EFFECT OF *SPIRULINA PLATENSIS* PRETREATMENT ON ISOPROTERENOL INDUCED HYPERLIPIDEMIA IN RATS

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Summary

The antihyperlipidemic activity of *Spirulina platensis* (family: Oscillatoriacae, cynobacteria/blue green algae) was evaluated in isoproterenol-induced hyperlipidemic rats. In the present study, *Spirulina platensis* powder suspended in water was administered orally to rats for 21 days at the dose of 450 mg/kg/day followed by subcutaneous administration of isoproterenol (200 mg/kg/day for 2 days). Isoproterenol, a synthetic catecholamine and β-adrenergic agonist has reported to cause oxidative stress with subsequent increase in serum lipid profile after subcutaneous administration.
The clinical biochemistry parameters like serum cholesterol, triglycerides, HDL-cholesterol, atherogenic index, VLDL and LDL cholesterol of the rats were determined for the evaluation of antihyperlipidemic activity of *Spirulina platensis.* The minimum alteration in clinical biochemistry parameters were observed in *Spirulina platensis* treated animals as compared to control animals indicating its antihyperlipidemic potential.

Key words: *Spirulina platensis*, Hyperlipidemia, High-density lipoprotein, Low-density lipoprotein, cholesterol, Isoproterenol.

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Cardiovascular disorder has become the leading cause of death worldwide. Epidemiological studies established a direct relationship of serum cholesterol and coronary artery disease [1]. Hyperlipidemia is characterized by increased triglyceride-rich lipoproteins, postprandial lipemia and decreased high-density lipoprotein (HDL) cholesterol [2]. The efforts of lowering lipid levels in different studies indicated lower morbidity and mortality in coronary heart disease which commensurate with reduction of serum cholesterol [3].

Spirulina platensis (family: Oscillatoriacae) is a blue green algae comprising of vital nutrients such as proteins, lipids and carbohydrates. It also contains essential elements like Zinc, Magnesium, Manganese, Selenium, and some vitamins like β–carotene, riboflavin, cyanocobalamine, α-tocopherol and α–linoleic acid. All these minerals, nutrients and vitamins promote physical health, improve the non specific resistance of the body, prevent the disturbances in homeostasis of the human system and promote revival of physiological functions after debilitating diseases. It also exhibits antioxidant activity [4, 5].

Isoproterenol, a synthetic catecholamine and β-adrenergic agonist has reported to cause oxidative stress in the myocardium, resulting in infarct-like necrosis of heart muscle [6]. The pathophysiological changes following isoproterenol administration were compared to human myocardial infarction [7].

Mathew *et al*, reported an altered lipid metabolism in myocardial necrosis following isoproterenol administration [8]. The present study investigated the beneficial effect of *Spirulina platensis* pretreatment in isoproterenol-induced hyperlipidemia in rats.

Methods

Plant product

Spirulina platensis was received as a gift sample in the form of spray dried powder from M/s Parry Nutraceuticals, Ltd., Chennai, Tamilnadu, India. It has also other constituents like pigments, vitamins and minerals.

The composition of the spray dried green powder of *Spirulina platensis* (Constituent/100 g) was as follows:

Major constituents	
Proteins: 60-69% Crude fibres: 9.3%	Fats: 6.7% Carbohydrates: 16%
Vitamins	
Beta carotene: 3,20,000 I.U. Cynocobalamine: 65.7 mg Inositol: 0.018 mg Pyridoxin HCl: 0.39 mg Thiamine: 0.118 mg	Biotin: 0.22 mg Folic acid: 17.6 mg Niacin: 6.69 mg Riboflavin: 1.78 mg Tocoferol (E): 0.733 I.U.
Minerals	
Calcium: 131.5 mg Sodium: 41.2 mg Magnesium: 191 mg Potassium: 1540 mg	Iron: 58 mg Chloride: 440 mg Zinc: 3.9 mg

Animals

Wistar albino rats of either sex (180-220 g) randomly bred in the registered animal house facility of M.U.I.C.T., Mumbai, India were used for the study. The animals were housed in standard environmental conditions of temperature (22±5 ^{0}C) and humidity (55±15 %) with 12-h light-dark cycles. They were fed with commercial pelleted feed (M/s D. S. Trading Co., Mumbai) and water *ad libitum.* All the procedures were performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi, India [9]. The Institutional Animal Ethics Committee of M.U.I.C.T., Department of Pharmaceutical Sciences, approved the experimental protocol.

Experimental Design

The rats were divided into two groups of 12 animals each. Group 1 was treated as a control and received purified water (10 ml/kg/day for 21 days) by oral route. Group 2 received suspension of *Spirulina platensis* powder in water at the dose of 450 mg/kg/day, orally for a period of 21 days. At the end of 21 days, the rats from group 1 were divided in subgroups I and II and animals of group 2 were divided in subgroups III and IV. The treatments received in different groups were as follows.

I) Control group II) Control with Isoproterenol (200 mg/kg, s.c.), III) *Spirulina platensis* (450 mg/kg) and IV) *Spirulina platensis* (450 mg/kg) with isoproterenol (200 mg/kg, s.c.)

Induction of Hyperlipidemia

To induce hyperlipedemia, animals from group II and IV received Isoproterenol (Sigma, USA, 200 mg/kg, s.c.) twice at an interval of 24 hours [10].

Blood Collection

After the experimental period (i.e. 24 hour after second administration of isoproterenol) the blood was collected through retro-orbital plexus under light ether anesthesia in the eppendorf tubes. The serum was separated by spinning in temperature-controlled centrifuge (Superspin R-V/F_M, Plasto Crafts, India). The serum from group I and III were also separated similarly.

Serum Lipid profile

The serum cholesterol, triglycerides and HDL cholesterol were estimated in serum by using kits procured from Merck Ecoline®, Mumbai, India. The serum total lipids were determined by using commercially available kit manufactured by Span Diagnostics Ltd., Sachin, Surat, India. The LDL cholesterol and VLDL were calculated using Friedwalds formula [11] and method of Godkar [12]**.**

VLDL = Triglyerides/5

LDL cholesterol = Cholesterol – (HDL Cholesterol + VLDL/5)

Statistical analysis

The data were expressed as the mean ± S.E.M. obtained from the number of experiments (n=6). One-way ANOVA with Tukey's test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software [13], San Diego California USA, www.graphpad.com. The statistical significance was accepted at $p<0.05$.

Results

Effect of *Spirulina platensis* on cholesterol, triglycerides and total lipids

The serum concentration of cholesterol, triglycerides and total lipids was decreased significantly ($P<0.01$) in *Spirulina platensis* pretreated (21 days) groups as presented in table 1 compared to isoproterenol treated control.

Table 1 Effect of *Spirulina platensis* pretreatment (450 mg/kg, p.o.) on cholesterol, triglycerides and total lipids.

Groups	Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total Lipids (mg/dl)
I	Control	103.4±6.26	65.8±4.91	451.8±14.38
II	Isoproterenol control (200 mg/kg s.c.)	135.0±4.63	114.1±5.43	485.6±13.85
III	*S. platensis* (450 mg/kg, p.o.)	104.5±6.32 [a]	61.3±4.87 [b]	452.0±14.32
IV	*S. platensis* (450 mg/kg, p.o.) + isoproterenol (200 mg/kg, s.c.)	109.4±5.21 [a]	82.2±5.16 [a]	455.6±13.37 [b]

Values are expressed as mean ± S.E.M. from 6 animals. Statistical analysis by one-way ANOVA followed by Tukey's test using Graphpad Prism software; [a]$P < 0.01$ and [b]$P < 0.05$ compared to isoproterenol control (200 mg/kg, s.c.).

Effect of *Spirulina platensis* on LDL and HDL cholesterol and LDLc/HDLc risk factor

The serum concentration of LDL cholesterol was elevated due to isoproterenol administration in group II but it was not increased in III and IV as the *Spirulina platensis* pretreated group. HDL cholesterol was also increased significantly (P<0.01) in *Spirulina platensis* treated groups compared to isoproterenol control. The increase in HDL cholesterol and decrease in LDL cholesterol results in reduction of cardiovascular risk factor LDLc/HDLc in *Spirulina platensis* treated group compared to isoproterenol control. The results are mentioned in table 2.

Table 2 Effect of *Spirulina platensis* pretreatment (450 mg/kg, p.o.) on HDL cholesterol, LDL cholesterol, and LDLc/HDLc risk factor.

Groups	Treatment	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Risk factor LDLc/HDLc
I	Control	20.6±1.45	34.6±1.45	1.7±0.19
II	Isoproterenol control (200 mg/kg s.c.)	14.2±1.46	36.4±1.69	2.6±0.26
III	*S. platensis* (450 mg/kg)	22.2 ±1.23 [a]	44.2 ±1.25 [a]	2.0±0.13
IV	*S. platensis* (450 mg/kg) + isoproterenol (200 mg/kg, s.c.)	21.5±1.38 [a]	35.1±1.55 [b]	1.6±0.15 [b]

Values are expressed as mean ± S.E.M. from 6 animals. Statistical analysis by one-way ANOVA followed by Tukey's test using Graphpad Prism software; [a]P < 0.01 and [b]P <0.05 compared to isoproterenol control (200 mg/kg, s.c.).

Effect of *Spirulina platensis* on atherogenic index and VLDL fraction

The calculated VLDL fraction of lipid profile was lessen in *Spirulina platensis* treated group subsequently the atherogenic potential also reduced significantly (p<0.01) compared to isoproterenol control. The atherogenic index was calculated from the following formula,

$$\text{Atherogenic Index [14]} = \frac{\text{Cholesterol} - \text{HDL Cholesterol}}{\text{HDL Cholesterol}}$$

Table 3 Effect of *Spirulina platensis* pretreatment (450mg/kg, p.o.) on atherogenic index and VLDL fraction.

Groups	Experimental Groups	Atherogenic index	VLDL fraction (mg/dl)
I	Control	4.2±0.57	13.1±0.58
II	Isoproterenol control (200 mg/kg s.c.)	7.4±0.99	22.8±0.68
III	*S. platensis* (450 mg/kg)	3.8±0.30 [a]	12.2±0.57 [b]
IV	*S. platensis* (450 mg/kg) + isoproterenol (200 mg/kg s.c.)	4.2±0.37 [a]	16.4±0.63 [b]

Values are expressed as mean ± S.E.M. from 6 animals. Statistical analysis by one-way ANOVA followed by Tukey's test using Graphpad Prism software; [a]P < 0.01 and [b]P<0.05 compared to isoproterenol control (200 mg/kg, s.c.).

Discussion

The levels of lipid profile and atherosclerosis risk is reduced in animals pretreated with *Spirulina platensis* for 21 days are presented in table 1, 2 and 3. *Spirulina Platensis* pretreatment and subsequent exposure to isoproterenol results in minimum alteration of cholesterol, triglycerides, Low and high-density lipoprotein.

In isoproterenol administered rat group II, cholesterol and triglycerides, LDL and VLDL fractions increased significantly with a decrease in HDL cholesterol. In groups IV rats (*Spirulina platensis* + isoproterenol treated) the alterations in the levels of cholesterol and triglycerides, LDL, HDL and VLDL fractions were minimum. HDL alters the balance of unesterified cholesterol between plasma and cells by increasing its utilization in lecithin cholesterol acyl transferase system to form cholesterol ester, which moves rapidly back into cells [15].

The twice isoproterenol (200 mg/kg s.c.) administration results in significant increase in serum cholesterol, triglycerides, total lipids and LDL cholesterol. Increased levels of circulating cholesterol fractions and its accumulation in heart tissue are well associated with cardiovascular damage [8]. Hypertriglyceridemia was seen in isoproterenol-treated rats, condition observed in ischemic heart disease, and is due to a decrease in the activity of lipoprotein lipase in the myocardium resulting in decreased uptake of triglycerides from circulation [9]. The administration of isoproterenol mainly raised LDL cholesterol and decreased HDL cholesterol level in serum [16].

Spirulina platensis affect on lipoprotein lipase activity and hepatic triglyceride lipase activities in post-heparin plasma were studied in fructose-induced hyperlipidemic rats [17]. The presence of novel protein C-phycocyanin (C-PC) present in *Spirulina platensis* may involve in the inhibition of both jejunal cholesterol absorption and ileal bile acid reabsorption [18]. It is found that the aqueous extract of *Spirulina platensis* may inhibit the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity [19]. C-PC, one of the major phycobiliproteins of *Spirulina platensis* (a blue-green alga), which is involved in first increased cyclic GMP/VASP Ser157 phosphorylation and subsequently inhibits protein kinase C activity, resulting in inhibition of both P47 phosphorylation and intracellular Ca^{2+} mobilization, and secondly may inhibit free radicals (such as hydroxyl radicals) released from activated platelets, which ultimately inhibits platelet aggregation. This maintains blood homeostasis in experimental animals [20].

The multicomponent spray dried powder of marine algae, *Spirulina platensis* contains substantial amount of gamma-linolenic acid (GLA) may prevent accumulation of cholesterol and is also established clinically [21]. One of the earlier studies on the reduction of serum cholesterol by *Spirulina* was that done on rats by Devi and

Venkataraman. Since then several workers have confirmed antihyperlipidemic studies involving animals and humans [22].

Spirulina platensis has been proved beneficial in treatments like hepatoprotective effect in anti-tubercular drug induced hepatotoxicity in rats [23]. The effect of *Spirulina platensis* powder was successfully evaluated for cholesterol-induced hypercholesterolemia by Japanese scientist in rats [24]. The spray-dried powder contributes to the treatment in hyperlipidemic condition and in combination of many important medicinal plants it can be recommended for the wide use.

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