Hypoglycemic potential of some seaweeds from Karachi coast of Pakistan

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Abstract: Hypoglycemic activity of ethanol extracts and polysaccharides of seaweeds were studied in Sprague Dawley rats. Based on primary screening of ethanol extracts of 10 seaweeds, three *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* were selected for further study and polysaccharides were also extracted from them. Ethanol extracts and polysaccharides were administered to alloxan-induced diabetic rats and glucose level, liver and cardiac enzymes were determined. Antidiabetic activity of ethanol extract of three seaweeds *S. swartzii*, *S. indica* and *S. variabile* caused more than 30% reduction in blood glucose after 6 hours atleast on one dose level (2 mg/200 g and/or 10 mg/200 g body weight) in normal and alloxan diabetic rats. Polysaccharides of these three seaweeds also showed anti-diabetic activity after 6 hours atleast on one dose level (1mg/200 g and/ or 2mg/200g body weight) in normal and alloxan diabetic rats. Seaweed extracts and their polysaccharides caused slight alteration in liver and cardiac enzymes like *Serum Glutamate Oxaloacetate Transaminase* (SGOT), *Serum Glutamate Pyruvate Transaminase* (SGPT) and *Alkaline Phosphatase* (ALP).

Keywords: Seaweeds, ethanol extract, polysaccharides, hypoglycemia, diabetes, liver enzymes

INTRODUCTION

Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population (WHO, 2016). It is chronic disorder caused by inherited and/or acquired deficiency in insulin production by pancreas or by the ineffectiveness of the insulin produces (Berry *et al*., 2007), resulting in increased concentration of glucose in blood which damage body systems particularly blood vessels and nerves (Nagappa *et al*., 2003). Modern medicines used for the treatment of diabetes have side effects, including blood and skin diseases, gastrointestinal problems, coma and disturbed kidney and liver function (Larner, 1985; Poncelet, 2003; Sharifuddin *et al.,* 2015).

Seaweeds are rich in dietary fibers, unsaturated fatty acids, and polyphenolic compounds. Many of these seaweed have been reported to be beneficial to human health including in managing diabetes (Lamela *et al*., 1989; Sharifuddin *et al.,* 2015). Seaweed intake decreased fasting sugar in patients with type 2 diabetes mellitus (Kim *et al.,* 2008). *Sargassum ringgoldianum* showed significant lowering effect on streptozotocin-induced diabetic mice (Lee and Han, 2012). The antidiabetic activity of another brown seaweed *Ascophyllum nodosum* was reported due to the presence of polysaccharides and polyphenols (Zhang *et al*., 2007). The antifungal and nematicidal (Ara *et al.,* 1998), cytotoxic (Ara *et al.,* 1999; Ayesha *et al.,* 2010), antibacterial (Ara *et al.,* 2002a), hypolipideamic (Ara *et al.,* 2002b; Ruqqia *et al.,* 2015) and hepatoprotective (Hira *et al.,* 2016) activities of some seaweeds from Karachi Coast have been reported. The present work describes the hypoglycemic activity of ethanol extract and polysaccharide components of some seaweed collected from Karachi coast.

MATERIALS AND METHODS

Collection of seaweeds and preparation of ethanol extracts

Seaweeds belonging to Chlorophyta, *Caulerpa racemosa* (Fork.) J. Ag., *Colpomenia sinuosa* ((Roth.) Derb. et Sol., *Iyengaria stellata* (Borg.) Borg., and *Padina pavonia* (L.) Lamour, Phaeophyta, *Sargassum binderi, S.swartzii* (Turn.) C. Ag., *S. tenerrimum* J. Ag., *Spatoglossum variabile* fig. et De Notar, *Stoechospermum marginatum* (C.Ag.) Kutz. and *Stokeyia indica* Thivy et Doshi were collected from coastal area (Buleji beach) of Karachi in different seasons at low tide. Ethanol extracts of these seaweed were obtained using method described by Hira *et al.,* (2017).

Extraction of polysaccharides from seaweeds

The polysaccharides were extracted using the slightly modified method of Lamela *et al*. (1989). Accordingly 25 gm of seaweed was mixed with 1.0 liter deionized water

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and extracted for 4 hours on water bath with constant stirring. This extract was pooled and mixed with 95% ethanol (4 vol.) to precipitate polysaccharide (Lamela, *et al*., 1989; Su and Hassid, 1962). The polysaccharides were dissolved in water and dialyzed against water for 2 days. The non-dialyzed portions was collected and lyophilized and used.

Hypoglycemic activity of seaweeds

i). Animals

All tests were carried out in healthy adult male albino rats of Sprague Dawley strain purchased from HEJ Research Institute of Chemistry, University of Karachi, weighing 120-180g. The animals were kept in standard hygienic condition and provided with normal routine pellet diet and water.

ii). Primary screening for hypoglycemic activity in normal rats

Primary screening of ten seaweeds viz., *Caulerpa racemosa*, C*olpomenia sinuosa, Iyengaria stellata*, *Padina pavonia, Sargassum tenerrimum*, *S.swartzii, S.binderi, Spatoglossum variabile*, *Stoechospermum marginatum* and *Stokeyia indica* was performed in normal rats. Animals were divided in three groups, each group was comprised of four rats. Seaweed extracts at the dose of 2mg/200g and 10mg/200g body weight were fed orally to animals once in 24 hours. Control animals were given 1.0 ml distilled water through the same route. Animals of all three groups were sacrificed after 24 hours of administration of extracts.

iii). Antidiabetic activity of selected seaweeds in normal and alloxan-induced diabetic rats

a). Normal rats

The rats were divided into five groups (I-V) and kept on fasting for 12 hrs before giving the seaweed extracts or polysaccharides. Group I was served as control, while group II & III were given ethanol extracts at the dose of 2mg/200g body weight and 10mg/200g body weight, dissolved in 1.0ml of distilled water orally. Polysaccharides were administered orally @ 1mg/200 g body weight and 2mg/ 200g body weight respectively to animals of group IV and V. Control animals received 1.0 ml of distilled water through the same route. The animals were sacrificed after 1, 2, 4 and 6 hrs. There were four replicates of each treatment per dose per interval.

b). *Alloxan-induced hyperglycemic rats*

Hyperglycemia was induced by injecting alloxan-monohydrate at the rate of 120mg/kg body weight for 4 consecutive days intraperitoneally, dissolved in saline (Akhtar and Ali, 1985). Blood glucose levels were estimated on the fifth day with the help of glucometer (Glucotrend Roche, serial no. 05146127001). The animals were then kept on fasting for 12 hrs and alloxan-induced hyperglycemic rats were divided into five groups (I-V). Group I treated as control and received distilled water orally. Group II & III were given seaweed extract at the dose of 2mg/200mg body weight and 10mg/200g body weight respectively dissolved in 1.0ml of distilled water through oral routes, while polysaccharides @ 1mg/ 200g body weight and 2mg/200g body weight were administered to the rats of group IV&V respectively and sacrificed after 1, 2, 4 and 6 hours. Serum were used for the analysis of biochemical parameters.

Biochemical analysis

Glucose in the serum was determined by GOD-PAP method by using kit (Tinder, 1969) and absorbance was measured at 546 nm. The concentration of liver and cardiac enzymes Serum Glutamate Oxaloacetate Transaminase (SGOT) was estimated using Bergmeyer, *et al*., (1986a) method, while Serum Glutamate Pyruvate Transaminase (SGPT) was analyzed by kit of Boehringer Mannheim (Bergmeyer *et al*., 1986b). The alkaline phosphatase (ALP) was estimated by Deutsche Gesellschaft fur Kinische Chemie (DGKC) method by using kit (Ecoline Germany) and absorbance was measured at 340 nm..

Analysis of data

Data were analyzed and subjected to analysis of variance (ANOVA) followed by Least Significant Difference (LSD) (Armitage and Berry, 1994).

Ethical approval

All experiments were carried out with the approval of Institutional Research Ethical Committee.

RESULTS

In preliminary screening ethanol extracts of seaweeds *Spatoglossum variabile, Stokeyia indica, Sargassum swartzii* and *Padina pavonia* significantly lowered the blood glucose level in normal rats after 24 hours when used @ 2mg/200 g body weight. Whereas at high dose of 10mg/200g body weight, *Stoechospermum marginatum, Caulerpa racemosa, Sargassum tenerrimum, Caulpomenia sinuosa* and *Iyengaria stellata* induced significant hypoglycemic effects (table 1). Ethanol extracts of three selected seaweeds viz., *Sargassum swartzii*, *Stokeyia indica*, and *Spatoglossum variabile* exhibited significant reduction in serum glucose level atleast one dose level in normal rats (table 2). Similarly in alloxan-treated rats *S. swartzii* and *S. indica* significantly decreased serum glucose level atleast one dose level after 6 hours (table 2). Ethanol extracts of seaweeds caused slight alteration in liver and cardiac enzymes like SGPT, SGOT and ALP (tables 3-5).

Polysaccharides of *S. swartzii, S. indica*, and *S. variabile* were also exhibited serum glucose lowering effects atleast at one dose level (1mg/ 200 g body weight and or 2 mg/200 g body weight) in normal rats (table 6). In alloxan

Table 1: Effect of ethanol extracts of seaweeds on serum glucose (mg%) of normal rats after 24 hours.

Treatments	Doses	
	2 mg/ 200 g b.wt.	10 mg/200 g b.wt.
Control	99.7	99.7
Caulerpa racemosa	133.5	75.6
Colpomenia sinuosa	131.5	84.6
Iyengaria stellata	94.2	84.4
Padina pavonia	94.2	83.1
Sargassum tenerrimum	108.1	96.8
S. swartzii	54.9	62.6
S.binderi	91.1	96.0
Spatoglossum variabile	64.5	86.4
Stoechospermum marginatum	133.6	83.7
Stokeyia indica	60.9	49.7

$LSD_{0.05}$ Treatments = 2.2[1] Dose = 0.95[2]

Table 2: Effect of ethanol extracts of brown seaweeds *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* on serum glucose (mg%) in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	99	95	99	94	320	294	270	260
S. variabile 2 mg/200 g b.wt.	64	73	56	51	262	273	193	306
S. variabile 10 mg/200 g b.wt	86	88	101	84	233	299	211	258
S. indica 2 mg/200 g b.wt.	64	62	92	44	287	272	216	301
S. indica 10 mg/200 g b.wt.	88	52	63	43	200	244	187	171
S. swartzii 2 mg/200 g b.wt.	60	86	65	58	200	184	201	215
S. swartzii 10 mg/200 g b.wt.	64	77	82	54	295	332	344	324

$LSD_{0.05}$ Treatments = 4.03[1], Hours = 3.05[2], Rat Model = 2.15[3]
[1]Means values of treatments in columns showing differences of LSD values are significantly different at $p<0.05$
[2]Means values in rat models in rows showing differences of LSD values are significantly different at $p<0.05$
[3]Means values at different time intervals in rows showing differences of LSD values are significantly different at $p<0.05$

Table 3: Effect of ethanol extracts of brown seaweeds *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* on SGPT (IU/L) liver and cardiac enzyme in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	51	51	53	46	69	56	46	64
S. variabile 2 mg/200 g b.wt.	58	65	55	52	51	61	73	103
S. variabile 10 mg/200 g b.wt	62	56	51	62	88	63	42	47
S. indica 2 mg/200 g b.wt.	57	54	54	49	48	43	50	51
S. indica 10 mg/200 g b.wt.	63	30	53	28	48	33	64	46
S. swartzii 2 mg/200 g b.wt.	36	48	47	46	50	45	54	52
S. swartzii 10 mg/200 g b.wt.	40	49	56	52	63	92	103	93

$LSD_{0.05}$ Treatments = 1.84[1], Hours = 1.39[2], Rat Model = 0.98[3]

treated rats the polysaccharides of these three seaweeds reduced serum glucose levels, however *S. variabile* @ 1mg/ 200g body weight did not show constant effect on glucose levels whereas dose of 2 mg/ 200g body weight produced significant and gradual reduction in serum glucose levels at all the time intervals. Polysaccharides of *S. swartzii* showed better results in normal rats as compared to allaxon-induced diabetic rats, whereas polysaccharides of *S. variabile* and *S. indica* produced more or less similar effects in both normal and allaxon-induced diabetic rats (tables 7-9).

DISCUSSION

In the present study ethanol extracts of seaweed *Stokeyia indica*, *Spatoglossum variabile* and *Sargassum swartzii* exhibited significant reduction in the elevated level of blood glucose in normo-glycemic and alloxan-induced diabetic rats. The hypoglycemic effect of extracts assessed in non-diabetic rats might be extra-pancreatic action either by reducing or inhibiting glycogen break down, elevating glycogenesis and gluconeogenesis in liver or by penetrating glucose in the muscles, adipose

Table 4: Effect of ethanol extract of brown seaweeds *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* on SGOT (IU/L) liver and cardiac enzyme in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	53	53	52	53	61	33	51	69
S. variabile 2 mg/200 g b.wt.	51	42	36	32	30	39	42	85
S. variabile 10 mg/200 g b.wt	41	45	42	44	94	76	37	61
S. indica 2 mg/200 g b.wt.	52	48	53	44	52	48	29	40
S. indica 10 mg/200 g b.wt.	41	28	43	28	23	50	37	51
S. swartzii 2 mg/200 g b.wt.	43	46	53	56	32	47	57	54
S. swartzii 10 mg/200 g b.wt.	44	48	51	58	65	70	86	62

$LSD_{0.05}$ Treatments = 1.53[1], Hours = 1.16[2], Rat Model = 0.82[31]
[1]Means values of treatments in columns showing differences of LSD values are significantly different at p<0.05
[2]Means values in rat models in rows showing differences of LSD values are significantly different at p<0.05
[3]Means values at different time intervals in rows showing differences of LSD values are significantly different at p<0.05

Table 5: Effect of ethanol extract of brown seaweeds *Spatoglossum variabile Stokeyia indica* and *Sargassum swartzii* on ALP (IU/L) liver enzyme in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	139	132	121	102	214	212	215	253
S. variabile 2 mg/200 g b.wt.	64	95	155	65	225	220	211	298
S. variabile 10 mg/200 g b.wt	145	148	155	151	183	215	130	201
S. indica 2 mg/200 g b.wt.	66	101	128	120	197	227	231	241
S. indica 10 mg/200 g b.wt.	141	63	99	76	148	142	212	222
S. swartzii 2 mg/200 g b.wt.	131	128	124	104	187	123	164	145
S. swartzii 10 mg/200 g b.wt.	129	129	122	102	288	302	264	214

$LSD_{0.05}$ Treatments = 4.12[1], Hours = 3.12[2], Rat Model = 2.20[3]

Table 6: Effect of polysaccharides of brown seaweeds *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* on serum glucose (mg%) in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	99	95	99	94	320	294	270	260
S. variabile 1 mg/200 g b.wt.	96	77	70	67	323	333	377	395
S. variabile 2 mg/200 g b.wt	93	80	76	60	226	231	212	198
S. indica 1 mg/200 g b.wt.	93	85	113	73	182	164	122	117
S. indica 2 mg/200 g b.wt.	58	50	81	100	241	246	233	198
S. swartzii 1 mg/200 g b.wt.	65	75	86	47	270	278	290	207
S. swartzii 2 mg/200 g b.wt.	83	77	80	69	237	293	190	184

$LSD_{0.05}$ Treatments = 2.71[1], Hours = 2.05[2], Rat Model = 1.45[3]
[1]Means values of treatments in columns showing differences of LSD values are significantly different at p<0.05
[2]Means values in rat models in rows showing differences of LSD values are significantly different at p<0.05
[3]Means values at different time intervals in rows showing differences of LSD values are significantly different at p<0.05

tissues or other organs (Lesabre, 1981). Although different biological activities have been reported from seaweeds but hypoglycemic potential of seaweeds has now receiving attention. Different red and brown seaweeds especially *Sargassum*, *Cystoseria* (brown), *Corallina* and *Pterocladia* (red) species have been reported to possess blood sugar and lipid lowering activities (Bezanger-Beauquesne, 1982: Lee and Han, 2012; Kim *et al.,* 2008; Selvaraj and Palanisamy, 2014).

Maeda *et al*, (2007), demonstrated that fucoxanthin from marine plants improve insulin resistance and decreases blood glucose levels in mice. Many macromolecules and polysaccharides from seaweeds have been reported to possess hypoglycemic and hypocholesteromic activities (Guven *et al*., 1979; Lamela *et al*., 1985). There may be several possible mechanism of action of these seaweed extracts. Seaweeds may stimulate insulin secretion from beta cells of pancreas with controlled insulin and

Table 7: Effect of polysaccharides of brown seaweeds *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* on SGPT (IU/L) liver and cardiac enzyme in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	51	51	53	46	69	56	46	64
S. variabile 1 mg/200 g b.wt.	54	46	46	39	97	77	84	86
S. variabile 2 mg/200 g b.wt	62	62	45	43	51	53	54	51
S. indica 1 mg/200 g b.wt.	60	55	54	52	60	56	54	43
S. indica 2 mg/200 g b.wt.	33	46	68	41	75	65	52	52
S. swartzii 1 mg/200 g b.wt.	37	50	85	45	54	69	52	50
S. swartzii 2 mg/200 g b.wt.	66	62	53	55	54	68	81	59

$LSD_{0.05}$ Treatments = 1.19[1], Hours = 0.90[2], Rat Model = 0.63[3]

Table 8: Effect of polysaccharides of brown seaweeds *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* on SGOT (IU/L) liver and cardiac enzyme in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	53	53	52	53	61	33	37	69
S. variabile 1 mg/200 g b.wt.	49	45	50	41	79	65	53	51
S. variabile 2 mg/200 g b.wt	39	51	48	49	66	48	40	37
S. indica 1 mg/200 g b.wt.	77	68	58	42	51	45	37	40
S. indica 2 mg/200 g b.wt.	29	34	34	39	78	43	38	44
S. swartzii 1 mg/200 g b.wt.	63	43	64	140	34	48	46	33
S. swartzii 2 mg/200 g b.wt.	41	41	37	38	40	47	53	55

$LSD_{0.05}$ Treatments = 1.47[1], Hours = 1.11[2], Rat Model = 0.78[3]

[1] Means values of treatments in columns showing differences of LSD values are significantly different at $p<0.05$

[2] Means values in rat models in rows showing differences of LSD values are significantly different at $p<0.05$

[3] Means values at different time intervals in rows showing differences of LSD values are significantly different at $p<0.05$

Table 9: Effect of ethanol extract of brown seaweeds *Spatoglossum variabile, Stokeyia indica* and *Sargassum swartzii* on ALP (IU/L) liver enzyme in normal and alloxan induced diabetic rats model

Treatments	Normal rats				Allaxon diabetic rats			
	1 hr.	2 hrs.	4 hrs	6hrs	1 hr.	2 hrs.	4 hrs.	6 hrs.
Control	139	132	121	102	214	212	215	253
S. variabile 1 mg/200 g b.wt.	168	175	198	205	250	243	232	217
S. variabile 2 mg/200 g b.wt	194	190	229	216	253	267	211	196
S. indica 1 mg/200 g b.wt.	174	172	70	61	186	191	206	203
S. indica 2 mg/200 g b.wt.	146	107	104	99	154	218	210	193
S. swartzii 1 mg/200 g b.wt.	141	124	138	191	217	211	213	231
S. swartzii 2 mg/200 g b.wt.	140	138	182	193	220	205	208	216

$LSD_{0.05}$ Treatments = 5.90[1], Hours = 5.90[2], Rat Model = 4.17[3]

[1] Means values of treatments in columns showing differences of LSD values are significantly different at $p<0.05$

[2] Means values in rat models in rows showing differences of LSD values are significantly different at $p<0.05$

[3] Means values at different time intervals in rows showing differences of LSD values are significantly different at $p<0.05$

glucagon hormone along with decreased glucose-6-phosphatase, fructose-diphosphatase, pyruvate-carboxylase, phosphoenol-pyruvate carboxy-kinase and enhanced glucokinase activity (Serap, *et al*., 2009). Presumably, seaweed protects the β-cells from the alloxan effect or stimulate glucose utilization by peripheral tissues (Farjou and Al-Lami, 1988; Bakirel *et al*., 2008). The ethanol extract of *Ascophyllum nodosum* was found to inhibit intestinal alpha-glucosides and stimulate basal glucose uptake in to 3T3-L1 adipocytes. The alpha-glucosides inhibition was found due to the presence of polyphenolic component in the extract (Zhang *et al.,* 2007). Similarly seaweed polysaccharides has been reported to activity and prevent diabetic nephropathy (Da and Viswanathan, 2017). It would suggest that seaweed and its polysaccharide should be given due attention for the treatment of diabetes mellitus without causing any adverse effect on patients.

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