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Hypolipidemic potential of sterol containing fractions of *Jolyna laminarioides*: A brown alga

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Abstract: Solvent fractions (*n*-hexane, cholorofrom, methanol) and fractions containing sterols of *Jolyna laminarioides* was evaluated in triton-induced and high-fat-diet induced hyperlipidemic rats. Oral administration of *J. laminarioides* significantly reduced the elevated level of serum total cholesterol, triglycerides and LDL-c, both in triton induced and high fat diet induced hyperlipidemic rat models with increased serum HDL-c. Chloroform: methanol fraction (2:1) and *n*-hexane fraction containing sterol showed promising results in reducing LDL-c. The methanol fraction showed hypolipidemic effect by increasing HDL-c (90%). The extracts and fractions of the seaweed also decreased the increased level of cardiac and hepatic marker enzymes beside lowering lipid profile. *J. laminarioides* exhibited high anti-hyperlipidemic effects both in triton induced and high fat diet induced hyperlipidemic rats.

Keywords: *Jolyna laminarioides*, hypolipidemic, solvent fractions, sterol, triton-induced, high-fat-diet-induced.

INTRODUCTION

According to an estimate 17.7 million people were died in 2015 from cardio vascular diseases (CVDs), including 7.4 million due to coronary heart disease and 6.7 million were due to stroke (WHO, 2017). Elevated level of fasting total cholesterol is known as hyperlipidemia which may or not be due to elevation of triglyceride (Nelson, 2013). The abnormalities of lipoproteins are also responsible for hyperlipidemia and are the main cause of atherosclerosis and related cardiovascular diseases (Fuster *et al.,* 2011; Leopold and Loscalzo, 2008). It is unsurprising that South Asian population including Pakistan suffers from the highest prevalence rates of coronary artery disease (CAD) as compared to any other country throughout the world and the national mortality from CAD is 410/10,000 (Iqbal *et al.,* 2012). The primary consideration in therapy for atherosclerosis is to reduce the incidence of cardiovascular diseases and hyperlipidemia by increasing the anti-atherogenic high density lipoprotein cholesterol and reducing the elevated plasma lipid parameters (Shamim *et al.,* 2010). Cardiovascular disease is clinically dependant on lipid lowering drugs but are reported to have some side effects (Cardoso *et al.,* 2015).

Jolyna laminarioides, Guimaraes, belongs to Phylum Phaeophyta, and class Scytosiphonaceae (Shameel, 2008). It is yellow to dark brown in colour, grows in association with *Ulva fasciata* and *Melanothamnus* specie, mostly found on lime stone in mid-littoral zone (Nizamuddin and Farooqi, 1968). Khan *et al.* (2011) isolated a new compound named as jolynamine from the same seaweed. *J. laminarioides*, found at the Buleji beach, Karachi, Pakistan, demonstrated bioactivities such as anti-bacterial, antifungal, phytotoxic, insecticidal and nematicidal activities (Rizvi 2010). Ayesha *et al.* (2010) reported cytotoxic activity in the ethanol extract of *J. laminarioides*. In our previous study, we have reported hypolipidemic potential of ethanol extract of *J. laminarioides* and presence of sterol in it (Ruqqia *et al.,* 2015). The present report describes the hypolipidemic activity of *n*-hexane, chloroform and methanol fractions of ethanol extract. Since presence of sterol in seaweeds has been suggested to be a possible mechanism of hypolipidemic activity (Patterson 1977), solvent fractions of *J. laminarioides* containing sterol was also evaluated for hypolipidemic potential.

MATERIALS AND METHODS

Collection of seaweed and preparation of solvent fractions *Jolyna laminarioides*, was collected from Karachi coast: Buleji beach, washed, dried under shade and ethanol extract was prepared and identified by Dr. Aisha Begum, Department of Botany, University of Karachi. The ethanol extract (10 g) was separated into *n*-hexane, chloroform and methanol soluble fractions as described earlier (Hira *et al*., 2017).

Extraction of sterols from seaweed

Sterol was extracted from dry powder of seaweed *J. laminarioides* (50g) by using Soxhlet apparatus. The seaweed powder was mixed with 200ml of hexane and extracted for 8 hours. The hexane fraction (Fr. I) obtained was concentrated on rotary vacuum evaporator. The residue was further extracted with the mixture of chloroform and methanol (2:1) (Fr. II) for 8 hours and was concentrated. The presence of sterols in fractions (Fr.

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I & II) was determined by Thin layer chromatography (TLC), chloroform: methanol (9.5:0.5) was used as mobile phase and compared with cholesterol as standard. The ceric sulphate positive spots of sterol was identified.

Experimental animals

Hypolipidemic activity of solvent fractions of *J. laminarioides* was examined in normal, triton-induced and diet-induced hyperlipidaemic rats models. Adult male Albino rats (Dawley Sprauge), body weight 150-200g were used for the study. The animals were housed in uniform hygienic conditions and kept on a standard pellet diet and water *ad libitum*.

Normal rats model

The rats were divided into two groups control group and seaweed treated group. Each group contain six rats and had free access on water and diet. Rats in seaweed treated group were fed with solvent fractions (*n*-hexane, chloroform or methanol) of seaweed *J. laminarioides* (10 mg/200 g b.wt.) in water separately for 12 days. Distilled water were given to rats of control group. On day 12, all the rats were given overnight fasting and decapitated for serum collection and lipid parameters and liver and cardiac enzymes were estimated.

High fat diet induced hyperlipidemic rat model

In this model rats were fed with high diet (1% cholesterol, 0.5% cholic acid and 5% coconut oil in normal diet) to induced hyperlipidemia ((Ruqqia *et al.*, 2015). Rats of group I fed high fat diet for 12 days while group II rats received seaweed fraction in water (once daily for 12 days) and fed high fat diet. Control group received distilled water. On day 12, rats of all groups were sacrificed after 12 hours fasting, blood was collected and lipid profile, cardiac and liver enzymes were estimated in serum.

Triton model of hyperlipidemia

In this model hyperlipidemia was induced by a single dose (at 400mg/kg body weight by intraperitoneal injection) of Triton WR-1339 (Sigma Chemicals Company, St. Louis, MO, USA). Solvent fractions dissolved in distilled water were given orally at 10 mg /200g body weight simultaneously with triton in triton plus seaweed treated group. Rats of control group and triton control group received distilled water. Animals were sacrificed after 18 hours and lipid profile, cardiac and liver enzymes in serum were estimated. (Friedwald *et al.,* 1972).

Estimation of lipid profile and enzymes

Lipid profile and enzymes were determined on blood chemistry analyzer (Merck, Microlab-300) using kits (Merck/Ecoline, Germany). Kits of Merck was used for the estimation of total cholesterol (Tc) and HDL-cholesterol, while serum triglycerides (TG) level was determined by GPO-PAP method. Friedwald formula (1972) was used to calculate the LDL-c. Alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined using kits of Ecoline, Germany.

STATISTICAL ANALYSIS

One way ANOVA was used for data analysis, and significant level at $p<0.05$ was calculated using Duncan's multiple range test using software CoStat, Cohort Software, CA, USA.

Ethical approval

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

RESULTS

Effect of solvent fractions of Jolyna laminarioides in normal rats

Solvent fractions showed lowering effect on lipid profile in normal rats. Hexane fraction lowered the total cholesterol by 30.67%, triglyceride by 30.8% and LDL-cholesterol by 50.5%. Whereas chloroform fraction reduced total cholesterol by 20.1%, triglyceride by 15.2% and LDL-cholesterol by 6.1%. Methanol fraction reduced total cholesterol by 25.3% and LDL-cholesterol by 29.4%. All the fractions also reduced HDL-cholesterol (table 1). Test groups received solvent fractions also showed decrease in liver and cardiac enzymes levels compared to control group (table 1).

Effect of solvent fractions of Jolyna laminarioides in diet-induced hyperlipidemic rats

A significant ($p<0.05$) increased in serum lipid profile, like total cholesterol, triglyceride, LDL-choleterol and enzymes: LDH, ALP and ASAT were observed in rats group fed with high fat diet as compared to normal control group (table 2). The oral administration of hexane, chloroform, and methanol fractions of seaweed *J. laminarioides* at the dose of 10 mg/200 g b.w. for 12 days prevented the increase in serum total cholesterol, triglycerides, LDL-cholesterol and increased the low level of HDL-c. Treatment with hexane fraction in diet induced hyperlipidaemic rats significantly decreased the total cholesterol (37%), triglyceride (21.6%) and LDL-cholesterol (69%), beside increasing HDL-c (19.2%). Treatment with chloroform fraction was also found effective in increasing the HDL-c (36%) and reducing LDL-c (58.3%) while the methanol fraction showed less lowering effects as compared to hexane and chloroform fractions. Solvent fractions of *J. laminarioides* (*n*-hexane, chloroform, methanol) significantly decreased ($p<0.05$) the elevated level of liver and cardiac enzymes as compared to high fat diet control group. However hexane fraction demonstrated highest activity as compared to chloroform and methanol fraction (table 2).

Table 1: Hypolipidaemic effect of solvent fractions of seaweed *Jolyna laminoroides* on lipid profile and on liver and cardiac enzymes (LDH), (ALP), (ASAT) and (ALAT) in normal rats at 10 mg/200g b.w.

TREATMENTS	Total cholesterol	Triglyceride	HDL-cholesterol	LDL-cholesterol (u/l)	LDH (U/L)	ALP (U/L)	ASAT (U/L)	ALAT (U/L)
Control	$83^{a}\pm4.3$	$92^{a}\pm2$	$35.3^{a}\pm2.5$	$29.2^{a}\pm2.4$	$339^{a}\pm9$	$241^{a}\pm3$	$100.3^{a}\pm1.5$	$47.6^{a}\pm3.2$
Hexane fraction	$57.6^{c}\pm2.8$	$63.6^{c}\pm2.5$	$30.3^{b}\pm2.5$	$14.6^{b}\pm1.9$	$261.3^{b}\pm5.5$	$166^{d}\pm1$	$68.6^{d}\pm2.5$	$33^{c}\pm1$
	(-30.6%)	(-30.8%)	(-14.1%)	(-50%)	(-22.9%)	(-31.1%)	(-31.6%)	(-30.6%)
Chloroform fraction	$66.3^{b}\pm4$	$78^{b}\pm6$	$23.3^{c}\pm1.1$	$27.4^{a}\pm3.9$	$207.3^{c}\pm2.5$	$179.6^{c}\pm0.5$	$76^{d}\pm6$	$42^{b}\pm3.6$
	(-20.1%)	(-15.2%)	(-33.9%)	(-6.1%)	(-38.8%)	(-25.4%)	(-24.2%)	(-11.7%)
Methanol fraction	$62^{bc}\pm5.5$	$91.6^{a}\pm6.1$	$23^{c}\pm1$	$20.6^{b}\pm4.7$	$268^{b}\pm7$	$194^{b}\pm1.5$	$92^{c}\pm2$	$52^{ab}\pm4.3$
	(-25.3%)	(-0.4%)	(-33.9%)	(-29.4%)	(-20.9%)	(-19.5%)	(-8.2%)	(9.2%)

Values are means± standard deviation (n=6)
Means values in column having same super script letters are not significantly ($p<0.05$) different according to Duncan's Multiple Range Test Values in parenthesis are showing percent change

Table 2: Hypolipidaemic effect of solvent fractions of seaweed *Jolyna laminoroides* on lipid profile and on liver and cardiac enzymes (LDH), (ALP), (AST) and (ALT) in triton-induced and high-fat-diet induced hyperlipidaemic rats at 10 mg/200g b.w.

TREATMENTS	Total cholesterol	Triglyceride	HDL-cholesterol	LDL-cholesterol (u/l)	LDH	ALP	AST	ALT
Triton induced rat model Control (normal)	$68.6^{e}\pm1.5$	$72^{e}\pm1.0$	$33^{bc}\pm1.5$	$22^{e}\pm0.2$	$181^{c}\pm2.1$	$84^{e}\pm2.0$	$73.3^{e}\pm4.1$	$32^{b}\pm1.0$
Triton (control)	$137^{a}\pm1.0$	$187^{a}\pm1.0$	$25.3^{d}\pm0.8$	$74.3^{a}\pm1.7$	$272^{a}\pm1.5$	$136^{b}\pm3.5$	$155^{b}\pm2.6$	$34.3^{a}\pm1.2$
Triton+Hexane fraction	$95.3^{d}\pm4.0$ (-30.4 %)	$169^{b}\pm2.0$ (-9.6)	$26^{cd}\pm1.0$ (+4%)	$36^{d}\pm3.7$ (-52.1%)	$247^{b}\pm1.5$ (-9.1%)	$97^{d}\pm1.7$ (-28.5)	$82^{d}\pm1.0$ (-47%)	$25.3^{c}\pm1.5$ (-26.2%)
Triton+Chloroform fraction	$113.3^{c}\pm0.8$ (17.5%)	$162^{c}\pm3.0$ (-13.3)	$33.7^{b}\pm2.1$ (+33%)	$47.3^{d}\pm2.4$ (-36.5%)	$272^{a}\pm2.0$ (0%)	$141^{a}\pm2.9$ (-3.6%)	$160^{a}\pm3.5$ (-3.2%)	$31^{b}\pm1.0$ (-9.6%)
Triton+Methanol fraction	$130.3^{b}\pm1.5$ (-5.1%)	$125.3^{d}\pm3.5$ (-33%)	$48^{a}\pm8.0$ (+90%)	$57.3^{b}\pm6.9$ (-22.9%)	$270^{a}\pm4.0$ (-0.6%)	$121^{c}\pm1.0$ (-11%)	$91^{c}\pm2.0$ (-41.3%)	$27^{c}\pm1.0$ (-21.3%)
High fat diet-induced rat model								
Control (normal)	$72.3^{d}\pm2.5$	$65^{bc}\pm1.5$	$44.3^{a}\pm3.0$	$15^{e}\pm1.2$	$377^{b}\pm1.0$	$134^{d}\pm4.0$	$85^{b}\pm1.5$	$34^{a}\pm2.5$
High fat diet control	$105^{a}\pm1.0$	$77^{a}\pm1.5$	$29.6^{d}\pm2.0$	$60^{a}\pm1.2$	$451^{a}\pm2.0$	$276^{a}\pm2.0$	$116^{a}\pm0.5$	$35^{a}\pm2.0$
HFD+Hexane fraction	$66^{e}\pm1.0$ (-37%)	$60^{bc}\pm5.2$ (-21.6)	$35.3^{c}\pm1.5$ (19.2%)	$18.6^{d}\pm0.8$ (-69%)	$267^{d}\pm5.5$ (-41%)	$124^{e}\pm1.5$ (-55%)	$57^{d}\pm3.0$ (-51%)	$17^{c}\pm2.0$ (-52%)
HFD+Chloroform fraction	$77^{c}\pm2.0$ (-27%)	$56.3^{c}\pm4.0$ (-26.5%)	$40.3^{b}\pm0.5$ (36%)	$25^{c}\pm3.0$ (-58.3%)	$330^{c}\pm5.4$ (-27%)	$187^{c}\pm2.6$ (-32.2%)	$73^{c}\pm2.0$ (-37%)	$27.3^{b}\pm1.5$ (-22%)
HFD+Methanol fraction	$83^{b}\pm1$ (-21%)	$67^{ab}\pm7.5$ (-10.4%)	$73^{bc}\pm1.1$ (26%)	$32^{b}\pm2.3$ (-47%)	$308^{cd}\pm2.0$ (-32%)	$202^{b}\pm2.5$ (-27%)	$84^{b}\pm3.5$ (-27%)	$30^{b}\pm2.5$ (-15.4%)

Values are means± standard deviation (n=6)
Means values in column having same super script letters are not significantly ($p<0.05$) different according to Duncan's Multiple Range TestValues in parenthesis are showing the % change as compared to triton control and high fat diet control

Table 3: Hypolipidaemic effect of fractions of seaweed *Jolyna laminoroides* containing sterol on lipid profile and on liver and cardiac enzymes LDH, ALP, ASAT and ALAT in normal rats at 10 mg/200g b.w.

TREATMENTS	Total cholesterol	Triglyceride (mg%)	HDL-cholesterol	LDL-cholesterol (u/l)	LDH	ALAT	ASAT	ALP
Control (normal)	$71^{b}\pm1$	$74^{e}\pm1$	$34^{a}\pm1$	$22.2^{c}\pm0.9$	$183^{c}\pm3.2$	$83.6^{d}\pm1.0$	$75^{c}\pm4.0$	$32^{a}\pm1.0$
HFD+ Fr. I (hexane fraction containing sterol)	$85^{a}\pm3$ (+19.7%)	$131.3^{a}\pm2.0$ (+77.4%)	$25.3^{b}\pm2.5$ (-25.5%)	$33.4^{a}\pm2.4$ (+51.4%)	$254.3^{c}\pm3.5$ (+38.7%)	$83^{d}\pm2$ (-0.7%)	$64^{b}\pm3$ (-15.3%)	$26.3^{b}\pm1.5$ (-17.8%)
HFD+ Fr. II (chloroform: methanol fraction containing sterol)	$86.3^{a}\pm0.5$ (+21.5%)	$122.3^{b}\pm1.5$ (+65.3%)	$34.3^{a}\pm1.5$ (+0.8%)	$27.5^{b}\pm2.2$ (+23.8%)	$305^{a}\pm1$ (+66.6%)	$88.3^{c}\pm2.8$ (+5.6%)	$65^{b}\pm1$ (-14%)	$17^{d}\pm2$ (-46.8%)

Effect of solvent fractions of Jolyna laminarioides in triton-induced hyperlipidemic rats

Intravenous administration of triton WR-1339 caused a significant increased ($p<0.05$) in serum total cholesterol, triglyceride, and LDL-c and decreased HDL-c in triton control group as compared to control group. Treatment with fractions of seaweed *J. laminarioides* resulted in lowering in total cholesterol, triglycerides, LDL-c and increasing in HDL-c as compared to triton control group. Treatment with hexane fraction was very effective in lowering LDL-c (52.1%) beside other lipid profile. Methanol fraction was found very effective in increasing HDL-c and demonstrated maximum increase in HDL-c (90%) (table 2).

The solvent fractions showed varying degree of enzyme lowering effect on cardiac and liver enzyme when compared with control of triton. Hexane fraction showed significantly higher effect in lowering the enzymes, LDH (9.1%), ALP (28.5%), ASAT (47%) and ALAT (26.2%) in comparison with chloroform and methanol fractions. Methanol fraction was found more efficient in lowering ASAT and ALAT level, whereas ALP level was controlled moderately. Chloroform and methanol fraction exhibited no or negligible effect on LDH level (table 2).

Effect of solvent fractions of Jolyna laminarioides containing sterols in normal rats

The solvent fractions Fr.I and Fr.II increase the total cholesterol and triglyceride in normal rats as compared to control rats. However this elevation was found within the normal range. Fractions Fr.I and Fr.II also increased LDH enzyme (table 3).

Table 4: Hypolipidaemic effect of fractions of of seaweed *Jolyna laminoroides* containing sterol on lipid profile and on liver and cardiac enzymes LDH, ALP, ASAT and ALAT in high-fat-diet induced hyperlipidaemic rats at 10mg/200g b.w.

TREATMENTS	Total cholesterol	Triglyceride (mg%)	HDL-cholesterol	LDL-cholesterol (u/l)	LDH	ALAT	ASAT	ALP
High fat diet rat model Control (normal)	69^{d} ±1.5	72^{d} ±1.0	33^{b} ±1.5	22^{c} ±0.2	183^{c} ±3.2	83^{d} ±1.0	75^{c} ±4.0	32^{a} ±1.0
High fat diet (control)	133.3^{a} ±3.1	115^{a} ±2.9	32^{b} ±1.0	58.4^{a} ±3.0	317.3^{a} ±1.5	143.3^{a} ±1.5	93^{a} ±1.0	28^{b} ±2.5
HFD+ Fr. I (hexane fraction containing sterol)	106^{b} ±0.6 (20.4%)	105^{b} ±2.0 (-8.6%)	33^{b} ±1.0 (3.1%)	52^{b} ±0.3 (-11%)	229^{b} ±2.1 (-28%)	108^{b} ±1.0 (-24.5%)	71^{c} ±1.2 (-23.6%)	15.3^{d} ±2.1 (-45.3%)
HFD+ Fr. II (chloroform: Methanol fraction containing sterol)	88^{c} ±2.5 (-33.9%)	95.3^{c} ±3.5 (-17.1%)	50^{a} ±1.7 (56%)	18.6^{c} ±1.4 (-68.1%)	153^{d} ±2.1 (-51.7%)	92^{c} ±2.1 (-35.7%)	83^{b} ±1.0 (-10.7%)	21^{c} ±1.0 (-25%)

Values are means± standard deviation (n=6)
Means values in column having same super script letters are not significantly ($p<0.05$) different according to Duncan's Multiple Range Test
Values in parenthesis are showing the % change as compared to triton control and high fat diet control

Table 5: Hypolipidaemic effect of fractions of seaweed *Jolyna laminoroides* containing sterol on lipid profile and on liver and cardiac enzymes LDH, ALP, ASAT and ALAT in triton-induced hyperlipidaemic rats at 10mg/200g b.w.

TREATMENTS	Total cholesterol	Triglyceride (mg%)	HDL-cholesterol	LDL-cholesterol (u/l)	LDH	ALAT	ASAT	ALP
Control (normal)	72^{d} ±2.0	73^{d} ±2.0	33.6^{c} ±1.5	24^{b} ±3.5	183^{d} ±1.0	84.3^{b} ±4.0	75^{d} ±2.6	33^{a} ±1.0
Control (triton)	171.3^{a} ±2.0	305^{a} ±3.5	51^{b} ± 1.0	60^{a} ±0.8	303^{a} ±3	93^{a} ±2.6	133^{a} ±1.5	31^{ab} ±1.0
Triton+Lipocore (0.7 mg/200 g b.w.)	122^{c}±2 (-28.7%)	182.3^{b}±2 (-40.1%)	54.3±3 (+6.4)	31.2^{d}±3.4 (-47.4)	253.6±20 (-16.7%)	86.6^{c}±1.5 (-6.8%)	128.3^{b}±0.5 (-3.7%)	21^{d} ±1 (-32.2%)
Triton+Fenuget (0.7 mg/200 g b.w.)	114^{d}±2.6 (-33.4%)	191.3^{c}±1.5 (-37.1%)	50^{b}±3 (-1.9%)	25^{d}±2.9 (-58.3%)	300^{a} ±1 (-1.0%)	96.6^{a} ±2 (+3.8%)	127^{bc} ±1 (-4.7%)	24^{c} ±1 (-22.5%)
Triton+ Fr. I (Hexane fraction containing sterol)	124^{c} ±1.5 (-27.8%)	193^{c} ±1.5 (-36.5%)	59^{a} ±3.0 (15.6%)	26^{c} ±2.3 (-56.6%)	214^{c} ±2.0 (-29.3%)	84.6^{b} ±2.5 (-9.0%)	125^{b} ±1.5 (-6.0%)	28^{c} ±1.5 (-9.7%)
Triton+ Fr. II (chloroform: methanol fraction containing sterol)	128^{b} ±0.5 (-25.5%)	235^{b} ±2.0 (-22.8%)	46^{a} ± 3 (-9.8%)	35.1^{b} ±1.9 (-41.6%)	255^{b} ±2.0 (-15.8%)	85^{b}±1.0 (-8.6%)	121^{c} ±1.0 (-9.2%)	31^{bc} ±1.5 (0%)

Values are means± standard deviation (n=6)
Means values in column having same super script letters are not significantly ($p<0.05$) different according to Duncan's Multiple Range Test
Values in parenthesis are showing the % change as compared to triton control

Effect of solvent fractions of Jolyna laminarioides containing sterols in diet-induced hyperlipidemic rats

The oral administration of Fr. I and Fr.II fractions of J. laminarioides at the dose of 10 mg/200gm b.w for 12 days with an atherogenic diet prevented the increase in serum triglycerides, LDL-cholesterol, total cholesterol and increased the HDL-cholesterol. Sterol containing fraction Fr. I & Fr. II showed pronounced hypolipidemic effect in high fat diet model (table 4). Fr. II effectively lowered LDL-cholesterol (68.1%) and increased HDL-cholesterol (56%). Fr. I showed very small change in the concentration of HDL-cholesterol (3.1%) and HDL-cholesterol (-11%) in high fat diet model. Fractions I & II of *J. laminarioides* also reduced the elevated level of liver and cardiac enzymes than high fat diet control group (table 4).

Effect of solvent fractions of Jolyna laminarioides containing sterols in triton-induced hyperlipidemic rats

The sterol containing fractions, fraction I (Fr.I) and fraction II (Fr.II) significantly lowered the elevated level of total cholesterol, triglycerides and LDL-cholesterol. Fr.I & Fr. II both were found very effective in lowering LDL-c by 56.6% and 41.6% and increasing HDL-c by 15.6% and 9.8% respectively in triton model (table 5). Both fractions showed significant effect on total cholesterol and triglyceride. Reference drugs lipocore and fenoget (at 0.7mg/200g b.wt.) also showed significant hypolipidemic activity. Fr. I and Fr. II showed moderate to low effect in lowering the enzymes in comparison with their corresponding control group (table 5).

DISCUSSION

Seaweeds are a rich source of diverse compound having great potential for diverse applications in many field including cardiovascular-health (Cardoso *et al.,* 2015). In this study, in normal rat model *n*-hexane fraction of *J. laminarioides* played a potential role in lowering of total cholesterol and LDL-c. while its chloroform and methanol fraction were effective in increasing HDL-c. Hypolipidemic activity of ethanol extract and its solvent fractions have been reported by us earlier (Ara *et al.,* 2002, 2005, Ruqqia *et al.,* 2015). Lowering of lipid profile by the seaweed extract may be due to the presence polyphenol in extract and fractions which caused antioxidant activity (Tariq *et al.,* 2011). Enzymes like LDH, ALP, ASAT and ALAT are reliable markers of heart and liver function. These enzymes are present normally in small amount and elevated due to the hyperlipidemia (Balamuruga and Ignacimuthu, 2011). In this study *n*-hexane fraction and fractions containing sterols also reversed the elevated level of these enzymes in hyperlipidaemic rats.

The main sterol extracted from plants, animal and fungi is cholesterol and ergosterol (Sanchez-Machado *et al.,* 2004). Heilbron *et al.,* (1935) for the first time investigated the presence of sterols in seaweeds. In our study presence of sterols in chloroform: methanol (Fr.II) of seaweeds *J. laminarioides* was determined by thin layer chromatography (TLC). The extracted sterol fractions were investigated for their antihyperlipidemic potential at the dose of 10 mg/200g b.w. Fr.I and Fr.II of seaweeds *J. laminarioides* demonstrated hypolipidemic effect in high fat diet induced and triton-induced hyperlipidemic rat model. Fr.I and Fr.II reduced Total cholesterol and triglyceride, but the reduction was less than the reference drug fenoget. Both the fractions caused improvement in HDL-c as compared to the normal control and the reference drugs. Sterols has been reported from brown algae (El Shoubaky and Salem, 2014). Sheikh (1992) reported hydroxyl sterol such as saringosterol, three unsaturated sterols such as stigmasta-5, 23-dien-3β-ol, 24-methyl-cholesta-5 ,25-dien-3β-ol and 24-methylene cholesterol in *J. laminoriodes*. In our study the hypolipidemic activity of *J. laminarioides* may be due to presence of these sterol., In our study sterol containing fractions of *J.laminarioides* decreased LDL-c more than 15%. Katan *et al.,* (2003) reported that the daily intake of about 4g of phytosterols, decreases the LDL-c level by 10-15%

Most of the previous study reported the hypolipidemic activity of seaweed due to polysaccharides (Jiao *et al.,* 2011). Porphyran, a sulphated polysaccharide from *Pyropia,* a red alga showed hypolipidemic activity in mice (Cao *et al.,* 2016). Similarly, Huang *et al.,* (2010) reported hypolipidemic effect of fucoidan from *Laminaria japonica* in hyperlipidemic rats by lowering the total cholesterol, triglyceride and LDL cholesterol with increased in HDL cholesterol. In this study we have found that sterol containing fractions of *J.laminoriodes* has great potential against hyperlipidemia induced by high fat diet and also by triton. Qudeer *et al*., (2016) has been reported the anti-pherlipidemic effect of fucosterol from marine algae.

CONCLUSION

The plant stanols and sterols have been suggested as safe and effective alternative for the management of cholesterol level (Katan *et al.,* 2003). The recent introduction of satnol and sterol enriched foods in many part of the word is an important development and may be considered as alternative to statin drugs (Gylling *et al.,* 2014). Seaweeds containing sterols may be one of the choice.

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