}{\text{Dry weight of algae}} * 100$$

Alpha-amylase assay in vitro

Alpha-amylase inhibition activity of all samples was determined based on the spectrophotometric assay using glucor as the reference compound [11].

The sample of HSSP was dissolved in distilled water to give different concentrations of 50, 100, and 200 mg/ml. The α-amylase solution was prepared by mixing of 50 mg of α-amylase in 100 ml of 40mM phosphate buffer, pH 6.9. Positive control glucor was obtained by dissolving in phosphate buffer. The assay was conducted by mixing 80 ml of sample, 20 ml of α-amylase solution, and 1ml of 2-chloro-4-nitrophenol-a-Dmaltotrioside (CNPG3) (2.25 mM). The mixture was incubated at 37°C for 5 min. The absorbance was measured at 405 nm spectrophotometrically. Similarly, a control reaction was carried out without the sample/acarbose. Percentage inhibition (PI) was calculated based on this expression:

$$PI = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{control}}} * 100$$

Animals and treatments

The assays of the present study were conducted on adult male Wistar rats, weighing 180± 20 g, which were obtained from the local Central Pharmacy, Tunisia. All rats were kept in an environmentally controlled breeding room (temperature: 20± 2 °C; humidity: 60 ±5%; 12 h dark/light cycle) where they had standard diets and free access to tap water. The experimental protocols were conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax, Tunisia and approved by the Committee of Animal Ethics. Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared alloxan solution in normal saline at a dose of 150 mg/kg body weight [14].

The injection of alloxan can cause fatal hypoglycemia due to a reactive massive release of pancreatic insulin. For this reason, the rats were also orally given 5-10 ml of a 20% glucose solution after 6 h. The animals were then kept with free access to 5% glucose solution for the next 24 h to prevent severe hypoglycemia. After 2 weeks, rats with moderate diabetes having glycosuria checked by urine test strip and hyperglycemia (i.e. with blood glucose levels superior to 2 g/L) were chosen for the experiment, as well blood was drawn from a small cut in the tail and blood glucose level was measured at the end of each day with the aid of a one touch glucometer (Bionime, Pharmatec, Tunisia).

Before treatment, the rats were divided into 4 groups of eight animals each as follows:

- Group 1: control rats, considered as referent non-diabetic rats (Cont).

- Group 2: surviving untreated diabetic rats (glycemia was superior to 2 g/L) (Diab).
- Group 3: diabetic rats treated with glucor by gastric gavage route (10 mg/kg of body weight/daily during 30 days) (Diab+Glu).
- Group 4: diabetic rats treated with HSSP by gastric gavage route (200 mg/kg of body weight/daily during 30 days) (Diab+HSSP).

For the toxicity study, six groups of rats were used (eight animals per group). Group 1 received NaCl 0.9% orally in a volume of 10 ml/kg of body weight (bw) and served as control. Groups 2, 3, 4, 5 and 6 were administrated with HSSP at the doses of 100, 400, 800, 1000 and 2000 mg/kg bw respectively orally in a volume of 10 ml/kg. After the oral administration of HSSP, animals were observed individually at least once during the first 30 min, periodically during the first 48 h [6].

At the end of the experimentation and for oral glucose tolerance test (OGTT), three rats from each group received glucose 2 g/kg/ bw by gastric gavage route. Blood samples were collected from tail vein under light anesthesia at time 0, 30, 60, 90, 1200 and 90 min subsequent to glucose (2 g/kg body weight) received a fasting blood glucose level were measured.

Four weeks later, the rats were weighed and killed by decapitation, and their trunk blood collected. The serum was prepared by centrifugation (1500g, 15 min, 4°C), frozen

and stored at -20 °C until analysis. The kidney and liver were removed and cleaned of fat.

All samples were stored at -80 °C until use.

For histological studies, pieces of pancreas, liver and kidney tissues were removed

and fixed in 10% formaldehyde solution. The washed tissues were dehydrated in increasing gradient of ethanol and finally cleared in toluene. The tissues were then embedded in molten paraffin wax and the sections were cut at 5 mm thickness and stained with haematoxylin

and eosin. The slides were photographed with an Olympus U-TU1X-2 camera connected to an Olympus CX41 microscope (Tokyo, Japan).

Biochemical analysis

The liver and the pancreas of each rat were excised and homogenized in Tris- Buffered Saline (TBS), pH 7.6 and centrifuged (5000 g, 20 min). The supernatant of liver homogenate was frozen and stored for further use in the profile lipid assay. The analyses of serum lipase, serum lipids level of triglycerides (TGs), total cholesterol (T-Ch), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were measured using the corre-sponding commercial kits (Biolabo, France) on an automatic biochemistry analyzer (BS 300, China) at the biochemistry laboratory of Hedi chaker Hospital (Sfax, Tunisia). Serum levels of Creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bili) activities and creatinine, uric acid and urea rates

were measured in frozen aliquots of serum by standardized enzymatic procedures using commercial kits from (Biolabo, France) on an automatic biochemistry analyzer (Vitalab Flexor E, USA) at the biochemistry laboratory of Hedi chaker Hospital (Sfax, Tunisia). After the homogenization of the liver and pancreas in a phosphate buffer, the lipid peroxidation in the liver and pancreas of control and all treated groups of animals was measured by the quantification of thiobarbituric acid reactive substances (TBARS) determined by the method of Yoshioka et al. The activity of SOD was assayed by the spectrophotometric method of Marklund and Marklund [26] and expressed as U/mg protein. The GPX activity was measured by the method described by Pagila and Valentine [33] and expressed as mmoles GSS/ (min mg protein). The CAT activity was assayed by the calorimetric methods at 240 nm and expressed as mmoles of H_2O_2 consumed/ (min mg protein), as described by Aebi [3]. The level of total protein was determined by the method of Lowry et al. [23] using bovine serum albumin as the standard at 660 nm.

Spectroscopic analysis

Fourier transform-infrared (FT-IR) spectrometry

The characteristic organic groups of HSSP were analyzed using FTIR-System Perkin-Elmer 1750 DX Infrared Spectrometer. In brief, the freeze-dried sample (1 mg) was mixed with KBr and tabulated into pellet. The FT-IR spectra of sample were recorded from 4000 to 400 cm^{-1}.

X-ray diffraction

The X-ray diffraction (XRD) pattern of HSSP was measured at room temperature by a (SIEMENS D5000) diffractometer, equipped with a ‘Scintillation counter’. The data were collected in the 2θ range 5–100° with a step size of 0.02° and a counting time of 0.78 s/step.

Analytical methods

Hydrolysis of the polysaccharide

Hydrolysis of the sample was made by a partially modified version of the method described by O’Rourke et al. [31]. Polysaccharide sample (50 mg) was dissolved in 2 M trifluoroacetic acid (TFA) and incubated for 2, 6 and 24 h at 100 °C. The hydrolysis tube was cooled at room temperature. The reaction mixture was then neutralized to pH 7 by adding NaOH (1 M) and the sample was mixed thoroughly by vortexing for 10 s and filtered.

The resulting acid hydrolysates were analyzed by TLC and by GC/MS. For the TLC analysis,

10 µg of the hydrolyzed samples were spotted on a silica gel plate and a mixture of chloroform: acetic acid: water (6:7:1, v/v) was used as developing solvent. Detection of sugar was performed using 5% (v/v) sulfuric acid in ethanol. Solution was sprayed on the plate and the color was developed at 110 °C for identifying sugars in HSSP samples.

Monosaccharide composition

Determination of monosaccharide composition was carried out by GC-MS (gas chromatography-mass spectrometry) and was commonly used in the analysis of polysaccharide composition including the type and mole ratio of monosaccharide after hydrolysis of the HSSP with 2 M TFA at 100 $^{\circ}$C for 24 h as previously reported. The obtained hydrolysate was lyophilized and then silylated by a mixture of (pyridine hexamethyldisilazane-trimethylchlosilane, 9:3:1, v/v/v) using 50 µl per mg of dried hydrolysate. Analysis of the obtained trimethylsilyl sugars' derivatives was performed on a

Varian 3800 chromatograph, equipped with a fused silica capillary column (30 m_0.25 mm) coated with DB-225MS (Durabond) and a Varian Saturn 2000 ITD spectrometer operating under the following conditions: the injected volume was 1 µl; the detector and the injector's temperature were set at 320 $^{\circ}$C, the column temperature was set 1 min at 100 $^{\circ}$C ramped from 100 to 260 $^{\circ}$C at 4 $^{\circ}$C min^{-1} and 10 min at 260 $^{\circ}$C. Helium was used as carrier gas at 1ml min^{-1}.

2.8. Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical significance was assessed by the Fisher's test. $p < 0.05$ was considered statistically significant.

Results And Discussion

Biological study

In vitro α-amylase inhibitory assay

One therapeutic approach for treating Type 2 diabetes is to decrease postprandial hyperglycemia [28]. The ability of a drug or diet to retard the production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes such as α-amylase is one of the therapeutic approaches for decreasing postprandial hyperglycemia.

The bioactivity of HSSP was tested *in vitro* using α-amylase inhibitory assay. As shown in Table 1,HSSP showed strong *α*-amylase inhibitory activity under *in vitro* conditions. A concentration-dependent inhibitory activity against α-amylase was observed for the HSSP used at doses of 50, 100 and 200 mg/ml with IC_{50} value of 47.58 mg/ml as compared to glucor with IC_{50}=17.26 mg/ml.

Postprandial hyperglycemia plays an important role in the development of type 2 diabetes mellitus and complications associated with the disease such as micro- and macro-vascular diseases has been proposed as independent risk factor [32]. Therefore, control of postprandial hyperglycemia is suggested to be important in the treatment of diabetes and prevention of cardiovascular complications [19]. Inhibiting glucose uptake in the intestines may help diabetic patients to control the blood glucose level in

the postprandial state hence substances that inhibit amylase have been studied and some have been developed as drugs to treat diabetes mellitus [16].

Therefore, our extract can be considered a new natural source possessing properties for the fight against type 2 diabetes. To show equal preference for α-amylase enzyme it is always necessary to do the corresponding in vivo activity. Thus proof of concept needs to be demonstrated in preclinical animal studies and it was essential to confirm the in vivo experiments action following oral administration to live animals.

Effect of HSSP on pancreas β-cells architecture and α-amylase activity in serum, pancreas, intestine and blood glucose level

The pancreas is an important organ for blood sugar regulation. Research has shown that insulin resistance is closely associated with an impaired pancreatic β-cells function [9]. As reported in Fig. 1, administration of alloxan to rats provoked a massive damage and death of pancreas β cells. Consequently, a significant increase in the α-amylase activity by 57.26%, 114.15%, and 132.1% in the serum, mucosal small intestine and the pancreas, were observed respectively in the untreated diabetic rats, which leads to an increase in the glucose rate by 240.17% as compared to untreated diabetic rats (Fig. 2). However, the administration of HSSP corrects partially the damage in the β cells, which leads to a decrease in α-amylase activity by 38.79%, 45.43% and 58.07% in the serum, small intestine and pancreas, respectively. Consequently reduction of blood glucose rate by 67.97%.

Alloxan produces free radicals in the body in which it causes pancreatic damage by preventing insulin secretion which induces a significant increase in serum glucose concentration of rats. This hyperglycemia is due to disorders in the use of glucose by tissues or to the increase of gluconeogenesis.

These results performed by OGTT in conscious fasted rats after HSSP administration. In fact, these results clearly showed that acute oral administration of HSSP to surviving diabetic rats reduced significantly the peak of blood glucose concentration 60 min after glucose administration as compared to untreated diabetic rats (Fig. 3).

This which clearly shows a very active use of glucose by peripheral tissues, explained by an increase in glucose tolerance in these tissues when animals are treated with some marine algae.

Effect of HSSP on liver function and antioxidant capacity on diabetic rats

This study showed that hyperglycemia provoked liver toxicity and stress; evidenced by a significant decrease in the SOD, CAT and GPX activities in liver tissues of diabetic rats (Fig. 4). Moreover, the liver toxicity and dysfunction were showed by the increase in blood liver indices such as AST, ALT and LDH in diabetic rats and confirmed by the apparition of fatty cysts in the hepatic tissues (Fig. 5).

In diabetic rats treated by HSSP, a significant protective action was showed. In fact, the administration of HSSP increases the antioxidant capacity in the liver tissues (increase in SOD, CAT and GPX activities by

240.25%, 299.42% and 120%, respectively and decrease in TBARS rate by 46.39% as compared to untreated diabetic rats). This increase in the antioxidant capacity prevents liver toxicity showed the decrease in the activity of AST, ALT and LDH in the blood and was confirmed by histological observation (Fig. 5).

This antihyperglycemic action is due to sulfated polysaccharides isolated from marine algae that are known to be important antioxidants for the prevention of pancreatic oxidative damage, which is an important contributor in diabetes mellitus [15].

It is believed that oxidative stress plays an important role in chronic complications of diabetes. Therefore, alleviation of oxidative stress is essential for preventing or reversing diabetic complications [20] and by a compound with antioxidant activity combined with inhibitory activities against α-glucosidase and α-amylase should be a more effective anti-diabetic agent.

In this study, indeed to the hypoglycemic action, HSSP stimulates the activity of CAT, SOD, and GPx, reduces the lipid peroxidation and suggests a compensatory response to oxidative stress as it reduces the endogenous H_2O_2 production thus diminishing the toxic effects due to this radical or other free radicals derived from secondary reactions [4-21]. This antioxidant action of HSSP on diabetic rats can prevents liver toxicity and damage showed by the decrease in the liver cells indices toxicity such as AST, ALT and LDH and also prevents the accumulation of lipid in liver tissues, evidenced by histological analysis.

Effect of HSSP on kidney function on diabetic rats

Results of this study revealed that hyperglycemia induced kidney toxicity, evidenced by the increase in albumin, creatinine and urea in blood by 19.94%, 35.36% and 111.57%, respectively as compared to untreated diabetic rats (Table 2). This can be explained by the accelerated degradation of hepatic and plasma proteins [15] or the degradation of somebody protein compounds due to the administration of alloxan or dietary compounds that can be degraded into amino acids and then into urea.

Our study showed that chronic hyperglycemia induces renal toxicity. The concentration of albumin, urea and creatinine is often considered as a clinical parameter to detect the toxic effects related to the treatment of some compounds on the kidneys in experimental animals [6]. Also hyperglycemia induced stress oxidant and kidney cells damage showed by a decrease in the SOD, CAT and GPX activities by 43.65%, 44.51% and 90.62%, respectively and an increase in TBARS rate by 108.54% (Fig. 6). This toxic effect of diabetes on kidney was confirmed by histological analysis (a capsular space shrinkage and glomerular hypertrophy). However, the administration of HSSP normalized all these perturbations (Fig. 7).

Effect of HSSP on lipid profile on diabetic rats

Results of this study revealed that hyperglycemia associated with increase in the blood TC, TG, LDL-C and decreased in HDL-C concentration was in accordance with previous study [15]. However, treatment

with HSSP normalized all the lipid profile parameters.

The data in the table 2 showed that the supplement of HSSP normalizes lipid profile. In fact, the administration to HSSP to surviving diabetic rats decreases LDL-C and TG by 33% and 26%, respectively and increases HDL-C by 45% as compared to untreated diabetic rats.

The HSSP leads to a significant improvement in the lipid profile similar to that observed in diabetic rats treated with glucor. The increase in the level of HDL-C known as good cholesterol [27] is due mainly to their beneficial effect on cardiovascular complications, mainly atherosclerosis [10]. This antihyperlipidemic activity attributed to sulfated polysaccharides, major constituent of extract as sulfated polysaccharides enhance the negative charges of cell surface so as to affect the aggradation of cholesterol in blood, thus decreasing the cholesterol in serum [7].

Spectroscopic analysis

FT-IR spectrometric analysis

The characteristic absorption of HSSP was identified by the FT-IR spectra (Fig. 8). FTIR spectrum elucidates the structural information of polymers and determines their major functional groups. The infrared spectrum of HSSP displayed a broad stretching intense characteristic peak at 3315 cm^{-1} for the hydroxyl group [22]. A weak band at 2967 cm^{-1} was attributed to the C-H stretching and bending vibration. The absorption band centered at 1725- 1641 cm^{-1} were caused by C=O asymmetric stretching vibration. The absorption at 1446 cm^{-1} was assigned to C–OH deformation vibration. The well defined peak at 1278 cm^{-1} attributed to the presence of sulfate ester groups (S=O) [34] and the sharp band at 753 cm^{-1} (C-S-O) suggested that the majority of sulphate groups occupy position 2 and/ or 3 (equatorial position) [13]. Each particular polysaccharide has a specific band in the 1193- 1137 cm^{-1} region; this region was dominated by ring vibrations overlapped with stretching vibration of pyranosyl ring (C-O). A band at 1085 cm^{-1} of HSSP was found and corresponded to C-O-H deformation vibration. The relatively strong absorption peak at 924 cm^{-1} of HSSP reflected the absorption of the furan ring.

The X-ray diffraction determination

The X-ray diffraction was used to determine the structure and degree of crystallinity of the

HSSP. The diffracted intensities were recorded from 10° to 80° at 2 theta angles (Fig. 9). The results of X-ray diffractograms of HSSP suggest that HSSP was a semi-crystalline polymer with major crystalline reflection at 31.3°. Similar results were obtained by Maud Lemoine and William Helber [17] which reported that the sulfated polysaccharide was extracted from red algal cell wall as a dense network of semi-crystalline fibers. This structural arrangement is known to directly affect various properties, including tensile strength, flexibility, solubility, swelling or opaqueness of the bulk polymer. In fact, physical properties are dependent on the degree of order within the material [25].

Monosaccharide composition of HSSP

Sugar composition of HSSP has been carried out preliminarily by TLC analysis (Fig. 10). Indeed, after acid hydrolysis of HSSP, the retention time of acid-hydrolyzed HSSP sample was exactly the same as the monosaccharides such as glucose, arabinose, xylose, and galactose in the TLC analysis. A total hydrolysis of HSSP liberated glucose, arabinose, xylose, and galactose as a final hydrolysis product since the R_f value of acid-hydrolyzed HSSP was identical to that of glucose, arabinose, xylose, and galactose under our solvent ascending condition. To confirm the results obtained by CCM we applied a more efficient analytical technique to the study of the chemical composition, GC-MS. As a result, the chromatogram obtained makes it possible to determine the monosaccharide composition of the HSSP by reductive hydrolysis method (GC-MS) (Fig. 11).

The GC–MS analysis of the crude polysaccharide fractions revealed the presence of different carbohydrate moieties in varied proportions (Table 3). The acid hydrolysis of HSSP showed that the ribose was the most abundant sugar (14.76%), followed by mannose, lyxose, glucose, xylose, talose, galactopyranose and arabinose (11.12%, 8.88%, 8.64%, 7.68%, 6.84%, 6.08% and 5.91% respectively). It has been reported that glucose, xylose, arabinose, galactose and mannose were associated with antioxidant activities [24]. In this study, HSSP was assumed to be responsible for the antioxidant activity; hence it was used for further experiments. In addition, the monosaccharides composition of sulfated polysaccharide isolated from brown seaweed *Lobophora variegata* showed that it is a polysaccharide composed of galactose, fructose, and xylose shows 36.8%, 29.2% and 0.1%, respectively [24]. Another study had shown a sulfated galactofucan with high-level galactose (22%) and fructose of brown seaweed *Adenocys tisutricularis* was obtained [35]. However, galactofucans often contain xylose in the monosaccharide composition from minor to significant. Cole *et al*. [8] describe that sulfate groups of polysaccharides from brown seaweed have action on several signaling events because its S-domains can interact with a number of growth factors, chemokines and their receptors.

These results confirm the biological activities of sulfated polysaccharide that would be related to the characterization of monosaccharides such as mannose, lyxose, glucose, xylose, talose, galactopyranose and arabinose contents in our HSSP sample.

Conclusion

For the first time our results demonstrate that HSSP exhibited promising therapeutic effects on postprandial hyperglycemia. Thus, this study suggest that HSSP treatment exerts beneficial effects on glucose metabolism and improves oxidative stress in alloxan-induced diabetic rats; and thus it might play an important role in the prevention from the oxidative stress related complications. Further studies are needed to investigate the role of HSSP in the treatment of diabetes mellitus that include human subjects. Accordingly, HSSP can exploit new pathways for the treatment and prevention of diabetes and related disease and can be considered as potential therapeutic agents for the treatment of diabetes. Additionally,

this study provides the possible pharmacologic rationale to the medicinal use of HSSP in the development of andiabetic drugs.

Declarations

Author contribution Amel Ben Gara, Nadia Hammami, Rim Chaaben, Abdelfattah El Feki, Francesco Paolo Pattie, Karima Belghith and Imen Dahech conceived and designed the experiments. Amel Ben Gara, Nadia Hammami and Rim Chaaben performed the experiments. Amel Ben Gara, Abdelfattah El Feki, Francesco Paolo Pattie and Karima Belghith analyzed the data. Amel Ben Gara wrote the manuscript. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Tables

Table 1-3 are available in the Supplemental Files section.

Figures

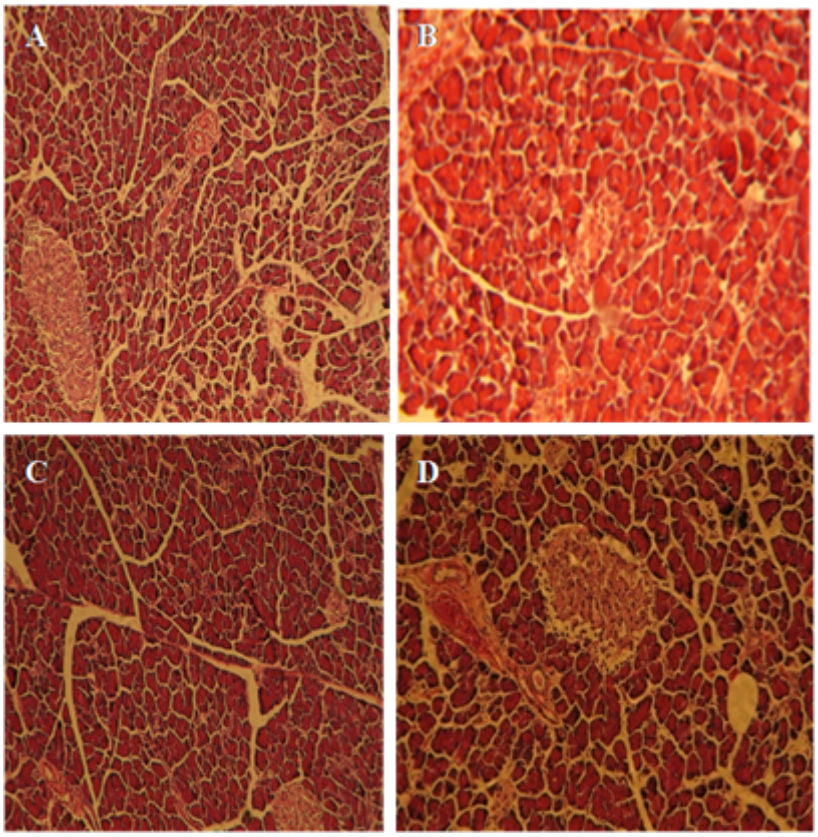

Figure 1

Histological study of pancreas from (A) normal control rats showing normal architecture ; (B) alloxan-diabetic rats, massive destruction of β-cells ; (C) alloxan-diabetic rats, treated with glucor ; (D) alloxan-diabetic rats treated with HSSP, a partial protector effect of β-cells from pancreas was observed (H&E 100×).

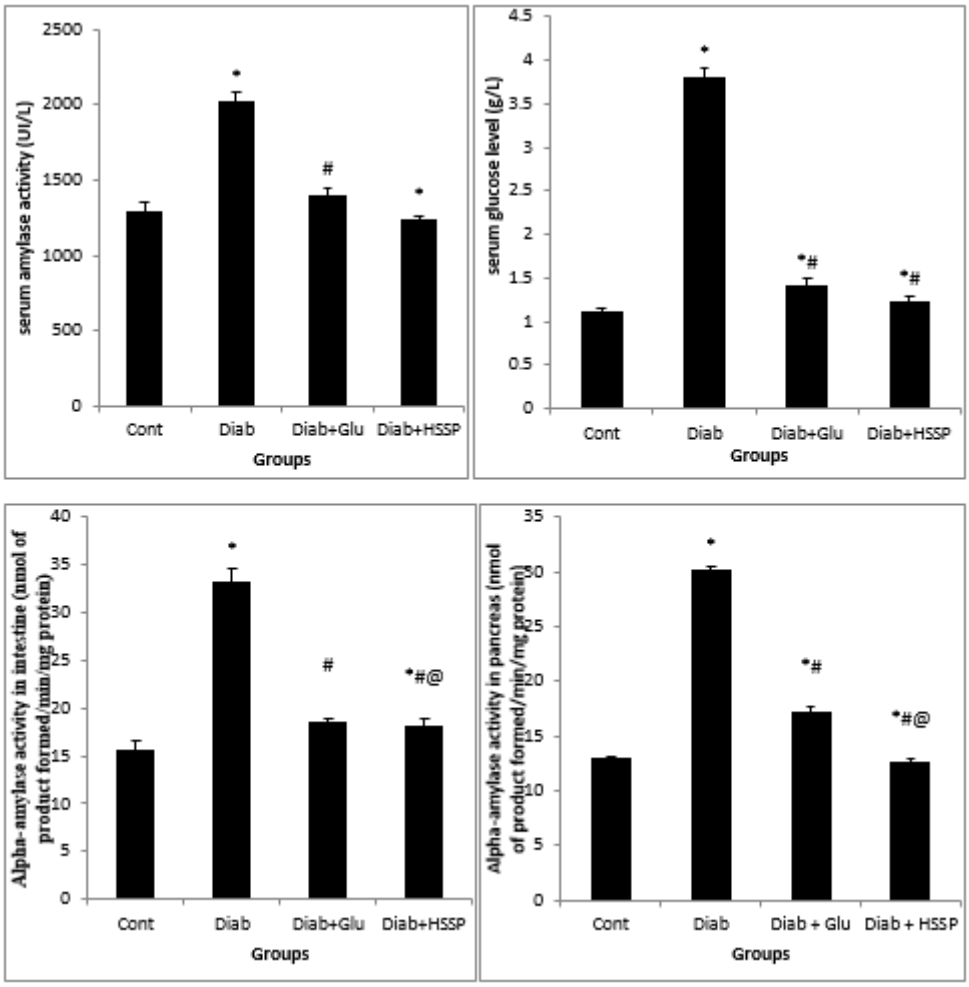

Figure 2

Inhibitory effect of HSSP on α-amylase activity in serum (a),) intestine (b), and pancreas (c), and blood glucose rate (d) on surviving diabetic rats. Values are given as mean ± SD for 6 rats in each group. Values are statistically presented as follows: $^{*}P < 0.05$ significant differences compared to controls. $^{\#}P < 0.05$ significant differences compared to diabetic rats. $^{@}P<0.05$ significant differences compared to diabetic rats treated with glucor.

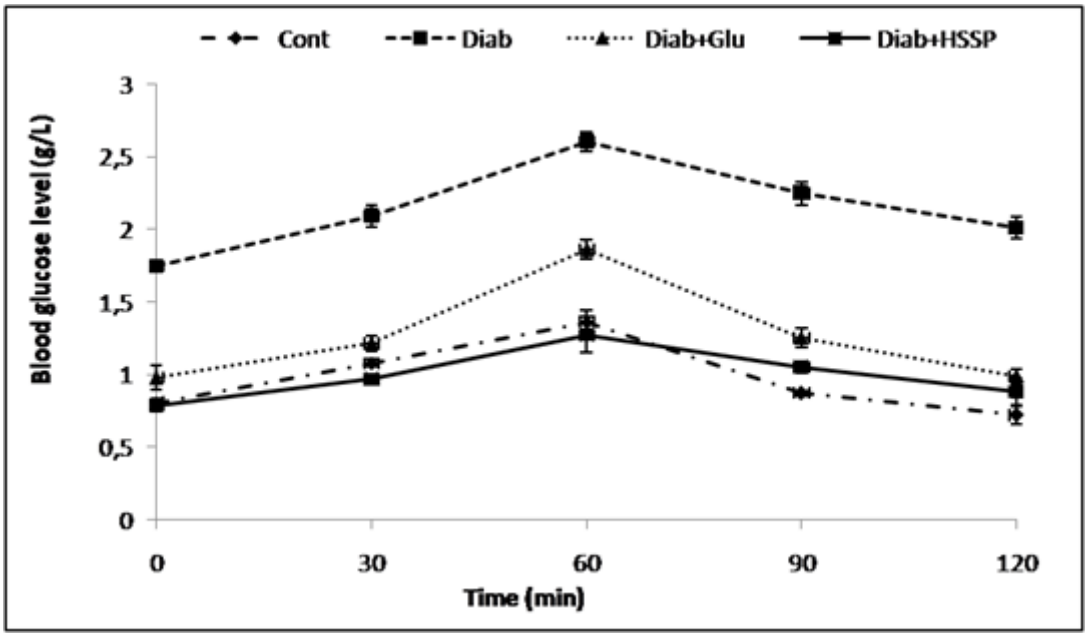

Figure 3

Oral glucose tolerance test on control and experimental groups of rats. . Glucose levels obtained from Cont: normal rats; Diab: alloxan-diabetic rats; Diab + Glu: alloxan-diabetic rats treated with glucor medicament; Diab + HSSP: alloxan-diabetic rats treated with HSSP. Data represent mean±SD (n = 6 for each group). Values differ significantly at $P < 0.05$.

Result of this study revealed that administration of HSSP reduced significantly peak glucose the same as ameliorated insulin sensibility in surviving diabetic rats.

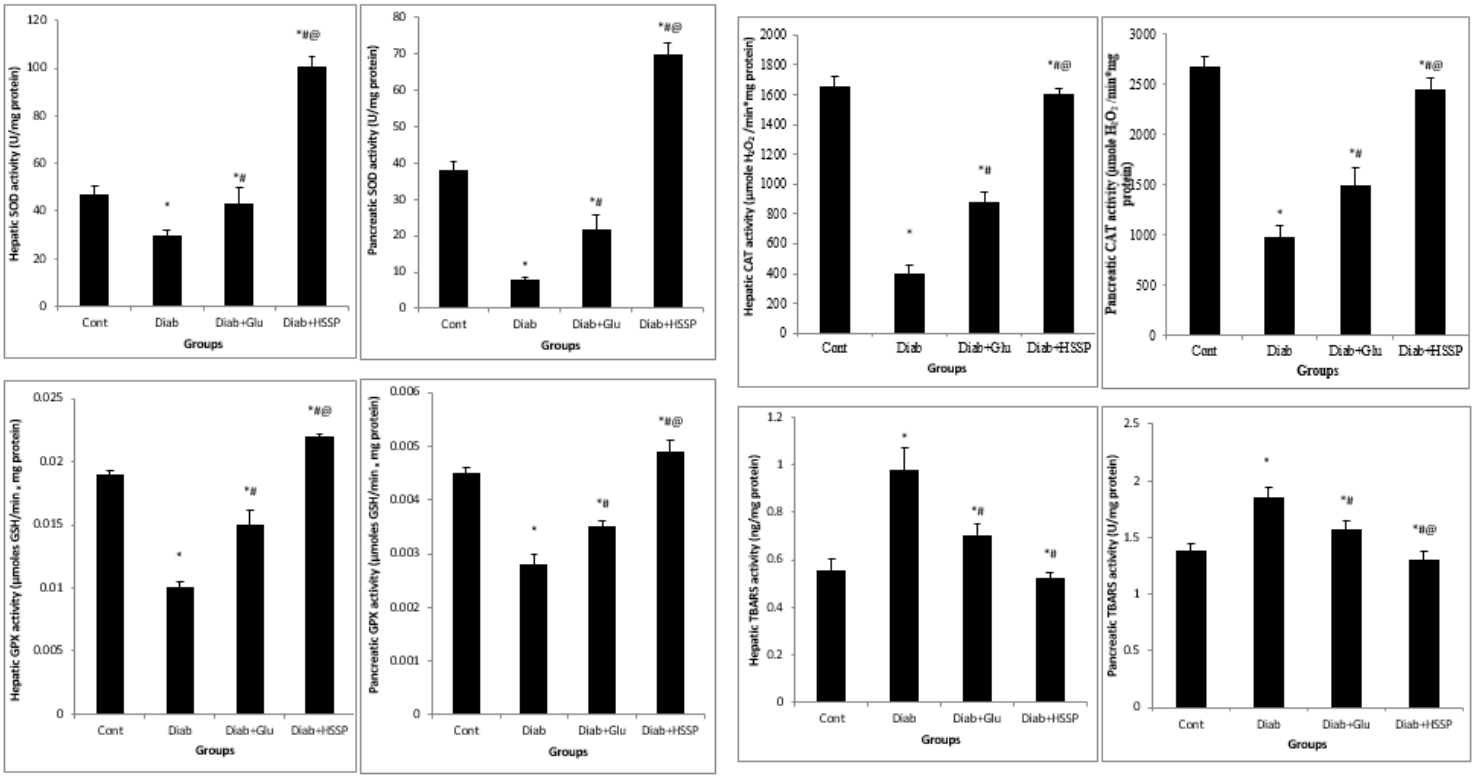

Figure 4

Activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and TBARS in liver and pancreas of controls, diabetic rats, and treated diabetic rats. Cont: normal rats; Diab: alloxan-diabetic rats; Diab+Glu: alloxan-diabetic rats given glucor; Diab+HSSP: alloxan-diabetic rats given extract of HSSP. Data represent mean±SD (n=6 for each group). Values differ significantly at $p<0.05$. $^{*}p<0.05$ compared with normal control rats. $^{\#}p<0.05$ compared with diabetic rats. $^{@}p<0.05$ compared with diabetic rats received glucor.

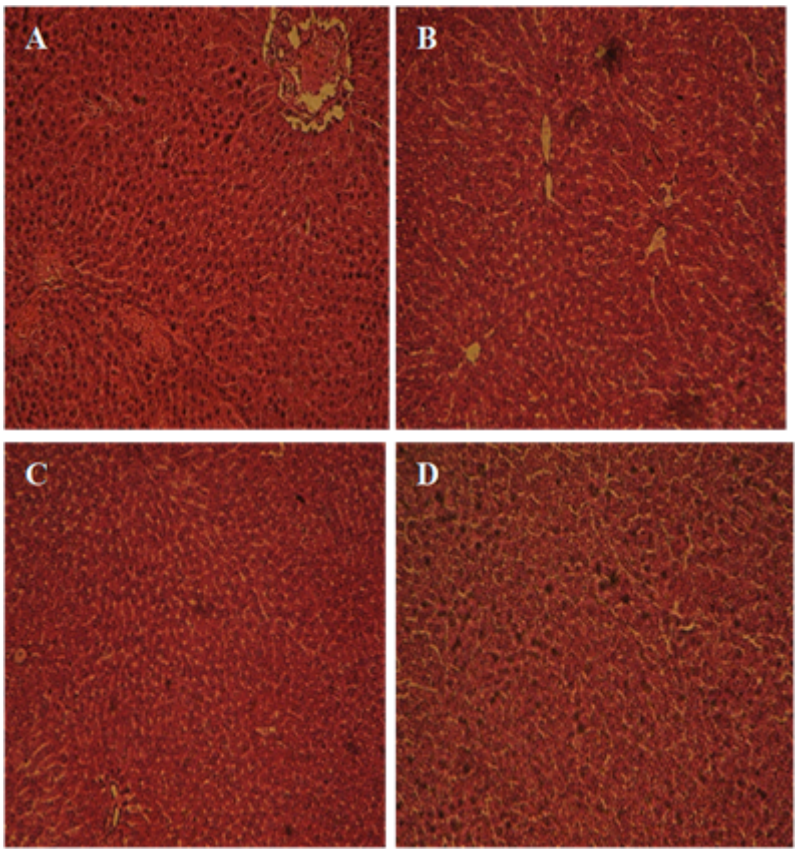

Figure 5

Histological comparison of liver from (A) normal control rats showing normal architecture ; (B) alloxan-diabetic rats, appearance of lipid accumulation in liver cells ; (D) alloxan-diabetic rats treated with glucor, and (C) alloxan-diabetic rats treated with HSSP, a decrease in the abundance of lipid accumulation in liver cells was showed (H&E 100×).

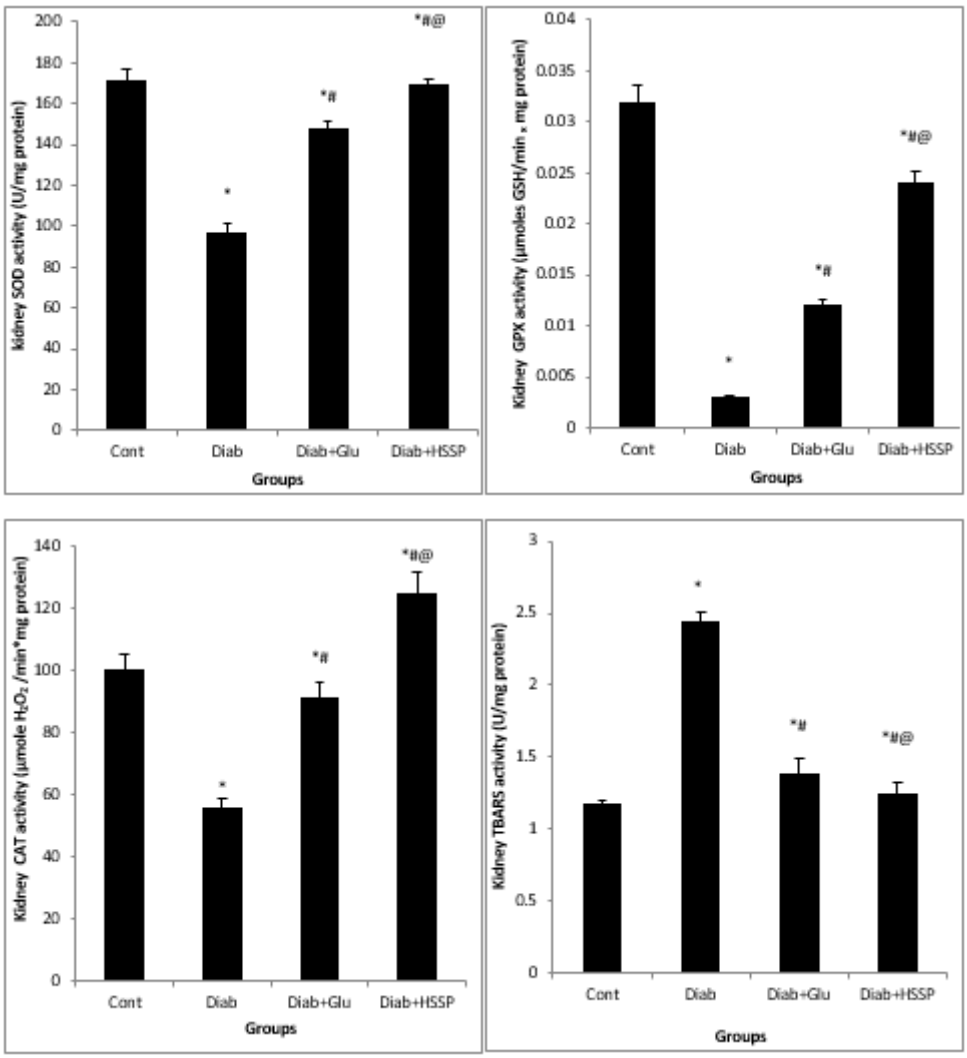

Figure 6

Activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and TBARS in kidney of controls, diabetic rats, and treated diabetic rats. Cont: normal rats; Diab: alloxan-diabetic rats; Diab+Glu: alloxan-diabetic rats given glucor; Diab+HSSP: alloxan diabetic rats given extract of HSSP. Data represent mean±SD (n=6 for each group). Values differ significantly at $p<0.05$. $^{*}p<0.05$ compared with normal control rats. $^{\#}p<0.05$ compared with diabetic rats. $^{@}p<0.05$ compared with diabetic rats received glucor.

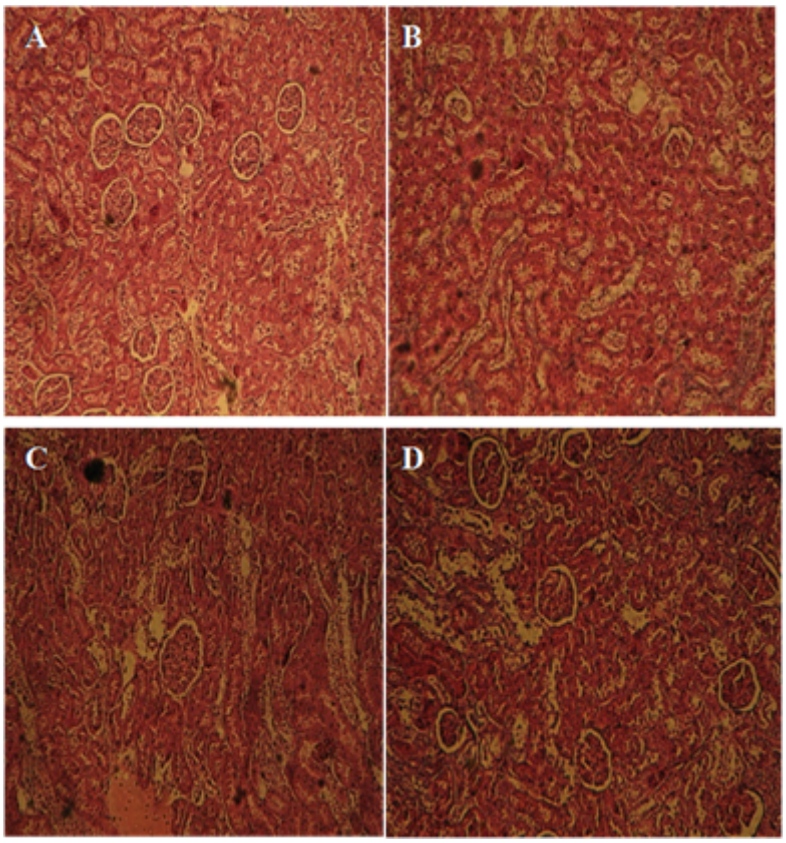

Figure 7

Histological comparison of kidneys from (A) normal control rats showing normal architecture; (B) alloxan-diabetic rats showed Bowman's space size and atrophy of glomerulus ; (C) alloxan-diabetic rats treated with glucor and (D) alloxan-diabetic rats treated with HSSP, a protector effect was showed (H&E 100×).

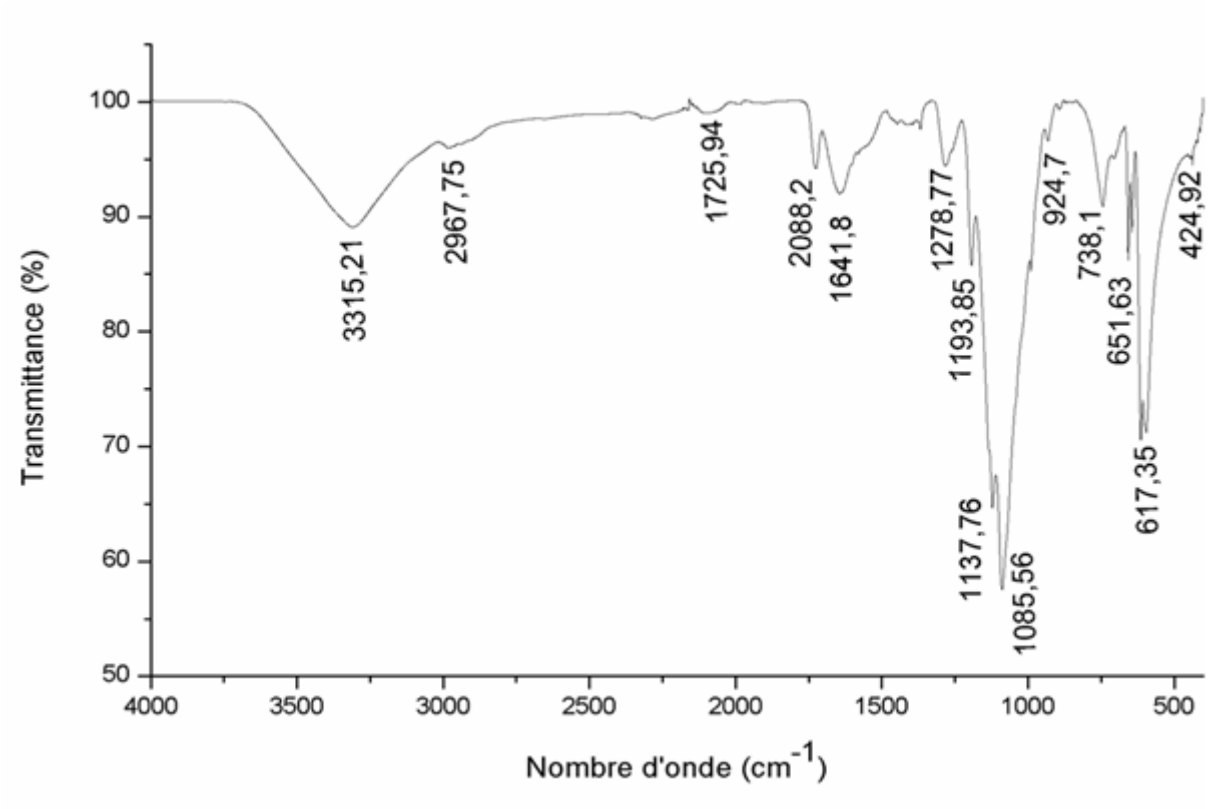

Figure 8

FT-IR spectra of *Hypnea spinella* sulfated polysaccharide.

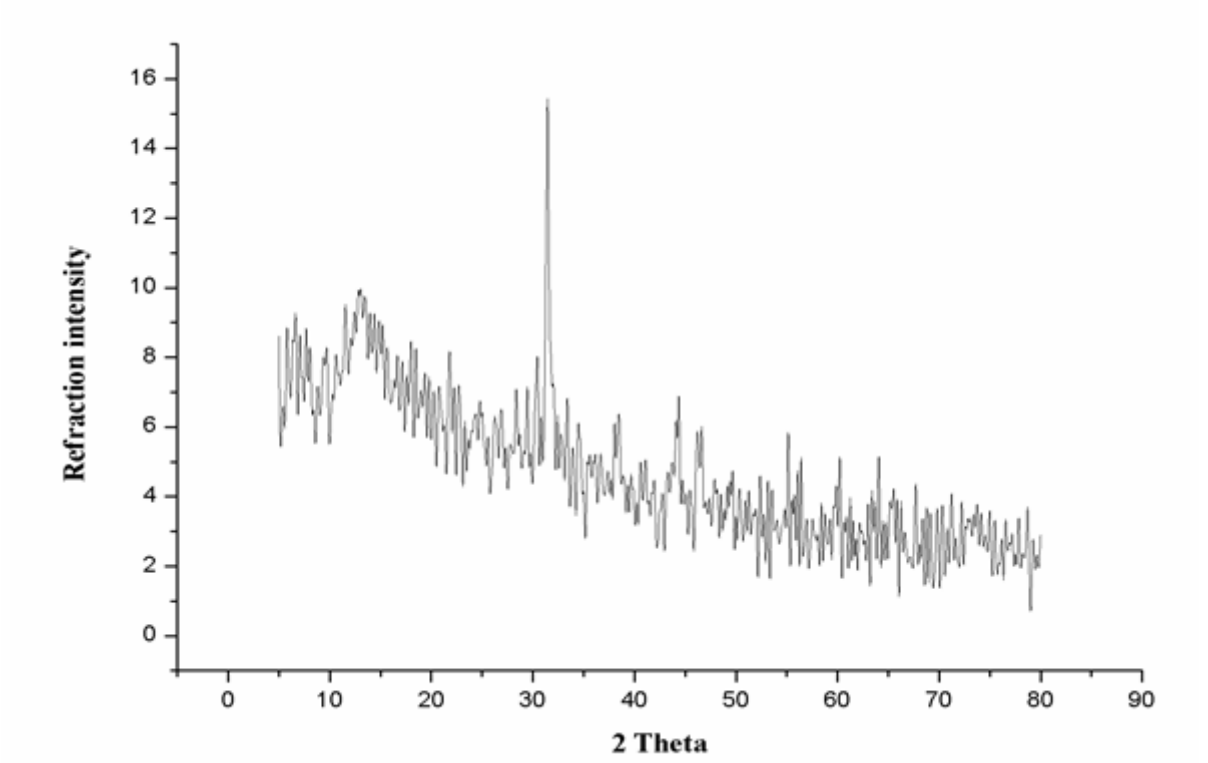

Figure 9

X-ray diffraction pattern of sulfated polysaccharide from *Hypnea spinella*.

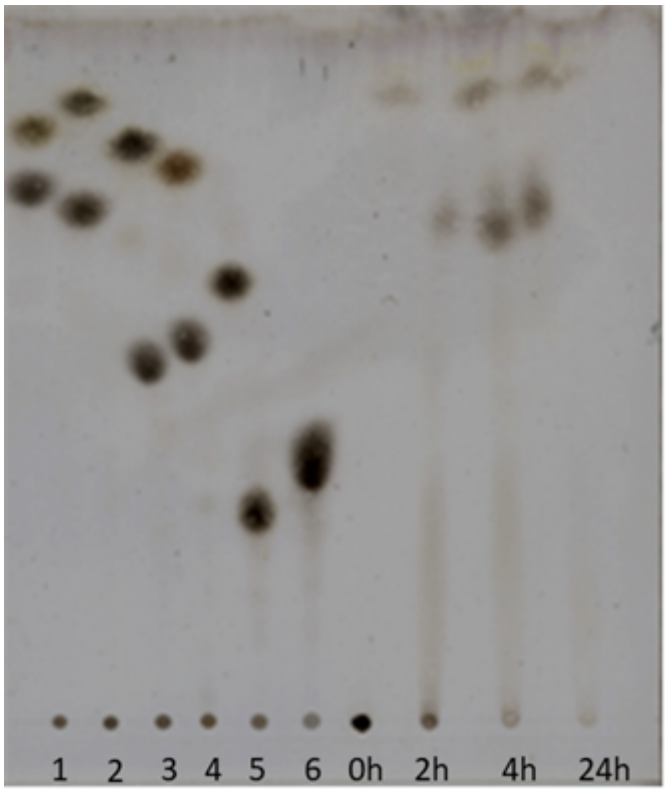

Figure 10

TLC showing the process of partial and total HSSP hydrolysis after the addition of trifluoroacetic acid. Standard sugars: 1, arabinose+glucose; 2, xylose+galactose; 3, fructose+cellubiose; 4, mannose+maltose; 5, sucrose+raffinose; 6, lactose. 0 h: partial HSSP hydrolysis; 2 h: HSSP hydrolysis after 2 h; 6 h: HSSP hydrolysis after 6 h; 24 h: total HSSP hydrolysis after 24 h.

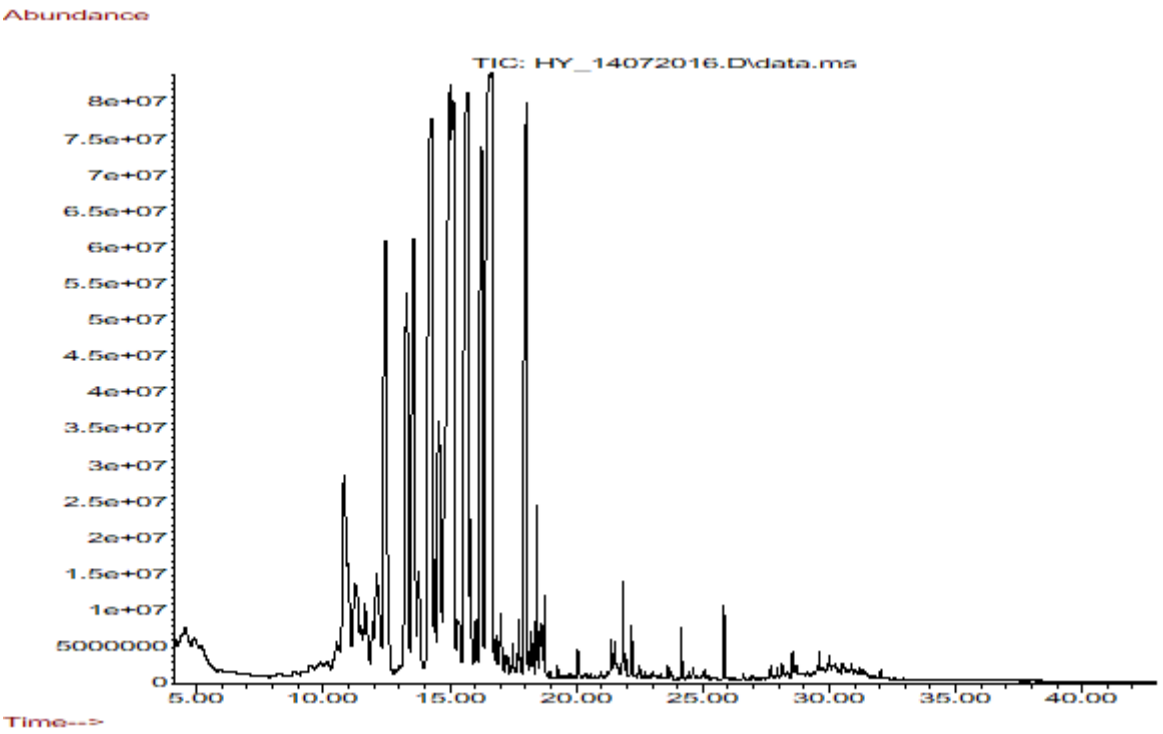

Figure 11

GC-MS of *Hypnea spinella* sulfated polysaccharide hydrolysate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.docx