
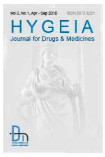

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Anti hyperlipidemic activity of *Spirulina platensis* in Triton x-100 induced hyperlipidemic rats.

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Abstract:

Hyperlipidemia is the greatest risk factor of coronary heart disease. The present study was designed to investigate the antihyperlipidemic activity of Spirulina platensis in Triton X-100 induced Hyperlipidemic rats. Spirulina platensis was administered at a dose of 0.5gm/day, (p.o) to Triton induced Hyperlipidemic rats. Fenofibrate is used as reference standard. The statistical analyses were carried out using one way ANOVA followed by Dunnet's multiple comparison test. S.platensis show a significant decrease in the levels of serum cholesterol, phospholipids, triglycerides, LDL, VLDL and significant increase in the level of serum HDL at the dose of 0.5gm/day (p.o) against Triton induced hyperlipidemic rats. Therefore it effectively suppressed the Triton induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

***Key words*:** *Spirulina platensis*, Hyperlipidemia, Triglycerides, lipoprotein, Triton X-100**.**

1. Introduction

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases (16). Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (5). Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease (17). Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (1). The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurance of further cardiovascular disease or cerebrovascular disease (4). Currently available drugs have been associated with number of side effects (15). The consumption of synthetic drugs leads to hyperuricemic, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (2).

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Certainly, a food, a nutritional supplement or a drug that has documented anti-viral activity as well as an ability to positively affect the immune response would be of considerable clinical interest.

Based on the existing research, *Spirulina* shows great potential in both of these related areas of disease resistance.Unique nutrients found within *Spirulina* once again play an important role in *Spirulina*'s function as an antiviral (8). *Spirulina*(Arthrospira) may have a beneficial effect in the prevention of cardiovascular diseases. Decreases in blood pressure and plasma lipid concentrations, especially triacylglycerols and low density lipoprotein-cholesterol have been demonstrated as a result of oral consumption of *Spirulina.* It has also been shown to indirectly modify the total cholesterol and high density lipoproteincholesterol values (11). A water extract from *Spirulina* may inhibit the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity (6). As the antihyperlipidemic activity of *Spirulina platensis* had not been elucidated in an exclusive Triton induced hyperlipidemic animal model, the present study has been designed to evaluate the lipid controlling activity of *S. platensis* in Triton induced hyperlipidemia in Wistar albino rats.

2. Materials and Methods

Spirulina, a fine dark blue-green spray-dried powder was obtained from the OFERR Nallayan Research Centre, Chennai, Tamil Nadu, India). The composition of the *Spirulina* used in our experiments was made up of proteins (65.38%), crude phycocyanin (15.37%), minerals (7.95%), total carotenoids (4.3 mg/g), β-carotene (1.67 mg/g) and total pheophorbide (0.02%).

2.1. *Chemicals*

Triton X-100(a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer) was obtained from Technicho lab chemicals, Coimbatore. Fenofibrate was obtained from Moral labs, Chennai. All other chemicals were of analytical grade and obtained locally.

2.2**. Preliminary Phytochemical analysis**

The Ethanolic extract of *Spirulina platensis* was subjected to preliminary phytochemical screening (10 & 7).

2.3**.** *Experimental Animals*

Wistar albino adult male rats weighing 200-250g were obtained from the animal house KMCH College of Pharmacy, Coimbatore, India. The animal were grouped and housed in polyacrylic cages (38x 23x 10 cm) with not more than five animals per cage and maintained under standard laboratory under standard laboratory conditions (temperature 25+2oC) with dark and light cycle (14/10 hour). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment.

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

3. Acute toxicity studies

Albino mice weighing 22-25 g selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD- 423 guidelines (acute class method) (3) .The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed for 14 days. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose.

If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/ kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

4. Antihyperlipidemic studies

4.1. Induction of Hyperlipidemia

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h (**9**).The animals were divided into four groups of five rats each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 100mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 5% CMC (p.o) for 7 days. The third group was administered a daily dose of *Spirulina platensis* 0.5g/day suspended in 5%CMC, p.o., for 7 days, after inducing hyperlipidemia. Fourth group was administered with the standard Fenofibrate 65mg/kg, p.o. for 7 days (13).

4.2. **Collection of blood**

On the 8thday, blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected (19).

4.3. **Liver lipid extraction**

The liver was homogenized in cold 0.15M KCl and extracted with CHCl3 CH3OH (2% v/v). This lipid extract was used for the estimation of lipid parameters (14).

4. 4. **Biochemical analysis**

The serum and liver extract were assayed for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) using standard protocol methods (18).

5. Statistical analysis

The results were expressed as mean + S.E.M. Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using Graph pad PRISM software version. P values < 0.05 were considered as statistically significant.

6. Result and Discussion

The cardiovascular benefits of Spirulina use are described in many papers. A review published in 2009 noted several reports suggesting that Spirulina (Arthrospira) may have a beneficial effect in the prevention of cardiovascular diseases. Decreases in blood pressure and plasma lipid concentrations, especially triacylglycerols and low density lipoprotein-cholesterol have been demonstrated as a result of oral consumption of Spirulina.

Spirulina has also been shown to indirectly modify the total cholesterol and high density lipoprotein cholesterol values (12). The ethanolic extract of *S.platensis* was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals.

The Phytochemical tests with the ethanol extract of *S.platensis* indicated the presence of carbohydrates, glycosides, terpenes, saponins, proteins and amino acids. Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values have been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The results are discussed under the lipid profile in serum and the lipid profile in liver. Lipid profile in serum and liver indicates that increased phospholipids (PL), triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 0.5g/day *Spirulina platensis*. LDL and VLDL levels were significantly increased in triton-injected animals to control rats. The results are shown in Tables 1and 2. The *S.platensis* markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that 0.5g/day of *S.platensis* treatment was effective in reduction of cholesterol, PL, TG, VLDL, LDL and HDL in a dose dependant manner.

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Table 1: Effect of *Spirulina platensis* on HDL, LDL, VLDL in Serum of Control and Experimental Rats

Groups	**Parameters**		
	HDL	**LDL**	**VLDL**
Group-I control	51±2.21	162.3±2.32	30.6±1.16
Group-II Triton treated	38.1±3.2*	198.21±3.21*	48.4±2.32**
Group-III Triton + *Spirulina* (0.5g/day)	47.3±1.21**	170.31±5.21**	34.2±2.51**
Group-IV Triton + Fenofibrate	49.1±2.2**	167.3±4.31**	32.4±1.61**

Values are in mean ± SE; Number of animals in each group = 5; *p < 0.05 Vs Group I; ** p <0.05 Vs Group II

Table 2: Effect of *Spirulina platensis* on Cholesterol, Triglycerides, Phospholipids in Serum of Control and Experimental Rats

Groups	**Parameters**	
	Cholesterol	**Triglyceride**
Group-I control	248.5±2.12	151±1.4
Group-II Triton treated	312.2±1.48*	194.2±2.21*
Group-III Triton + *Spirulina* (0.5g)	261.4±3.23**	159.3±1.31**
Group-IV Triton + Fenofibrate	254.3±2.21**	154.3±2.12**

Values are in mean ± SD; Number of animals in each group = 5; * p < 0.05 Vs Group I; ** p < 0.05 Vs Group II

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