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A N I N T E R N A T I O N A L J O U R N A L

Protective Effects of *Spirulina* on the Liver Function and Hyperlipidemia of Rats and Human

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ABSTRACT

In the present study, the effects of Spirulina *on subchronic treatments (two weeks) of hyperlipidemia and liver function of the rats and humans were investigated. The hyperlipidemia was induced in the rats using 25% of soya bean oil and 25% butter. The butter induced more hyperlipidemia than soya bean oil.* Spirulina *was used at the concentrations of 0, 2.5, 5.0 and 10 % of diet weight of the rats. The decrease in hyperlipidemia by* Spirulina *was dependent on its concentration in the diet. In case of human studies, about four g/day of* Spirulina *was taken via oral administration by Egyptian volunteers patients with hyperlipidemia.* Spirulina *decreased the levels of hyperlipidemia in these patients. The effects were dependent on the amount and number of administered dose of* Sprirulina. *The results suggested that the* Spirulina *treatment could induce marked reduction of aminotransferase through correcting lipid profile and increasing high density lipoprotein.*

Key words: *Spirulina*, Hyperlipidemia, Protective effects, Liver functions

INTRODUCTION

Hyperlipidemia is the presence of high, or abnormal levels of lipids and/or lipoproteins in the blood, or elevation of lipids in plasma. Several studies have shown that an intimate correlation exists between coronary diseases and high levels of lipoprotein **(**Shattat et al. 2010). Lipids, such as cholesterol and triglycerides, are insoluble in plasma and circulating lipid are carried on by lipoproteins that transport them to various tissues for energy utilization, lipid deposition, steroid hormone production, and bile acid production. Lipoprotein consists of esterified and unesterified cholesterol, triglycerides, and phospholipids, and protein, which consist mainly of apolipoproteins, or apoproteins **(**Rader et al. 1994).

Disturbance in lipid profile results in lipid disorders including 1) familial combined hyperlipidemia (FCH), caused by polymorphisms in the molecules and enzymes that participate in lipoprotein metabolism, such as ApoCII and ApoCIII and CETP (cholesterylester transferring protein) and acquired combined hyperlipidemia, which is common in the patients who suffer from other diseases from the metabolic syndrome (diabetes mellitus type II and hypertension). Excessive free fatty acid production by various tissues leads to increased VLDL synthesis by the liver. Initially, most of VLDL is converted into LDL (James et al. 2006).

Fatty liver, known as fatty liver disease (FLD) such as steatorrhoeic hepatosis, or steatosis hepatitis, is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis (Reddy and Rao 2006; Bayard et al. 2006). Fatty liver is often associated with alcoholic liver disease,

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hyperinsulinemia, and insulin-resistance. Accordingly, it is most often observed in alcoholics, obese persons, and diabetic patients. It is also frequently caused by pregnancy, malnutrition, chemical intoxication, drug and viral hepatitis, and intestinal bypass surgery (Riely 1987; Doherty et al. 1991; Guha-Mazumder 2001; Altlparmak et al. 2005; Adams and Talwalkar 2006).

Heart diseases remain the leading cause of death for both men and women of all races and ethnicities. It is expected that large proportion of elderly individuals would suffer from heart diseases. In men over the age of 65, for example, nearly one-half of all deaths are attributed to heart diseases. In this regard, the prevalence of hyperlipidemia is as high as 80-88% as compared to approximately 40-48% in age-matched controls without coronary diseases (Carroll et al. 2005; Boekholdt 2007). A variety of factors, often acting in combination, are associated with an increased risk for atherosclerotic plaques in coronary arteries and other arterial beds. Hypercholesterolemia is one of the major risk factors for heart diseases, including in those over the age of 65 (Lewington et al. 2007).

Spirulina is free-floating filamentous cyanobacteria characterized by the cylindrical, multicellular trichomes in an open left-hand helix which can be found in tropical and subtropical lakes in Africa, Asia and South and Central America (Vonshak 1997). It has high protein content, 60–70% of its dry weight, whose nutritive value is related to the quality of amino acid. It contains all essential amino acids, including leucine, isoleucine and valine, with reduced amounts of methionine, cysteine, and lysine when compared to the proteins of meat, eggs, and milk (Babadzhanov et al. 2004). It is, however, superior to typical plant protein, such as those derived from legumes (Babadzhanov et al. 2004). It also contains a relative high concentration of provitamin A, vitamin B12 and β-carotene, vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), vitamin C, vitamin D, and vitamin E. *Spirulina* have 4–7% lipids, essential fatty acids and ω-3 and ω-6 polyunsaturated fatty acids, including γ -linolenic acid, α-linolenic acid, linoleic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid (Sánchez 2007; Huang et al. 2007). *Spirulina* also is a rich source of several minerals, including potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc (Tokusoglu and Uunal 2003).

Administration of *Spirulina* has been found to lower the heart damage caused by chemotherapy **(**Khan et al. 2005), reduces the severity of strokes and improves recovery of movement after a stroke (Wang et al. 2005), and reverses age-related declines in memory and learning. *Spirulina* also has been found to prevent and treat hay fever through increase in immunological activities (Chen et al. 2005). In view of the above, this work aimed at investigating the protective effect of *Spirulina* on hyperlipidemia and liver function in a preclinical rat model as well as in human.

MATERIALS AND METHODS

Culture medium and growth conditions for *Spirulina platensis*

The strain of *Spirulina platensis* was kindly supplied from the culture collection of Mansoura University, Faculty of Science, Mansoura, Egypt. The medium used for the *Spirulina* cultivation was Zarrouk's medium (Zarrouk 1966). Erlenmeyer flasks (250 mL) contained 150 mL of Zarrouk's medium were sterilized in an autoclave at 1.5 atm. for 20 min. After cooling, the flasks were inoculated with 15 mL of the pre-culture organisms and incubated under continuous fluorescent light of 2500 lux. The cultures flasks were aerated with sterile air mixed with 3% CO_2 to accelerate cyanobacterial growth. The rate of gas was regulated by means of plastic valves (Zarrouk 1966).

Determination of dry weight

A definite volume of cyanobacterial suspension (200 mL) was centrifuged at 1,077xg for 10 min. The precipitated cells were washed two times with distilled water and dried overnight in an oven at 65˚C till constant weight. The data were given as g/100 mL.

Application on experimental animals

Adult male rats weighing 99-108 g were obtained from the Faculty of Science, Zoology Department, Tanta University, Tanta, Egypt. The rats were housed at 25°C and day and night light according to the time needed for each experiment.

Natural induction of hyperlipidemia
The rats were divided into three groups according the types of lipids sources (Young 2001): Group (1): this group was fed on normal standard diet to serve as a control group, which was housed throughout the work under the same conditions of other groups; Group (2): this group was fed on high rich oily diet with the oil percentage about 25% of total diet and housed for 15 days; Group (3): this group was fed on high rich butter (about 25% of total diet) and housed for 15 days. The groups fed on high rich fatty diet are listed in Table 1.

Table 1 - The different diets of high rich fats of food (25% Soybean oil and 25% Butter) and normal diet used for feeding of rats.

Composition	Food Group (1) (Control) gm	Food of Group (2) gm	Food of Group (3) gm
Soy protein	249.7	249.7	249.7
Vitamins mixture	10	10	10
Choline butyrate	2.5	2.5	2.5
Mineral Mixture	35	35	35
Soybean oil	100	250	-------
Butter	-----	------	250
Sucrose	602.8	452.8	452.8

Hyperlipidemia treatment using *Spirulina platensis*
The different groups of rats used for induction of hyperlipidemia were treated with dry *Spirulina* for 21 days at 0, 2.5, 5 and 10% of diet weight.

Blood sampling and serum preparation
The blood samples were collected in clean dry heparinized test tubes from the retro-orbital plexus using heparinized microcapillary tubes. The tubes were allowed to stand for 15 min to clot at room temperature and then centrifuged at 3500 rpm for 15 min using Heraeus Sepatech centrifuge (Labofuge 200), the plasma was separated, frozen at -20ºC and stored for further determination of the biochemical parameters (Walters and Gerarde 1970).

Application on human - Ethical committee number (FDA approval,Talk Paper #T81-18)
About 5 mL Blood samples were collected in venipuncture into heparinized syringe (Wu et al. 1989) from 20 Egyptian volunteer patients with history of hyperlipidemia, aged from 30 to 60 years (10 males and 10 females). Volunteers were supplied (4 g day^{-1}) oral uptake of dry *Spirulina*. Blood samples were collected in the morning after 12-16 h of fasting and prepared according to guidelines of the Lipid Research Clinic’s program Manual of Laboratory Operations.

Biochemical analyses
Cholesterol and triglycerides level were estimated according to Finley et al. (1978) and Buccolo and David (1973), respectively. High Density Lipoprotein (HDL) was determined by separation method based on the selective precipitation of apoliprotein B-containing lipoproteins (Very Light Lipoprotein, Low Density Lipoprotein and Lpa) by phosphotungsic acid/$MgCl_2$, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) according Burstein et al. (1980). Low Density Lipoprotein (LDL) was determined for human and experimental animals by using the following equation (Schumann and Klauke 2003):

[Cholesterol – HDL +Triglycride/5] = LDL mg/dl

After samples preparation, total and direct bilirubin were estimated according to Malloy and Evelyn, (1937). Alanine Aminotransferase (SGPT) and serum Aspartate Aminotransferase (SGOT) activities were determined according to the recommendation of the Expert panel of the IFCC (International Federation of Clinical Chemistry), without Pyridoxalphosphat activation according Schumann and Klauke (2003) and Schumann et al. (2002), respectively. The serum proteins and serum albumin levels of the rats groups and human were estimated according to Burtis (1999) and Rodkey (1964), respectively. Serum Alkaline Phosphatase was determined according to Fischbach and Zawta (1992).

Statistical analysis

All data were expressed as the mean of three replicates; ± standard error of the mean statistical analysis was performed using t test using SPSS 15 software.

RESULTS

Rats liver functions at zero time (start) and after 7, 14 and 21 days with normal diet

The results in Table 2 showed slight changes in different liver functions measured in the rats. These changes were insignificant as compared to the control group. The highest protein concentration was detected after 7 days of treatment with normal diet; it increased by 9.5% in comparison with zero time. The albumin level showed no change during the period of the experiment, except that there was a decrease at 7 days by 5%. SGPT showed increases at 7 and at 21 days by 6 and 9%, respectively in comparison with zero time. SGOT showed a slight increase in its level by 14 days. Alkaline phosphates level decrease at 21 days by 9% and after14 days by 16%.

Table 2 - Rats liver functions at zero time (start) and after 7, 14 and 21 days with normal diet (control).

Time (day)	Bil. T	Bil. D	Protein	Albumin	SGPT	SGOT	ALK
0	0.7±0.01	0.14±0.005	7.4±0.057	4.3±0.08	8.1±0.1	7.2±0.14	9.2±0.3
7	0.7±0.02*	0.14±0.005	8.13±0.27*	4.1±0.1*	8.2±0.2*	7.3±0.18*	9.5±0.2**
14	0.7±0.01*	0.15±0.005	7.36±0.20*	4.2±0.08*	8.4±0.2*	8.2±0.25*	7.7±0.1**
21	0.7±0.01*	0.14±0.005	7.43±0.20*	4.3±0.05*	8.1±0.2*	7.2±0.3*	8.4±0.2**

NB.:Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

Rats lipids profile at zero time (start) and after 7, 14 and 21 days with normal diet

The results in Table 3 showed serum lipids profile on day 0 and after 7, 14 and 21 days of control group fed on normal diet. Serum cholesterol level showed low insignificant decrease in its level at 21 days (3%) in comparison with the control. Triglycerides level (TRI) showed slight increase on days 7 and 21 by 2 and 3.7%, respectively, in comparison with the control rats. HDL showed low significant levels at 14, 21 days by 2.5% in comparison with the control. LDL showed low significant decrease at 21 days by 2.3% in comparison with the control rats.

Table 3 - Serum lipids profile of rats at zero time and after 7, 14 and 21 days with normal diet (control).

Time (day)	Cholesterol	TRI	HDL	LDL
0	169±4.932	95±2.88	43±1.527	105±1.7
7	169±3.7*	96.9±*2.9	43.66±0.88*	105.33±1.763*
14	166±3.05*	94.3±2.3*	42±1.154*	104±2.08*
21	164.6±3.9*	98.6±2.3*	42±1.52*	102.66±2.96*

NB.:Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

Liver function in rats after 15 days feeding on high fatty diets (Soybean oil and butter)

Total bilirubin showed increase by 4.3% with soybean diet and about 5.7% with butter diet in comparison with the rats fed on normal diet. SGPT showed highly significant increase in its level with soybean diet by 102.7% and with butter diet by 110.6% in comparison with the control at normal diet. SGOT showed increase in its level with soybean oil diet by 15.4% and with butter diet by 47.2% in comparison with the normal diet. AP level increased with soybean diet and butter diet by 6 and 4.1%, respectively in comparison with the normal diet (Table 4).

Data in Table 5 showed serum lipids in the rats after 15 days feeding on high fatty diets rich in soybean oil and butter. The cholesterol level increased with soybean oil diet by 17.5% and highly significant increase with butter diet by 104.1% in comparison with the control with normal diet. TR level showed highly significant increase in its level with soybean oil diet by 153% and also highly significant increase with butter diet by 157.9% as compared with the control. HDL showed a significant decrease in its level with soybean oil diet by 22.98% and a significant decrease with butter diet by 25.5% in comparison with the control. There was highly significant increase in level of low density lipoprotein (LDL) with soybean oil diet by 13.4% and also highly significant increase with butter diet by 153.4% in comparison with the control.

Table 4 - Liver function of rats after 15 days feeding by highly fatty diets (Soya bean oil and butter).

Food	Bil T	Bil D	Protein	Albumin	SGPT	SGOT	ALK
Normal	0.72±0.02*	0.14±0.01*	7.46±0.2*	4.26±0.145*	8.4±0.3*	7.1±0.2*	8.9±0.2*
Soya bean	0.74±0.01*	0.13±0.01*	7.56±0.2*	4.26±0.145*	17.1±0.4***	8.2±0.2***	9.5±0.3*
Butter	0.76±0.02*	0.15±0.003*	7.56±0.2*	4.30±0.12*	17.8±0.4***	10.5±0.3***	9.3±0.3*

NB.:Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

Table 5 - Lipids profile of rats serum after 15 days feeding by highly fatty diets (Soya bean oil and butter).

Food	Cholesterol	TRI	HDL	LDL
Normal	169±2.3*	76±2.01*	45±1.7*	109.3±3.1*
Soya bean	198.6±1.9***	192.66±1.5***	34.6±0.8**	124±2.08***
Butter	345±2.88***	196±2.08***	33.3±0.8**	277.3±1.4***

NB.:Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

The effect of different *Spirulina* concentrations on liver functions of rats naturally induced hyperlipidemia with 25% Soya bean oil through 21 days

Data presented in Table 6 showed that in the first week, the liver enzyme SGPT was decreased, while liver albumin increased. After 15 days, more decrease in liver enzyme (SGPT, 8.1u/L) was obtained; the liver albumin was nearly the same. The other changes were insignificant. After three weeks, the SGPT and ALK were decreased but liver albumin was increased.

Table 6 - The effect of the treatment with different *Spirulina* concentrations on liver functions of rats naturally induced hyperlipidemia with 25% Soya bean oil through 21 days.

Spirulina%	Bil T	Bil D	SGPT	SGOT	Protein	Albumin	ALK
Liver functions after one week of treatment							
0.0	0.72±0.01*	0.14±0.06*	17±0.6*	8.3±0.4*	7.2±0.2*	4.4±0.12*	8.16±0.2*
2.5	0.71±0.01*	0.13±0.06*	15.7±0.7*	7.6±0.4*	7.8±0.2*	4.3±0.12*	8.5±0.3**
5.0	0.70±0.01*	0.14±0.06*	13.7±0.4*	8.2±0.3*	7.3±0.3*	4.5±0.2*	8.13±0.2*
10	0.71±0.01*	0.13±0.0*	13.3±06**	8.2±0.3*	7.4±0.2*	4.6±0.03*	8.13±0.3*
Liver functions after two weeks of treatment							
0	0.72±0.01*	0.14±0.06*	17.5±0.6*	8.3±0.4*	7.2±0.2*	4.4±0.12*	8.16±0.2*
2.5	0.72±0.02*	0.14±0.006*	16±0.5*	8.1±0.2*	7.7±0.2*	4.5±0.1*	8.4±0.3*
5	0.72±0.01*	0.14±0.006*	12.1±0.4*	8.8±0.2*	7.6±0.2*	4.6±0.1*	8.2±0.3*
10	0.72±0.01*	0.14±0.006*	8.1±0.2**	8.1 ±0.2*	7.4±0.2*	4.6±0.1*	8.4±0.4*
Liver functions after three weeks of treatment							
0	0.72±0.01*	0.14±0.06*	15±0.6*	8.3±0.4*	7.2±0.2*	4.4±0.12*	8.1±0.2*
2.5	0.7±0.02*	0.14 0.006*	13±0.6*	7.01±0.1*	7.1±0.2*	4.5±0.1*	8.4±0.4*
5	0.7±0.01*	0.14±0.006*	12±0.6*	8.03±0.2*	7.2±0.2*	4.6±0.15*	8.3±0.4*
10	0.7±0.02*	0.13±0.006*	8.1±0.3*	7.47±0.2*	7.6±0.2*	4.7±0.15*	7.6±0.2*

NB.:Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

The effect of different concentrations of *Spirulina* on lipid

Table 7 shows the effect of different concentrations of *Spirulina* on serum lipids in the rats. After one week, the serum the levels of CHO, TRI, and LDL were decreased. A significant decrease was observed after the treatment with 10% *Spirulina.* The serum level of HDL was increased significantly as compared to the control rats. After two weeks, most of lipids were decreased by increasing the *Spirulina* doses. In the third week, serum HDL level increased and serum LDL decreased. The significant changes were observed in most lipids profiles, compared with the control. The serum LDL was decreased with highly significant change at P≤0.001.

Table 7 - The effect of three weeks treatment with different *Spirulina* concentrations on serum lipids profiles of rats naturally induced hyperlipidemia and fatty liver with Soya bean oil.

Spirulina %	**CHO**	**TRI**	**HDL**	**LDL**
		Serum lipids after one week treatment		
0	200±5.23*	190.33±4.91*	40±1.2*	115. 7±3.5*
2.5	197.7±7.8*	187.6±5.5*	40.7±1.2*	116±4.04*
5	183.3±7.6**	183±6.2*	40.7 ±1.2*	146.7 ±5.8**
10	183.7±5.5**	179±5.9**	43.7±1.7**	104.7 ±3.2**
		Serum lipids after two weeks treatment		
0	275.7±7.8*	210±5.2*	38.1±1.2*	84.7±2.6*
2.5	160±5.8***	119.8 ±2.6***	45±1.5**	93.7±3.3**
5	158±5.9***	91.3±9.2***	42±1.5*	95±3.8**
10	156±4.4***	71±2.1***	41±1.5*	107±3.4***
		Serum lipids after three weeks treatment		
0	285.3±5.8*	207. 7±6.7*	31.6±2.03*	276.3±4.9*
2.5	153.7±4.2***	175 ±18.03***	35. 6±1.4*	77±1.7***
5	151.3±4.7***	140±2.8***	41.667±1.4**	82±2.5***
10	151. 7±4.3***	127±4.04***	44±1.1**	100±2. 9***

Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05

The effect of different *Spirulina* concentrations on liver functions of rats naturally induced hyperlipidemia with 25% butter

Results in Table 8 demonstrated that total and direct bilirubin were slightly changed at 2.5, 5.0 and 10% *Spirulina* during three weeks. The liver enzymes were decreased and liver protein and albumin were increased. Alkaline phosphates increased only in the second week and decreased again in the third week. It was observed that the 10% concentration of *Spirulina* exhibited the better effect. The change of liver enzyme (SGPT) was highly significant at P≤0.001.

The effect of different *Spirulina* concentrations on lipids of rats naturally induced hyperlipidemia with 25% Butter through 21 days

Results in Table 9 showed that all the lipids profile decreased by increasing the *Spirulina* concentration, except HDL. Serum LDL decreased by 13% less than control at 10% *Spirulina* and HDL increased by 23% more than control. After two weeks of treatment, cholesterol and triglycerides were decreased by 39 and 50% as compared with the control, respectively. LDL was decreased by 43% as compared with the control. Three weeks later, all the lipids profiles were decreased, with the exception of HDL that increased by 53% more than the control. The changes in the third week were highly significant at P≤0.001.

Application on humans

Data present in Table 10 show liver function of 20 patients with the history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with *Spirulina*. Total bilirubin showed no change after one week of the treatment but after two and three weeks, there was decrease in total bilirubin by 0.1% in comparison with the control. Direct bilirubin showed decreases after one, two and three weeks of the treatment by 0.9%. Protein concentration showed no change after one week of the treatment but after two and three weeks, it increased by 0.8 and 2.4%, respectively. Albumin level showed no significant change after one, two and three weeks of *Spirulina* treatment. Alanine Aminotransferase (SGPT) increased after one week of the treatment by 5.3% but after two and three weeks, there was significant decrease by 12.6 and 16.0%, respectively. Aspartate Aminotransferase (SGOT) increased after one week of the treatment by 1.0%;

after two weeks, there was decrease by 2.5% and after three weeks, it increased again by 3.2%. Alkaline phosphatase level showed no changes after one week of the treatment but after two and three weeks, it decreased by 1.0 and by 8.1%, respectively.

Table 8 - The effect of treatment with different *Spirulina* concentrations on liver functions of rats naturally induced hyperlipidemia with butter during 21 days.

Spirulina%	Bil T	Bil D	SGPT	SGOT	Protein	Albumin	ALK
			Serum liver function after one week of treatment				
0	0.70±0.01*	0.15±0.01*	19.3±0.4*	11.8±0.5*	7.6±0.2*	4.4±0.2*	9.2±0.4*
2.5	0.70±0.01*	0.14±0.01*	17.2±0.4*	10.1±0.4*	7.7 ±0.2*	4.2±0.2*	8.2±0.2*
5	0.70±0.01*	0.14±0.01*	15.5±0.3**	8.3±0.4**	7.6±0.2*	4.4±0.2*	9.5±0.3*
10	0.70±0.02*	0.12±0.03*	13.2±0.4***	8.2±0.5**	7.5 ±0.2*	4.6±0.1*	8.1±0.2*
			Serum liver function after two weeks of treatment				
0	0.70±0.01*	0.13±0.003*	14.4±0.3*	8.9±0.3*	7.6±0.2*	4.7±0.1*	9±0.2*
2.5	0.69±0.01*	0.13±0.005*	13.1±0.3*	7.9±0.3*	7.7±0.1*	4.6±0.1*	10.2±0.3*
5	0.71±0.01*	0.15±0.006*	10.4±0.1**	9.06±0.3*	6.8±0.1*	4.6±0.1*	9.2±0.2*
10	0.71±0.01*	0.14±0.006*	7.6±0.2**	7.8±0.2*	7.6±0.2*	4.4±0.1*	9.06±0.3*
			Serum liver function after three weeks of treatment				
0	0.71±.0.2*	0.12±0.01*	17.3 ±0.6*	9.9±0.2*	7.2±0.2*	4.6±0.1*	9.2±0.5*
2.5	0.71±0.2*	0.12±0.01*	17.5±0.5*	8.05±0.1**	7.6±0.2*	4.7±0.1*	8.6 ± 0.6*
5	0.70±0.02*	0.11±0.03*	8.5±0.6***	9.2±0.3*	7.6±0.2*	4.9±0.1*	9±0.4*
10	0.70±0.01*	0.10±0.03*	8.6±0.2***	7.9±0.2**	7.7±0.2*	4.9±0.2*	7.9 ±0.1*

Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

Table 9 - The effect of three weeks treatment with different *Spirulina* concentrations on serum lipids profiles of rats naturally induced hyperlipidemia with butter.

Spirulina %	Cholesterol	TRI	HDL	LDL
		Serum lipids after one week of treatment		
0	358±7	210.33±8.95	30.7±1.2*	286.7 ±8.4
2.5	294±7***	206.66±7.3*	32±1.2*	219.33±6.9***
5	280. 7±7***	204±6.7*	32.66±1.2*	233.33±3.8**
10	270.7 ±5.8***	198±7.7**	38±1.2**	192.3±5.9***
		Serum lipids after two week of treatment		
0	189.4±4.02	162.5±4.3	41.5±1.8	107.3±4.1
2.5	182.5±3.9*	158.6±5.8*	42.2±1.6*	107±4.2*
5	181.0±6.3*	119.3±3.5***	42.2±1.5*	111.33±3.5*
10	116.3±2.3***	80.9 ±3.04***	43.3±1.5*	61.7±2.2***
		Serum lipids after three week of treatment		
0	279.7 ±7.9	212±6.01	30.7 ±0.9	204±4.5
2.5	151.7±4.4***	143.3±3.5***	41.8±1.6***	79.3±2.1***
5	142.7±4.3***	133.3±3.4***	45.6±1.2***	72.03±1.8***
10	122.3±4.3***	107.3±4.2***	47±1.1***	52±1.5***

Each value is the mean of 3 replicates ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05

Table 10 -Collective data of liver function of 20 patients with history of hyperlipidemia at zero time (without treatment) and after 7, 14 and 21 days of treatment with *Spirulina*.

Days	Bil D	Bil T	SGOT	SGPT	Protein	Albumin	Protein	Alk
0	0.15±0.2*	0.73±0.4*	47.7±11.4*	51.4±9.8*	7.06±0.1*	4.08±0.1*	7.06±0.1*	11.2±1.2*
7	0.14±0.1**	0.73±0.4*	48.1±9.7*	54.1±8.6*	7.06±3.2*	4.08±0.08*	7.06±3.2*	11.2±1.2*
14	0.13±0.1**	0.72±0.3*	46.5±9.7*	44.9±5.8**	7.1±0.1*	4.1±0.07*	7.1±0.1*	11.1±1.1*
21	0.13 ±1.4**	0.72±1.5*	49.2±6.7*	38.0±4.8**	7.2±0.1*	4.1±0.1*	7.2±0.1*	10.2±1.09*

NB.:Each value is the mean ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

As evident from Table 11, serum cholesterol level showed highly significant decrease after one, two and three weeks with *Spirulina* treatment (15.12, 23.9 and 28.44%, respectively). Triglycerides level showed highly significant decrease by 10.6, 20.5 and 31.6% after one, two and three weeks, respectively with the treatment. High density lipoprotein level increased significantly by 7.5, 22.0 and 28.0% after one, two and three weeks, respectively. Low density lipoprotein level showed highly significant decrease after one, two and three weeks and was 19.9, 32.2 and 37.7%, respectively.

Table 11 - Collective data of Serum lipids profile of 20 patients with history of hyperlipidemia at zero time (without treatment) and after (7, 14 and 21 days) of treatment with *Spirulina*.

Days	Cholesterol	TRI	HDL	LDL
0	257.9± 7.3*	155.2± 20.7*	36.8± 0.6*	190.4± 7.6*
7	218.9± 6.3***	138.7± 20.6***	39.5± 0.7**	154.2± 6.3***
14	196.2± 7.7***	123.4±2 0.6***	44.9± 2.6***	129.3± 5.1***
21	184.5± 7.09***	106.1± 16.4***	47.1± 1.3***	118.6± 5.6***

NB.:Each value is the mean ± standard error of the mean.
*** Highly significant at P≤0.001, ** Significant at P≤0.01, *Low significant at P≤0.05.

DISCUSSION

Spirulina is cultivated around the world and is used as a human dietary supplement as well as a whole food and is available in form of the tablet, flake, and powder. It is also used as a food supplement in the aquaculture, aquarium, and poultry industries (Wang et al. 2005). There is scientific and clinical evidence for its nutritional value and for its potential health benefits. These benefits attracted its use as a functional food in addition to its already established use as a dietary supplement (Lee 1997; Belay 2002).

The results of the present study showed low significant levels of functional changes in comparison with the control; on the other hand the serum lipids profiles changed after one week of treatment of naturally induced hyperlipidemia with soybean oil. Administration of different *Spirulina* concentrations resulted in significant decreases in total cholesterol, triglyceride and LDL and significant increase in HDL at 5 and 10% of *Spirulina*. A highly significant decrease in total cholesterol, triglyceride and LDL and highly significant increase in HDL at all *Spirulina* concentrations were obtained in the humans and rats. Significant reductions in the triglycerides, total cholesterol, and its fractions, except high density lipoprotein cholesterol (HDL-C) were observed after supplementation of *Spirulina* (Nakaya et al. 1988). Insignificant changes (p <0.05) of liver functions were obtained from soybean oil and butter. The serum cholesterol of the rats fed by oil and butter was increased. The butter gave the highest effect compared with the normal diet. The results showed that liver function and serum lipids profile changed after 15 days with feeding by highly fatty diets (soybean oil and butter); changes of SGPT and SGOT showed highly significant increases. Serum lipids profile (serum cholesterol level, triglycerides and low density lipoprotein) were significantly increased after 15 days after feeding with high fat diets, i.e., soybean oil and butter as compared with the normal diet (Torres-Durana et al. 1999).

In line with previous results (González de Rivera et al. 1993), the present results indicated that *Spirulina* prevented the dietary hypercholesterolemia and arteriosclerosis in the humans. Several reports also suggested that *Spirulina* could have a beneficial effect in the prevention of hypercholesterolemic cardiovascular diseases (Juárez-Oropeza et al. 2009).

Spirulina reduces the level of lipids by reducing cholesterol and serum triacylglycerol levels, where it increased the levels of HDL and decreased LDL. SGPT and SGOT were decreased by increasing the dose of *Spirulina* with time. Becker et al. (1986) and Nakaya et al. (1988) reported that *Spirulina* reduced serum cholesterol (4.5%), triacylglycerol and LDL when volunteers were given 4 g day^{-1} oral dose. In line with these studies, *Spirulina* (4.2 g day^{-1}) was added for eight weeks to the diet of 30 Japanese males with high cholesterol, mild hypertension, and hyperlipidemia. *Spirulina* resulted significant changes in cholesterol and blood pressure, lowered total cholesterol, increased HDL cholesterol, lowered triglycerides, and lowered systolic and diastolic blood pressure (Torres-Duran et al. 2007). A study with diabetic rats concluded that *Spirulina maxima* was effective in correcting the abnormal carbohydrate and lipid metabolisms caused by the excess of fructose within the body **(**Kulshreshtha et al. 2008).

In conclusion *Spirulina* reduced the level of lipids by reducing serum cholesterol and serum triacylglycerol levels. *Spirulina* increased the HDL and decreased LDL and corrected liver function, especially alanine aminotransferase (SGPT) and aminotransferase (SGOT).

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