Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.5.2017J6-278

Sulphated galactopyran derived from *Gracilaria opuntia,* a marine macroalgae restores the antioxidant metabolic enzymes during STZ induced diabetic rats

Lavanya Rayapu[1], Fasina Makkar[2], Anusree Maneesh[2], Kajal Chakraborty[2], Lokanatha Valluru[1*]

[1]*Department of Biotechnology, Dravidian University, Kuppam-517426, Andhra Pradesh, India*

[2]*Marine Biotechnology Division, Central Marine Fisheries Research Institute, Cochin, Kerala, India*

ARTICLE INFO

Article history:
Received 2 Dec 2016
Received in revised form 14 Dec 2016
Accepted 22 Dec 2016
Available online 28 Dec 2016

Keywords:
Marine macroalgae
Oxidative stress
Antioxidants
Streptozotocin
Diabetes

ABSTRACT

Objective: To screen the effect of sulphated galactopyran fraction isolated from *Gracilaria opuntia* (*G. opuntia*) (FM4) in streptozotocin (STZ) induced diabetic rats.
Methods: *In vitro* antioxidant assays of FM4 were estimated by DPPH, ABTS, hydroxyl free radical and Nitric oxide free radical activities. FM4 was purified and characterized by 1H-NMR spectra and FTIR as sulphated galactopyran. Diabetes was induced intraperitonially by single dose of STZ (55 mg/kg body weight). FM4 was administrated orally (80, 100, 125 mg/kg BW) to diabetic rats for 60 days. The enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione-S-transferase (GST), lipid peroxidase (LPx), glutathione reduced (GSH), vitamin-C (VIT-C) and vitamin-E (VIT-E) levels were estimated. Glibenclamide was used as standard drug.
Results: Our results demonstrated that the aqueous extract of *G. opuntia* possess free radical scavenging activity. During FM4 fraction treatment (100 mg/kg BW), the SOD, GPx, CAT, GST, GSH, VIT-C and VIT-E levels were significantly ($P < 0.05$) increased, and the LPx levels were decreased in different organs such as liver, kidney, brain and pancreas of diabetic rats.
Conclusions: The sulphated galactopyran fraction of the marine macroalgae (*G. opuntia*) possesses the antioxidant activity which might help in the prevention of oxidative damage that occurs during diabetes.

1. Introduction

Diabetes mellitus (DM) is a prevalent endocrine and metabolic disorder in the world in which carbohydrates, proteins and lipid metabolisms are erroneously regulated due to insulin hormone deficiency, which results in hyperglycemia[1,2]. Hyperglycemia leads to produce the reactive oxygen species by increased glycolysis, decreased antioxidant defences, auto oxidation of glucose, non enzymatic protein glycosylation and polyol pathway disruption[3].

Oxidative stress plays a vital role in cellular redox potential. The free radicals attack on fats, proteins, sugar, DNA *etc*., that affect the consecutive oxidation. Antioxidants play an important role in neutralizing the free radicals before they attach carbohydrates, lipids and proteins of the cell[4]. The antioxidant disfunctioning leads to several disorders such as cardiovascular diseases, rheumatoid arthritis, immune disorders including DM[5]. Insulin resistance leads to hyperglycemia that causes oxidative stress in which the β-cells were destructed and causes type-2 diabetes[6,7]. The destruction of pancreatic β-cells changes the antioxidant enzymes in various diabetic organs[8].

Although there are many anti-hyperglycemic drugs such as insulin and oral drugs (thiazolidinediones, biguanides and sulphonylureas) available in the market, they also produce many side effects[9]. In recent times, the food scientists, nutritional specialists and public have gained a great interest in natural antioxidants to reduce the chronic diabetic risk factors and to promote good health[10,11]. Over the decades several studies reveled that, marine algae have possessed high biological and potential medicinal importance. Red algae are rich source of different bioactive compounds such as polyphenols, carotenoids and oligosaccharides with various physiological benefits for human health[12]. *Gracilaria opuntia* (*G. opuntia*) is one of the members of Rodophyta belongs to the class Gracilariales. This Gracilariales group has more than 300 species in which 160 species are taxonomically accepted[13,14]. The *Gracilaria* species have carbohydrates, phycocolloids, agar α-(1,4)-3,6-anhydro-l-galactose and β-(1,3)-d-galactose with slight esterification in cell wall[15]. Along with these other polysaccharides are present in some of the species[16,17].

Reports regarding the anti-diabetic effect and clinical significance of red algae in diabetic rats are scanty in scientific

*Corresponding author: Dr. Lokanatha Valluru, Assistant Professor, Department of Biotechnology, Dravidian University, Kuppam-517425, Andhra Pradesh, India.
Tel: +91 9652840923 (L Valluru)
E-mail: lavanyarayapu05@gmail.com (L Rayapu); lokanath.valluru@gmail.com (L Valluru)

The journal implements double-blind peer review practiced by specially invited international editorial board members.

literature. Therefore, our study was aimed to screen the effect of sulphated galactopyran fraction isolated from *G. opuntia* (FM4) in streptozotocin (STZ) induced diabetic rats.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ), hydrogen peroxide, thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA), glutathione (GSH), trichloro acetic acid (TCA), NADPH, epinephrine, ascorbic acid, triton X-100, reduced glutathione (GR), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), nitro blue tetrazolium chloride (NBT), tetra butyl hydroperoxide were procured from Himedia (Bangalore, India) and Sigma Aldrich (St. Louis, Missouri, USA).

2.2. In vitro antioxidant assays

In the present study, *in vitro* antioxidant activity of red marine macroalgae *G. opuntia* (FM4) extracts were measured by DPPH assay[18], ABTS assay[19], hydroxyl radical scavenging assay[20] and nitric oxide scavenging assay[21].

2.3. Maintenance of animals

The study was carried out on male albino wister rats (*Rattus norvigicus*) weighing (190 ± 10) g, were selected as experimental animals, which were housed in isolated cages. The animals were purchased at Sri Venkateswara Enterprises Pvt. Ltd. (Bangalore, Karnataka, India) and maintained at room temperature (23 ± 2) ℃, humidity 45%–55% with 12 h light-dark cycle and allowed to free access of food and water. The study was approved by IAEC, PES Medical College, Kuppam, India (Vide No. PESIMSR/Pharma/IAEC/20/2014-2015/date 08.12.14).

2.4. Preparation of sulphated galactopyran fraction of G. opuntia (FM4)

The *G. opuntia* aqueous extract was prepared by extracting the dried seaweed powder (500 g) with hot water (2 L) at 80 ℃ for 3 h. The contents were cooled and solid residues were removed by centrifugation and it was lyophilized to get the crude extract (25 g). The aqueous extract of *G. opuntia* was concentrated in vacuum before being precipitated with alkanol (500 mL). The precipitate was lyophilized to get a dried oligosaccharide fraction of *G. opuntia* (FM4; ~□70%).

The precipitated oligosaccharide conjugate of *G. opuntia* was purified by anion exchange column chromatography using DEAE (diethylamineoethyl)-cellulose anion exchange resin. A glass column (25 × 4 cm) with a mesh sieve was mounted vertically on a stand and rinsed with water. DEAE-cellulose (3 g) was made into slurry with minimum amount of water and loaded into the column. The polysaccharide fraction (1 g) was dissolved in 2–3 mL of water to make a suspension and loaded into the previously packed column. The column was initially eluted with water to get the first fraction. This was followed by step-wise elusion with 0.1, 0.3, 0.5 and 1 M sodium chloride (NaCl) gradient until the absence of positive reaction of the phenol-sulphuric acid assay[22] in the test tubes containing eluted fractions. The eluted fractions were lyophilized to get the purified oligosaccharide fractions. The oligosaccharide enriched fraction was referred as sulphated galactopyran.

2.5. Preparation of STZ

55 mg of STZ was dissolved in 100 mM cold sodium citrate buffer (pH 4.5)[23].

2.6. Induction of hyperglycemia and treatment

After acclimatization of the rats to the lab conditions the treatment was started. By single intraperitonial (IP) administration of streptozotocin (STZ) (55 mg/kg BW) diabetes was induced in rats. Control rats received the buffer as vehicle alone. After inducing diabetes 10% glucose water was given in the night to rescue the rats from rapid hypoglycemic period that takes place instantly after the lysis of the pancreatic islets cells by STZ. After 48 h of development of diabetes in the rat's, glucose levels were checked in fasting by using accu-check meter (Roche Group, Mannheim, Germany) developed from the basic method of glucose oxidase[24] and HbA1c method[25]. Glucose levels more than 200 mg/dL and HbA1c levels more than 6.3% were measured as diabetic rats and used for experiment[23,25].

2.7. Experimental design

The treatment of sulphated galactopyran fraction of *G. opuntia* (FM4) was initiated after inducing diabetes with STZ in the rats. The animals were separated into 7 groups with 6 rats in each group. The experimental design is as follows:

Group 1: Control, Group 2: FM4 alone treated, Group 3: STZ, Group 4: STZ + Glb, Group 5: STZ + 80 mg/kg BW, Group 6: STZ + 100 mg/kg BW, Group 7: STZ + 125 mg/kg BW.

2.8. Sample collection for analysis

The blood samples were collected weekly once from the retro-orbital vein and used for the estimation of blood glucose levels by Accu-check method (Roche Group, Mannheim, Germany) developed from the basic method of glucose oxidase method[24] and estimation of glycosylated hemoglobin by HbA1c method[25].

2.9. Isolation of tissues

After treatment, rats were sacrificed by cervical decapitation and liver, kidney, brain and pancreas tissues were collected and rinsed of any adhering blood. The tissues fragments were homogenated in phosphate buffers saline for further studies. The unused tissues were stored at –40 ℃ refrigerator for further analysis.

2.10. Estimation of enzymatic and non-enzymatic antioxidant parameters

In the present study, in vivo antioxidant enzyme levels during red marine macroalgae *G. opuntia* (FM4) extracts treatment were analyzed in four organs such as liver, kidney, brain and pancreas. These tissues were used for estimation of enzymatic and non-enzymatic antioxidants such as Superoxide dismutase[26], catalase[27], glutathione peroxidase[28], glutathione-S-transferase[29], lipid peroxidation[30], vitamin-C[31], vitamin-E[32] and glutathione reduced[33] were estimated.

2.11. Statistical analysis

The obtained data was analyzed by using the statistical package of social sciences (SPSS, 16.0 version). Comparison between the control and experimental animals results were done by One-way ANOVA followed by Tukey's multiple comparison test. When $P < 0.01$, considered as significant difference. All the values were expressed as mean ± SD ($n = 6$).

3. Results

In the present study the *in vitro* antioxidant properties of *G. opuntia* were studied by using different assays. Table 1 shows the DPPH, ABTS, hydroxyl free radical and Nitric oxide scavenging activity at different concentrations (50–250 µg/mL). Our results demonstrates that the aqueous extract of *G. opuntia* possess free radical scavenging activity.

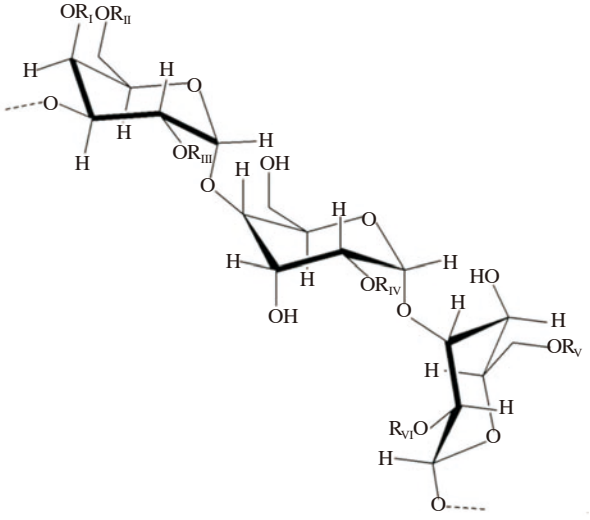

Figure 1. Proposed structure of sulphated galactopyran motif of *G. opuntia*.

The Oligosaccharide fraction of FM4 from the red marine macroalgae *G. opuntia* (FM4) was isolated by column chromatographic method and followed by step-wise elusion with sodium chloride gradient until the dearth of a positive reaction of the phenol-sulphuric acid assay[22]. The eluted fractions were lyophilized to get the purified oligosaccharide fractions. The carbohydrate enriched fraction referred as sulphated polygalactan. The structure of the sulphated polygalactan was elucidated by the extensive analysis of ^{1}H-NMR spectra. The polysaccharide fraction has resolved signals of anomeric protons (δ 4.4–5.5), methylene and methane hydrogens (δ 3.6–4.9) of the sulphated polygalactan moiety in ^{1}H-NMR spectra. Characteristic signal at δ 3.4 has revealed the region of additional number of alkoxy (-OCH_3) replacements in the sulfated polygalactans. In addition to the characteristic peaks for sulphated polygalactan units, xylose and anhydrogalactose units were in minor constituents in the fraction, which were often found in red seaweed polysaccharides. The presence of positively and negatively charged ions of sulphate groups in the sulphated galactan moiety has sustained the structure. The FT-IR spectra demonstrated a strong absorption band at 1 210-1 260 cm^{-1} corresponds to S=O groups (sulphate ester groups). Characteristic bands for –OH groups at 3 200–3 400 cm^{-1} has substantiated the structure of the sulphated polygalactan. The sulphated galactopyran motif of *G. opuntia* was designed based on the detailed NMR experiments (Figure 1).

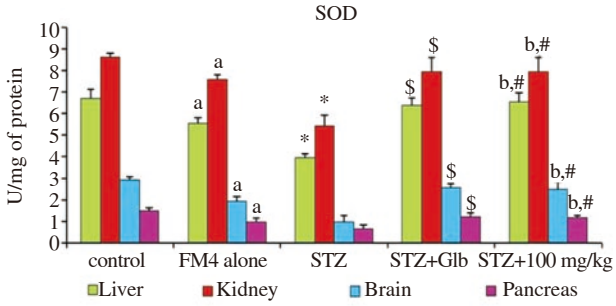

Figure 2. Analysis of sulphated galactopyran fraction of *G. opuntia* on SOD activity in liver, kidney, brain and pancreatic tissues of STZ induced diabetic rats.

The SOD levels were decreased in STZ-induced diabetic rats and the enzyme activity was significantly recovered by FM4 extract (100 mg/kg) treated groups compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between Healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between healthy control and diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ+Glb $P < 0.01$. [b]: Comparison between STZ+Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100 mg/kg). There is no significance between STZ+Glb and FM4 treated group (100 mg/kg).

In the present investigation the anti-diabetic properties in STZ-induced rats were screened by sulphated galactopyran fraction of *G. opuntia* (FM4) extract (Table 2). The diabetic rats were treated with three different concentrations of the FM4 extracts such as 80, 100 and 125 mg/kg BW. The current study demonstrated that all the three concentrations have showed recovery in altered blood glucose and HbA1c levels in diabetic rats. Among the experimental groups, FM4 with the concentrations 80 mg/kg BW (314.32% ± 16.35%, 12.47% ± 1.63%), 100 mg/kg BW (109.63% ± 16.35%, 5.38% ± 0.55%), 125 mg/kg BW (66.65% ± 20.86%, 3.95% ± 0.92%) showed significant ($P < 0.01$) reduction in glucose and HbA1c compared to diabetic groups (364.64% ± 52.47%, 14.15% ± 1.58%). 100 mg/kg BW treated group has shown the more significant ($P < 0.01$) reduction compared to other treated groups (Table 2).

Table 1
Free radical scavenging activities of *G. opuntia*.

Concentrations (µg/mL)	DPPH radical scavenging activity (% of inhibition)		ABTS radical scavenging activity (% of inhibition)		Hydroxyl radical scavenging activity (% of inhibition)		Nitric oxide scavenging activity (% of inhibition)	
	Aqueous extract of FM4	Ascorbic acid standard	Aqueous extract of FM4	Ascorbic acid standard	Aqueous extract of FM4	Ascorbic acid standard	Aqueous extract of FM4	Ascorbic acid standard
50	52.48 ± 0.66^a	36.24 ± 0.31	34.56 ± 0.62^a	36.90 ± 2.10	34.26 ± 1.90^a	8.10 ± 0.50	43.73 ± 0.06^a	49.52 ± 0.04
100	68.24 ± 0.26^b	42.21 ± 0.56	46.28 ± 1.10^b	21.30 ± 1.30	41.28 ± 1.60^b	11.26 ± 0.80	46.81 ± 0.12^b	69.97 ± 0.08
150	72.46 ± 0.48^c	48.39 ± 0.28^c	53.90 ± 1.60^c	52.62 ± 1.80	46.23 ± 1.20^c	18.25 ± 2.60	61.20 ± 0.56^c	77.85 ± 0.09
200	79.24 ± 0.52^d	52.86 ± 0.46	71.26 ± 0.89^d	65.90 ± 0.90	59.26 ± 1.20^d	25.32 ± 1.80	80.12 ± 0.15^d	93.36 ± 0.05
250	82.46 ± 0.36^e	57.28 ± 0.24	78.24 ± 3.20^e	74.26 ± 0.82	63.26 ± 1.80^e	33.24 ± 1.28	86.80 ± 0.05^e	96.26 ± 0.01

Values were performed in triplicates and represented as mean ± SD. Mean values followed by different superscript in a column are significantly different ($P < 0.05$).

Table 2
Effect of *G. opuntia* (FM4) extract on fasting Blood glucose and glycosylated haemoglobin (HbA1c) levels in STZ induced diabetic rats.

Groups	Treatment	Glucose levels (mg/dL)	HbA1c Levels (%)
Group 1	Control	103.38 ± 11.76	5.21 ± 0.82
Group 2	FM4 alone	105.44 ± 18.68[a]	5.31 ± 0.45[a]
Group 3	STZ	364.64 ± 52.47[*]	14.15 ± 1.58[*]
Group 4	STZ + Glb	129.40 ± 25.60[$]	6.10 ± 0.89[$]
Group 5	STZ + 80 mg/kg	314.32 ± 16.35	12.47 ± 1.63
Group 6	STZ +100 mg/kg	109.63 ± 16.35[b,#]	5.38 ± 0.55[b,#]
Group 7	STZ + 125 mg/kg	66.65 ± 20.86	3.95 ± 0.92

The data are expressed in mean ± SD, $n = 6$ in each group. [a]: Comparison between the control (Group 1) and FM4 alone treated group (Group 2). [*]: Comparison between the control group (Group 1) and diseased control group (Group 3). [$]: Comparison between diseased group (Group 3) and STZ + Glb group (Group 4) significance at $P < 0.01$. [b]: Comparison between STZ + Glb group (Group 4) and FM4-100 mg/kg treated group (Group 6) significance at $P < 0.05$. [#]: Comparison of diseased group (Group 3) and FM4-100 mg/kg treated group (Group 6).

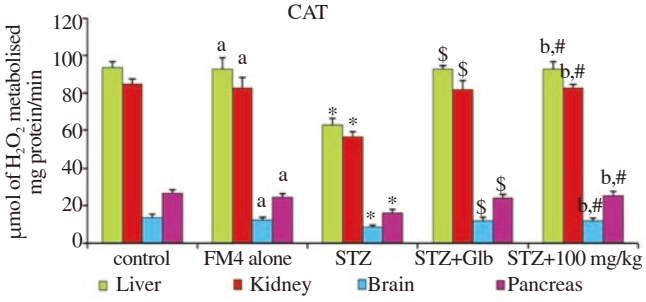

Figure 3. Estimation of CAT in liver, kidney, brain and pancreas of STZ induced diabetic rats treated with sulphated galactopyran fraction of *G. opuntia* (FM4).
CAT levels were reduced in STZ-induced diabetic rats. On treatment with the FM4 extracts (100 mg/kg) the CAT levels were increased in STZ-induced diabetic groups compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between healthy control and diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ + Glb $P < 0.01$. [b]: Comparison between STZ + Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100 mg/kg). There is no significance between STZ + Glb and FM4 treated group (100 mg/kg).

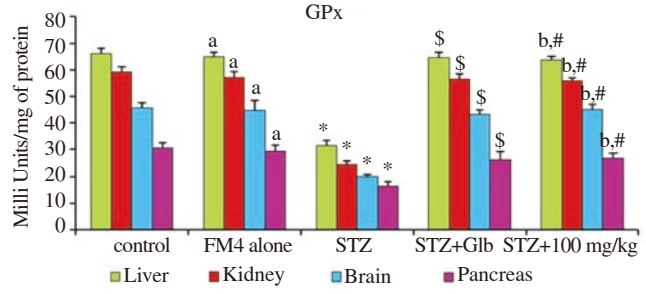

Figure 4. Effect of sulphated galactopyran fraction of *G. opuntia* on GPx in liver, kidney, brain and pancreas of STZ induced diabetic rats.
The GPx levels were decreased in STZ induced diabetic rats. FM4 extract (100 mg/kg) treatment was recovered the decreased levels of GPx in STZ-induced diabetic groups compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between Healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between Healthy control and Diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ + Glb $P < 0.01$. [b]: Comparison between STZ + Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100 mg/kg). There is no significance between STZ + Glb and FM4 treated group (100 mg/kg).

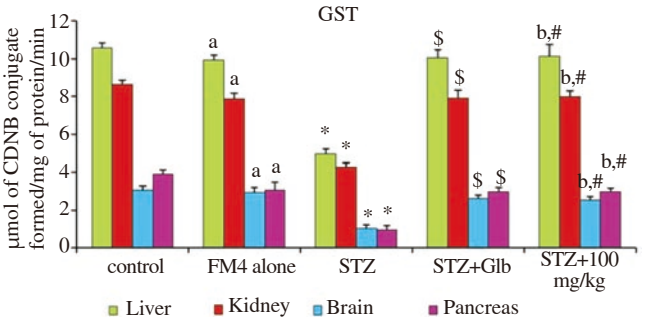

Figure 5. Analysis of GST in liver, kidney, brain and pancreas of STZ induced diabetic rats treated with sulphated galactopyran fraction of *G. opuntia* (FM4) extract.
The reduced GST levels in STZ-induced diabetic groups were significantly recovered after treating with the FM4 extracts (100 mg/kg) compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between healthy control and diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ + Glb $P < 0.01$. [b]: Comparison between STZ + Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100 mg/kg). There is no significance between STZ + Glb and FM4 treated group (100 mg/kg).

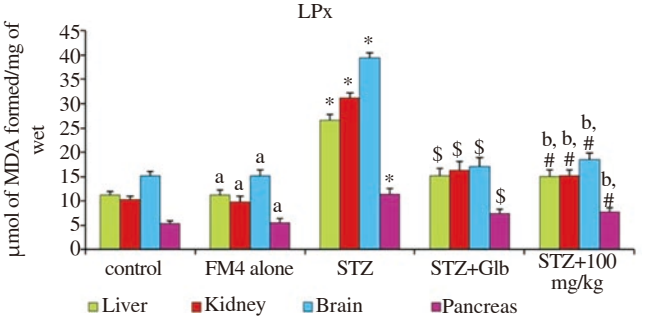

Figure 6. Estimation of LPx in liver, kidney, brain and pancreas of STZ induced diabetic rats treated with sulphated galactopyran fraction of *G. opuntia* (FM4). LPx levels were increased in STZ-induced diabetic rats and the enzyme activity was significantly decreased by FM4 extracts (100 mg/kg) treated in STZ-induced diabetic groups compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between healthy control and diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ + Glb $P < 0.01$. [b]: Comparison between STZ + Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100 mg/kg). There is no significance between STZ + Glb and FM4 treated group (100 mg/kg).

After inducing the diabetes, the levels of hepatic, renal, brain and pancreatic antioxidant contents were significantly ($P < 0.01$) altered in diabetic rats compared to normal rats. The enzymatic antioxidant such as SOD (3.93 ± 0.19; 5.42 ± 0.52; 0.98 ± 0.28; 0.65 ± 0.18) (Figure 2), CAT (63.08 ± 3.22; 56.42 ± 2.77; 8.58 ± 1.26; 16.32 ± 1.84) (Figure 3), GPx (31.42 ± 1.97; 24.42 ± 1.54; 19.86 ± 1.02; 16.26 ± 1.85) (Figure 4), GST (4.96 ± 0.13; 4.26 ± 0.12; 1.02 ± 0.11; 0.96 ± 0.08) (Figure 5) levels were significantly reduced where as LPx levels (26.58 ± 1.26; 31.26 ± 0.96; 39.36 ± 1.06; 11.40 ± 1.26) (Figure 6) were increased in diabetic rats. After treating with FM4 (100 mg/kg) extract the SOD (6.56 ± 0.40; 7.96 ± 0.66; 2.51 ± 0.32; 1.16 ± 0.12), CAT (92.66 ± 4.23; 82.68 ± 2.09; 11.96 ± 1.62; 25.51 ± 2.28), GPx (63.78 ± 1.12; 55.98 ± 1.01; 44.96 ± 1.89; 26.82 ± 1.84), GST (10.12 ± 0.28; 7.98 ± 0.23; 2.52 ± 0.19; 2.98 ± 0.12) and LPx

(14.96 ± 1.43; 15.21 ± 1.25; 18.60 ± 1.26; 7.76 ± 0.82) levels were recovered (Figures 2–6). The FM4 alone treated rats does not have any significant ($P < 0.01$) changes compared to the healthy control group.

The non-enzymatic antioxidants such as GSH (35.26 ± 1.15; 22.57 ± 1.21; 18.08 ± 0.42; 11.47 ± 0.96) (Figure 7), VIT-C (0.65 ± 0.12; 0.48 ± 0.09; 0.29 ± 0.09; 0.65 ± 0.09) (Figure 8) and VIT-E (3.94 ± 0.45; 2.03 ± 0.38; 0.87 ± 0.09; 0.64 ± 0.12) (Figure 9) levels were significantly ($P < 0.01$) reduced in diabetic rats. The VIT-C (1.37 ± 0.08; 1.08 ± 0.06; 0.77 ± 0.05; 0.98 ± 0.08), VIT-E (5.77 ± 0.48; 4.04 ± 0.42; 1.46 ± 0.16; 1.04 ± 0.16) and GSH (48.45 ± 1.28; 43.56 ± 0.98; 27.29 ± 1.53; 16.54 ± 1.26) levels were significantly ($P < 0.01$) recovered after treating with FM4-100 mg/kg BW compared to diabetes and other concentrations (80 and 125 mg/kg BW) (Figures 7–9).

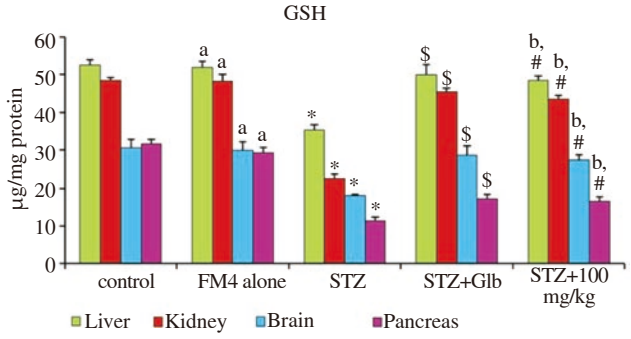

Figure 7. Effect of sulphated galactopyran fraction of *G. opuntia* on GSH in liver, kidney, brain and pancreas of STZ induced diabetic rats.

GSH levels were reduced in rats by inducing diabetes by STZ. On treating with FM4 extracts (100mg/kg) the GSH levels were increased in STZ-induced diabetes groups compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between healthy control and diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ + Glb $P < 0.01$. [b]: Comparison between STZ + Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100 mg/kg). There is no significance between STZ + Glb and FM4 treated group (100 mg/kg).

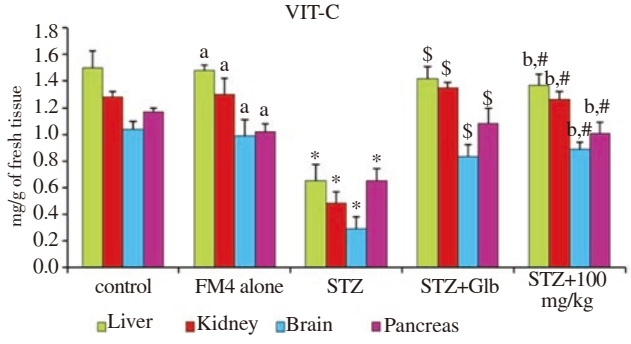

Figure 8. Estimation of VIT-C in liver, kidney, brain and pancreas of STZ induced diabetic rats treated with sulphated galactopyran fraction of *G. opuntia* (FM4).

VIT-C levels were reduced in STZ-induced diabetic rats and the enzyme activity was significantly increased by FM4 extracts (100 mg/kg) treated STZ-induced diabetic groups compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between Healthy control and Diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ + Glb $P < 0.01$. [b]: Comparison between STZ + Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100mg/kg). There is no significance between STZ + Glb and FM4 treated group (100 mg/kg).

Based on the glucose, HbA1c, enzymatic and non-enzymatic levels sulphated galactopyran fraction of *G. opuntia* (FM4) treated with the concentration 100 mg/kg BW has shown significant ($P < 0.01$) recovery in STZ induced diabetes in rats.

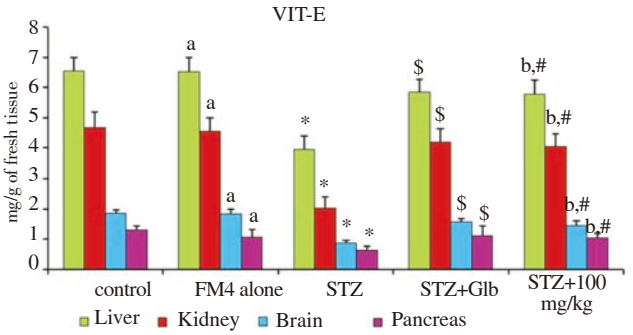

Figure 9. Effect of sulphated galactopyran fraction of *G. opuntia* on VIT-E in liver, kidney, brain and pancreas of STZ induced diabetic rats.

The reduced VIT-E levels in STZ-induced diabetic groups were significantly recovered after treating with the FM4 extracts (100 mg/kg) in STZ-induced diabetic group compared to STZ control group. Data were expressed in mean ± SD ($n = 6$). [a]: Comparison between healthy control and FM4 alone treated group $P < 0.01$. [*]: Comparison between healthy control and diabetic control (STZ) $P < 0.01$. [$]: Comparison between STZ and STZ + Glb $P < 0.01$. [b]: Comparison between STZ + Glb and FM4 $P < 0.01$. [#]: Comparison between STZ and FM4 (100 mg/kg). There is no significance between STZ + Glb and FM4 treated group (100 mg/kg).

4. Discussion

Macroalgae are used as nutraceutical substances due to the presence of many bioactive compounds and antioxidant substances[34]. The results of the antioxidant scavenging activity assays for *G. opuntia* has found to be exhibiting significantly ($P < 0.01$) higher DPPH, ABTS, hydroxyl and nitric oxide scavenging activities (Table 1). OH• has a short half-life and is the most reactive, known to be capable of abstracting hydrogen atoms from cell membranes and they bring about peroxidic reactions of lipids. Nitric oxide is a free radical that is generated when sodium nitroprusside reacts with oxygen to form nitrite, induces the inflammatory response and its toxicity multiplies if it reacts with O_2• radicals to form peroxynitrite[35]. The present results suggest that *G. opuntia* might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and peroxynitrite. The highly reactive hydroxyl radical (•OH) generated via a biologic Fenton reaction (hydrogen peroxide with Fe^{2+} and Cu^{2+} causes cytotoxic effect through the alteration of [Ca^{2+}] homeostasis[36].

Diabetes mellitus is a chronic metabolic disorder caused due to oxidative stress. The present study discussed about the anti-diabetic and antioxidant activity of sulphated galactopyran fraction of *G. opuntia* in streptozotocin-induced diabetic rats. The decrease in antioxidant status, protein glycation, enzymes inactivation and changes in structural functions of collagen basement membrane reveled that oxidative stress is occurred in DM[37]. Antioxidants present in our food plays an important role in decreasing the oxidative damage in our body. Free radicals are produced naturally in the cell by using the oxygen that can cause damage to the cell[38]. In antioxidant defensive system, vitamin-C plays an important role in protecting the lipids that undergo oxidation and reduces the apoptotic cell number[39]and oxidized vitamin-E is regenerated[40]. Vitamin-E is an non-enzymatic antioxidant that decreases the lipid peroxidation chain reaction. Vitamin-E plays an effective role in

lowering the glucose and HbA1c levels[41].

The insulin producing pancreatic β-cells were damaged due to STZ-induced hyperglycemia[42]. Thus our results demonstrated that the glucose levels in blood were significantly increased in diabetic rats and upon treatment with FM4 the levels were decreased (Table 2). This might be due to the regeneration of the pancreactic β-cells and secreting insulin. This has been previously reported in *Ecklonia stolonifera, Sargassum ringgoldianum* and *Sargassum polycustum* brown seaweeds that reduced the blood glucose levels in diabetic rats[43-45]. The reaction between the free amino group of Hb and glucose is a non-enzymatic reaction (glycosylation) that produces HbA1c as an end product[44]. FM4 administration exhibits a significant reduction in HbA1c levels, thereby increasing the levels of Hb in diabetic rats. The increase in HbA1c and decrease in Hb were directly proportional to the blood glucose levels. It has been reported that HbA1c levels were reduced in STZ and alloxan induced diabetic rats treated with *Spirulina* and *Ulva fasciata*[46].

The impaired antioxidant defense mechanism leads to decrease the SOD, CAT, GPx and GST levels that causes oxidative stress in DM[47]. SOD and CAT plays a major role in decreasing the cellular stress. Superoxide radicals are converted into H_2O_2 and O_2 because of SOD scavenging activity, while CAT reduces H_2O_2 and higher tissues were protected from the super active hydroxyl radicals[48]. The enzymatic antioxidant (SOD, CAT, GPx and GST) levels were significantly reduced in the organs (liver, kidney, brain and pancreas) of diabetic rats due to insufficiency of antioxidant defenses in hostility of ROS mediated damage (Figures 2–6). The decreased CAT and SOD activities may leads to H_2O_2 and O_2 production by the auto-oxidation of glucose. In maintaining the physiological levels of H_2O_2 and O_2, the CAT and SOD plays an important role by activating the O_2 radicals and eradicating the organic peroxides and H_2O_2 generated from the streptozotocin[5]. Upon treating with the FM4 the enzymatic antioxidant levels were recovered in diabetic rats. The increased levels of enzymes were recovered after treating with *Sargasum polycystum* and *Chlorella* was previously reported by Meena *et al.* and Amin *et al*.[49,50].

Figures 7–9 showed the reduced levels of VIT-C and E, the non-enzymatic antioxidants in diabetic rats. Vit-C and VIT-E levels were significantly ($P < 0.05$) increased in organs (liver, kidney, brain and pancreas) of diabetic rat treated with FM4. GSH has a versatile role in antioxidant defense mechanism. In GPx detoxification GSH acts as co-substrate and also acts as direct scavenger of free radicals. Due to oxidative stress in diabetes the GSH levels were decreased in various organs of rats. A Significant elevation was observed in FM4 treated diabetic rats. This may be due to decreased oxidative stress that leads to less degradation of GSH. Vijayvel *et al*. has reported that *Chlorella vulgaris* has reduced the non enzymatic levels in diseased rats[51]. The non-enzymatic levels were reduced upon treatment with *Sargasum polycystum*, *Spirogyra neglecta* in oxidative stress caused diseases in rats[52,53].

Lipid peroxidation is occurred due to the production of free radicals that leads to oxidative corrosion of polyunsaturated lipids. In plasma and tissues LPx will be in low concentration during the normal physiological conditions. This was increased due to cytotoxicity effects. The tissue damage occurred due to the production of oxygen free radicals that damages the cell membrane. This leads to increase in blood glucose levels and generates free radicals upon auto-oxidation[54]. In the current study, the LPx levels were increased significantly ($P < 0.05$) in liver, kidney, brain and pancreas of diabetic rats due to increase in the production of free radicals (Figure 6). The LPx levels were significantly decreased upon treating with FM4 in diabetic rats. Mohmoud *et al.* has reported in *Turbinaria ornata* and *Padina pavonia* has shown significant decrease in lipid peroxidation levels[55].

In conclusion, sulphated galactopyran fraction from *G. opuntia* (FM4) offers a capable therapeutic value in reducing the oxidative stress during diabetes. This efficacy may be mainly assigned to its antioxidant properties that showed a significant impact on increase of lipid peroxidation and in improvement of antioxidant defense systems in the selected tissues. Further studies are needed to resolve the main active ingredient that posses anti-diabetic effects.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

Dr. Lokanatha Valluru is highly thankful to DST- Science and Engineering Research Board (No. SR/S1/OC-96B/2013 Dt. 20.11.2013). Government of India, New Delhi, for providing financial assistance in the form of research grant. We also thankful to Dr. Kajal Chakraborty, CMFRI, Cochin for providing the marine macroalgae extracts.

References

[1] Nagarchi K, Ahmed S, Sabus A, Saheb SH. Effect of streptozotocin on glucose levels in albino Wister rats. *J Pharm Sci Res* 2015; **7**: 67-9.

[2] Nim DK, Shankar P, Chaurasia R, Goel B, Kumar N, Dixit RK. Clinical evaluation of antihyperglycemic activity of *Ocimum sanctum* in comparison with glibenclamide in the rat model of T2DM. *Int J Pharm Bio Sci* 2013; **4**: 699-706.

[3] Han LY. Pathogenesis of chronic hyperglycemia: from reductive stress to oxidative stress. *J Diabet Res* 2014; **2014**: 1-15.

[4] Jemai H, Sayadi S. Heart histopathology and oxidative features in diabetic rats and protective effects of Olieuropein. *Adv Biosci Biotech* 2015; **6**: 383-9.

[5] Fahmida A, Asiful MI, Kamal MA, Siew HG. Updates on managins type 2 diabetes mellitus with natural products: towards antidiabetic drug development. *Curr Med Chem* 2016; **23**: 1-37.

[6] Farouk KE, Hanan FA, Gamila HA, Rehab M, Safaa AS. Antidiabetic efficacy of *Dunaliella salina* extract in STZ-induced diabetic rats. *Int J Pharm Bio Sci* 2016; **7**: 465-73.

[7] Marlon EC. Beta cell dysfunction and insulin resistance. *Front Endocrinol* 2013; **4**: 1-12.

[8] Ahmed D, Vikas K, Amit V, Pushpraj SG, Hemant K, Vishal D, et al. Antidiabetic, renal/hepatic/pancreas/cardiac protective and antioxidant potential of methanol/dichloromethane extract of *Albizzia lebbeck* Benth. stem bark (ALEx) on streptozotocin induced diabetic rats. *BMC Complement Altern Med* 2014; **14**: 243.

[9] Balfour PC, Rodriguez CJ, Ferdinand KC. Blood pressure and cardiovascular effects of new and emerging antidiabetic agents. *Curr Hypertens Rep* 2014; **16**: 1-16.

[10] Valdes ST, Tostes MD, Anunciaçao PC, Da Silva BP, Sant'Ana HM. Association between Vitamin Deficiency and Metabolic Disorders Related to Obesity. *J Crit Rev Food Sci Nutr* 2016; doi: 10.1080/10408398.2015.1117413.

[11] Conklin AI, Monsivais P, Khaw KT, Nicholas JW, Forouhi NG. Dietary diversity, diet cost and incidence of type 2 diabetes in United Kingdom: a prospective cohort study. *PLoS Med* 2016; **13**: 1-16.

[12] Sumayya SS, Lawarence B, Manoj GS, Murugan K. Red algae diversity from thoothukudi and its therapeutic potentials: a search. *Indo Am J Pharm Res* 2016; **6**: 4173-78.

[13] Balaji V, Balaji M, Anjalakshi C, Cynthia A, Arthi T, Seshiah V. Diagnosis of gestational diabetes mellitus in Asian-Indian women. *Indian J Endocrinol Metab* 2011; **3**: 187-90.

[14] Skriptsova AV, Titlyanova TV, Titlyanov EA. Red algae of the genus *Gracilaria* in south of the Russian Far East. *Russ J Mar Biol* 2001; **27**: 38-52.

[15] Pal A, Kamthani MC, Kumar A. Bioactive compounds and properties of seaweeds - a review. *Open Acc Lib J* 2014; **1**: 1-17.

[16] Raul EC, Silvina RD, Fermin SM, Olga MA. Proteins and carbohydrates from red seaweeds: evidence for beneficial effects on gut function and microbiota. *Mar Drugs* 2015; **13**: 5358-83.

[17] Hemmingson JA, Furneaux RX, Murray-Brown VH. Biosynthesis of agar polysaccharides in *Gracilaria chilensis* Bird. *Carbohydr Res* 1996; **287**: 101-15.

[18] Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature* 1958; **181**: 1199-200.

[19] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad Biol Med* 1999; **26**: 1231-7.

[20] Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. 2nd ed. Oxford: Clarendon Press; 1989, p.186-9.

[21] Sreejayan N, Rao MNA. Nitric oxide scavenging by cucuminoids. *J Pharma Pharmacol* 1997; **49**: 105-7.

[22] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Calorimetric method for determination of sugars and related substances. *Anal Chem* 1956; **28**: 350-56.

[23] Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37919). *Cancer Chem Rep* 1963; **29**: 91-8.

[24] Hugget ASG, Nixon DA. Use of glucose oxidase, peroxidase and O-dianisidine in the determination of blood glucose and urinary glucose. *Lancet* 1957; **273**: 366-70.

[25] Karunanayake EH, Chandrasekaran NV. An evaluation of a calorimetric procedure for the estimation of glycosylated hemoglobin and the establishment of reference values of Srilanka. *J Nat Sci* 1985; **13**: 235-58.

[26] Misra HP, Fridovich I. The role of superoxide anion in the auto-oxidation of Epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; **247**: 3170-5.

[27] Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972; **47**: 389-94.

[28] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical roles as a component of glutathione peroxidase. *Science* 1973; **179**: 588-90.

[29] Habig WH, Pabst MJ, Jakoby WB. Glutathione-s-transferase. The first enzyme step in mercapturic acid formation. *J Biol Chem* 1973; **249**: 130-9.

[30] Hiroshi O, Nabuko O, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351-8.

[31] Omaye ST, Turnbull TD, Sauberlich HE. Selected method for the determination of ascorbic acid in animal cells, tissues and fluid. In: McCormic DB, Wright DL, editors. *Methods enzymol*. Vol. 62. New York: Academic Press; 1979, p. 3-11.

[32] Baker H, Frank O, DeAngelis B, Feingold S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr Rep Int* 1980; **21**: 531-6.

[33] Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim et Biophysic Acta* 1979; **582**: 67-78.

[34] Borbosa M, Patricia V, Andrade PB. Bioactive compounds from macroalgae in the new millennium: implications for neurodegenerative diseases. *Mar Drug* 2014; **12**: 4934-72.

[35] Bhattacharya S. Reactive oxygen species and cellular defence system. *Free Rad Hum Heal Dis* 2015; **2015**: 17-29.

[36] Jenifer P, Balakrishnan CP. *In vitro* antioxidant evaluation of *Gracilaria fergusonii* using various solvent extracts. *World J Pharm Res* 2016; **15**: 779-88.

[37] Nirmala A, Saroja S, Devi GG. Antidiabetic activity of *Basella rubra* and its relationship with the antioxidant property. *Bri Biotech J* 2011; **1**: 1-9.

[38] Khalil OA, Ramadan KS, Danial EN, Alnahdi HS, Ayaz NO. Antidiabetic activity of *Rosmarinus officinalis* and its relationship with the antioxidant property. *Afr J Pharm Pharmacol* 2012; **6**; 1031-6.

[39] Sadi G, Yılmaz O, Guray T. Effect of vitamin C and lipoic acid on streptozotocin-induced diabetes gene expression: mRNA and protein expressions of Cu-Zn SOD and catalase. *Mol Cell Biochem* 2008; **309**: 109-16.

[40] Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutri J* 2016; **15**: 1-22.

[41] Dal S, Sigrist S. The protective effect of antioxidants consumption on diabetes and vascular complications. *Disease* 2016; **4**: 1-51.

[42] Chen C, Yu LT, Meng QY, Xiang Z, Zhi YZ, Martin OA, et al. Recombinant Reg 3 β protein protects against streptozotocin-induced β-cell damage and diabetes. *Sci Rep* 2016; **35640**: 1-13.

[43] Kunihisa I. Antidiabetic and antioxidant effects of polyphenols in brown algae *Ecklonia stolonifera* in genetically diabetic KK-Ay mice. *Plant Foods Hum Nutr* 2008; **63**: 163-9.

[44] Mohammadi J, Naik PR. Evaluation of hyperglycemic effect of *Morus alba* in an animal model. *Ind J Pharmacol* 2008; **40**: 15-8.

[45] Motshakeri M, Mahdi E, Yong Meng G, Hemn Hassan O, Mohd Hair B, Suhaila M. Effects of brown seaweed (*Sargassum polycystum*) extracts on kidney, liver, and pancreas of type 2 diabetic rat model. *Evid Complement Alternat Med* 2014; **2014**: 1-11.

[46] Anitha L, Chandralekha K. Effect of supplementation of *Spirulina* on blood glucose, glycosylated hemoglobin and lipid profile of male non-insulin dependent diabetics. *Asian J Exp Biol Sc* 2010; **1**: 36-46.

[47] Snehal SP, Rajendra SS, Ramesh KG. Antihyperglycemic, antihyperlipidemic, and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats. *Indian J Experi Biol* 2009; **47**: 564-70.

[48] Ragini V, Prasad KVSRG, Bharathi K. Antidiabetic and antioxidant activity of *Shorea tumbuggaia* Rox. *Intern J Innov Pharm Res* 2011; **2**: 113-21.

[49] Meena B, Anbin Ezhilan R, Rajesh R, Sheik Hussain A, Ganesan B, Anandan R. Antihepatotoxic potential of *Sargassum polycystum* (Phaeophyceae) on antioxidant defense status in galactosamine-induced hepatitis in rats. *Afr J Biochem Res* 2008; **2**: 51-5.

[50] Amin A, Mohamed L, Mahmoud-Ghoneim D, Adeghate E, Mohamed AA, Maryam A, et al. Pancreas-protective effects of chlorella in STZ-induced diabetic animal model: insights into the mechanism. *J Diab Melli* 2011; **1**: 36-45.

[51] Vijayavel K, Anbuselvam C, Balasubramanian MP. Antioxidant effect of the marine algae *Chlorella vulgaris* against naphthalene-induced oxidative stress in the albino rats. *Mol Cell Biochem* 2007; **303**: 39-44.

[52] Hanumantha Rao BR, Armugam S, Devaki T. Hepatoprotective nature of seaweed alcoholic extracts on acetaminophen induced hepatic oxidative stress. *J Healt Sci* 2004; **50**: 42-6.

[53] Tarika T, Waristha T, Doungporn A, Yuwadee P, Rawiwan W. Evaluation of hepatic antioxidant capacities of *Spirogyra neglecta* (Hassall) Kutzing in rats. *Interdiscip Toxicol* 2013; **6**: 152-6.

[54] Malini P, Kanchana G, Rajadurai M. Antiperoxidative and antioxidant effect of ellagic acid on normal and streptozotocin induced diabetes in albino wistar rats. *Res J Pharm Biol Chem Sci* 2011; **2**: 33-5.

[55] Mahmoud AM, El-derby AM, Elsayed KMN, Abdella EM. Brown seaweeds ameliorate renal alterations in mice treated with the carcinogen azoxymethane. *Int J Pharm Pharm Sci* 2014; **6**: 365-9.