JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Individual and combined free radical scavenging response of *Spirulina* with combination of *Ginger* against HFD induced hypercholesterolemia

Rajesh Pandey and Surendra Singh
Algal Biotech Lab, Department of Post Graduate Studies and Research in Biological Sciences,
Rani Durgavati University, Jabalpur, (M.P.), India
rajeshrdu29@gmail.com

ABSTRACT

Spirulina is free-floating filamentous microalgae consumed as food and widely used as a nutraceutical food supplement worldwide. The nutritional value of *Spirulina* is well recognized with its unusually high protein and richness in vitamins, minerals, essential fatty acids, and other nutrients. Recently, great attention and extensive studies have been devoted to evaluating its therapeutic benefits on an array of diseased conditions including hypercholesterolemia, Preclinical studies with various animal models consistently demonstrate the hypolipidemic activity of *Spirulina.* Similarly, some plants have a hypocholesterolemic response which *Ginger* is well known. In the present investigation, *Spirulina* (microalga) and *Ginger* (plant) both alone and in combinations were evaluated against the HFD-induced hypercholesterolemic rat. Experiments were conducted in classified eight groups as n=4. Hypercholesterol induced on 40 days HFD administration and treatment was done through prepared individual and *Spirulina* and *Ginger* extract combination. GSH, SOD, TBARS, and Catalase antioxidants were examined from collected blood samples.

Spirulina exhibited a maximum decline of lipid per oxidation among individual therapies and in combinations, *Ginger* and *Spirulina* showed (13.63%), (21.05%) recovery action of Reduced Glutathione (mg/ml.), in combination 16.74% increasing response was accounted. Ginger (27.78%), *Spirulina* (31.6%), and a combination of both showed (22.37%) declined in beneficial response of Thiobarbituric acid

(g/ml). Likewise the SOD (unit/min/mg protein) level increases with *Ginger* (50%) and *Spirulina* (44.23%) and (18.75%) in combination. Catalase (mol/min/mg protein) increased the recovery response of *Ginger* (23.23%), *Spirulina* (21.95%), and in combination was shown (31.38%). The finding of the experiment suggested a potential free radical scavenging response of *Spirulina* with a combination of *Ginger* against HFD-induced hypercholesterolemia through alone and in combination therapy.

Key words: Antioxidant enzymes, *Spirulina*, Ginger, HFD, Hypercholesterol, and combination therapy

Introduction

Spirulina is free-floating filamentous microalgae consumed as food and widely used as a nutraceutical food supplement worldwide. Among functional foods, *Spirulina*, blue-green filamentous cyanobacteria of the genus *Arthrospira*, where *A. platensis* and *A. maxima* are the most common species cultured worldwide [1], has been considered by various international health organizations as one of "the greatest food on earth [2]. The nutritional value of *Spirulina* is well recognized with its unusually high protein content and its richness in vitamins, minerals, essential fatty acids and other nutrients. It has a well-balanced distribution of essential amino acids [3], *Spirulina* contain antioxidant and most of the organic compounds and enzymes that slow down oxidative damage, which results primarily from decreased oxygen states. This leads to the production of reactive oxygen species like superoxide radical anion, hydrogen peroxide, the hydroxyl free radical, and singlet oxygen [4]. The foremost enzymes that restrict oxidative damage are superoxide and catalase which are present in *Spirulina* along with polyphenols which act as superior antioxidants [5]. Some of the other antioxidants include water soluble ascorbate (vitamin C) and lipid-soluble α-tocopherol (vitamin E) and carotenoids such as astaxanthin.

The blue-colored pigment phycocyanin has been reported to have significant antioxidant, anti-inflammatory, hepatoprotective and broad-spectrum radical scavenging properties. This pigment can be easily extracted out from the cell and can be incorporated into food products. Pure phycocyanin can have higher therapeutic value for treatment of various disorders. Besides, being a natural compound it is least toxic. Studies show that phycocyanin stimulates production of white blood cells and red blood cells [6]. Carotenoids are the second most important group of pigments found in algae. They play a role as lipophylic antioxidants and they are thought to be responsible for the therapeutic property of carotene as anticancer agent [7]. Other species found are glutathione peroxidase. Mycosporine like amino acids, mainly considered as UV screening compounds, are also antioxidants that act as scavengers and quenchers of reactive oxygen species in algae [8].

Ginger

Recently, great attention and extensive studies have been devoted to evaluating its therapeutic benefits on an array of diseased conditions including hypercholesterolemia, Data from preclinical studies with various animal models consistently demonstrate the hypolipidemic activity of *Ginger*. hypoglycemia, hypolipidemia and hypocholesterolaemia properties In vivo clinical studies concerned to combined *Garlic* and *Ginger* impact showed hypolipidemic response [9]. Dyslipidemic actions of Ginger studies in case of induced diabetes through the preparation of water extract [10]. Crude extracts of *Ginger* rhizome were reported for potential antibacterial action [11]. Hypolipidaemic properties of *Ginger* in streptozotocin-induced diabetic conditions were reported [12]. Antioxidant properties studies with various ginger preparation [13, 14]. Antithrombotic and antiinflammatory action of ginger suggested a potential response through decreasing the status of lipid profile [15, 16]. *In the present investigation Spirulina (microalga) and Ginger (plant) both alone and in combinations were evaluated against the HFD-induced hypercholesterolemic rat.*

Material and Methods

Source of *Spirulina* and *Ginger*

Exponentially growing cells of *S. platensis* were harvested by filtration (screen-printing filter with pore size 305 nm (1400 pore/cm^2) and the biomass was oven dried at 55^{o}C. The dried biomass was collected, weighed, and used to feed the rats.

Ginger (rhizome) was collected from the Department of Aromatic and Medicinal plants, Jawaharlal Nehru Agriculture University, Jabalpur, India. The ginger was washed several times with tap water (Potable water). Then, it was peeled, rewashed using tap water, sliced into small sizes of 2-3mm diameter thick, and dried in a vacuum oven at 60-65°C for 72hrs [17]. The dried ginger was blended into powder using a kitchen blender. The powder obtained was then sieved using a sifter of pore size ≤ 300µm. The sieved ginger powder was put in plastic bags and stored at room temperature. Ginger extracts was obtained from ginger powder and ginger paste by modified methods [18].

Preparation of individual *Spirulina, Ginger* and their combination

Individual *Spirulina* prepared by mixing of 25g of *Spirulina* powder with 250ml of water to make a concentration of 100mg/ml solution.

Suspension of *Ginger* was prepared with certain modify method [15]. 500g of fresh *Ginger* was dried in shade and used for the aqueous extraction. *Ginger* rhizome was peeled on crushed ice and 25g was cut into small pieces and homogenized in 100ml cold (4^{o}C) distilled water.

Homogenization was carried out in blender at high speed (3000rpm) for 2-3min. The homogenized mixture was filtered through cheese cloth. The concentration of *Ginger* preparation was considered to be 250mg/ml on the basis of the weight of starting material (25g/100ml).

Combination of *Spirulina* with *Ginger* prepared through mixing of 25g of *Spirulina* powder with 100ml of aqueous extract of *Ginger*. Both were mixed with 400ml of distilled water to make a concentration of 50mg/ml each. Prepared suspensions and combination were stored (4^{o}C) in sterilized brown glass bottles tightly fixed and well labelled.

Source of experimental animals:

Healthy male Wistar albino rats, *Rattus norvegicus* (strain 2187), weighing about 180-200gm, age 6-8 week, brought from animal section of DRDE (Defence Research Development and Establishment), Gwalior, India.

Experimental groups:

Following eight groups of animals were subjected to different treatment schedules. Each group consisted of four animals (n=4).

Group I : Normal Control

Group II : Animals with continuous HFD

Group III : HFD Control

Group IV : Daily treatment of 250mg *Spirulina*/kg body weight.

Group V : Daily treated by 250mg *Spirulina* along with HFD.

Group VI : Daily treated by 250mg Ginger plant/kg body weight.

Group VII : Daily treated by 250mg Ginger plant/kg body weight along with HFD

Group VIII : Daily treated by 250mg Combination of *Spirulina* with Ginger/kg body weight.

Experimental normal and High Fat Rich Diets:

Two varieties of diet were used in the present part of study. The composition of both diets Control or Normal diet (NC) and High Fat Rich Diet (HFD) (Table 1)

Table 1: Constituents % in Normal control and high fat rich diet

Constituents	gm/Kg Normal Control diet	gm/Kg High fat Rich Diet
Wheat flour	225	**205**
Roasted Bengal gram powder	600	**526**
Skimmed milk powder	50	**50**
Casein	40	**40**
Refined oil	40	**40**
Salt mixture with starch	40	**40**
Choline mixture	5	**5**
Coconut oil	0	**40**
Cholesterol	**0**	**4**

Experimental conditions:

Complete process of investigation approved through the departmental animal ethical committee. All animals were housed in group of six and maintained under standardized condition (12/12 hour's light and dark cycle at 24°C with free access to pellet feed (Hindustan Liver Limited Mumbai, India) and water, adlibitum.

Induction of hypercholesterol and collection of blood sample:

Hypercholesterol induced in the course of regular 40 days administration of HFD, which comprised cholesterol, refined oil, skimmed milk powder, casein, coconut oil etc. The blood was drawn from the retro-orbital bleeding. The animals in all groups were kept on 4-5h fasting periods before collections of blood samples. The blood was collected by heparinised capillaries kept on heparinised tubes and in tubes containing EDTA (an anticoagulant, 2mg ml^{-1}) and without EDTA. Collected blood samples were centrifuged for 10min at 5000rpm at 4^0C. The serum were collected and stored at 4^0C for further exploration.

Antioxidant enzymes:

Enzymatic and non enzymatic antioxidant both were examined after haemolysate preparation which was clearly free from plasma and buffy coat through removal of whole blood with centrifugation at 2000rpm for 10min, at 4^0C. Haemolysate was prepared by mixing 1.9ml of cold distilled water to 0.1ml of PCV suspension. Prepared haemolysate was used for estimation of TBARS and Catalase, while for the estimation of SOD, the residual red cells were also haemolysed in adding 1.5 volume of cold distilled water. The lipids were removed by chloroform-ethanol extraction. The assessment of Reduced Glutathione (GSH) was performed [19]. TBARS was estimated [20]. Catalase and SOD were estimated through standard method [21,22].

Chemicals and Regents:

Epinephrines, Tetra Ethoxy Propane (TEP), NADPH, BSA, TBA, DTNB, Sodium metasulphate, Reduced Glutathione were purchased from Sigma Chemical Company (St. Louis, USA). Analytical grade of Phenazine metasulphate, Sodium azaide, Sodium hydroxide were used from Sisco Research Lab, Mumbai, India.

Result and Discussion:

The present investigation deals with the antioxidant status of combination and individual action of *Spirulina* and ginger on normal and hypercholesterolemic rats. Herbal drugs or formulations containing number of free radical scavenging agents are well known for their therapeutic activity. Prepared combination showed different levels of protection which is slightly differentiated from each other.

Combination impact of SP+GN on TBARS activity of HFD induced hypercholesterolemia

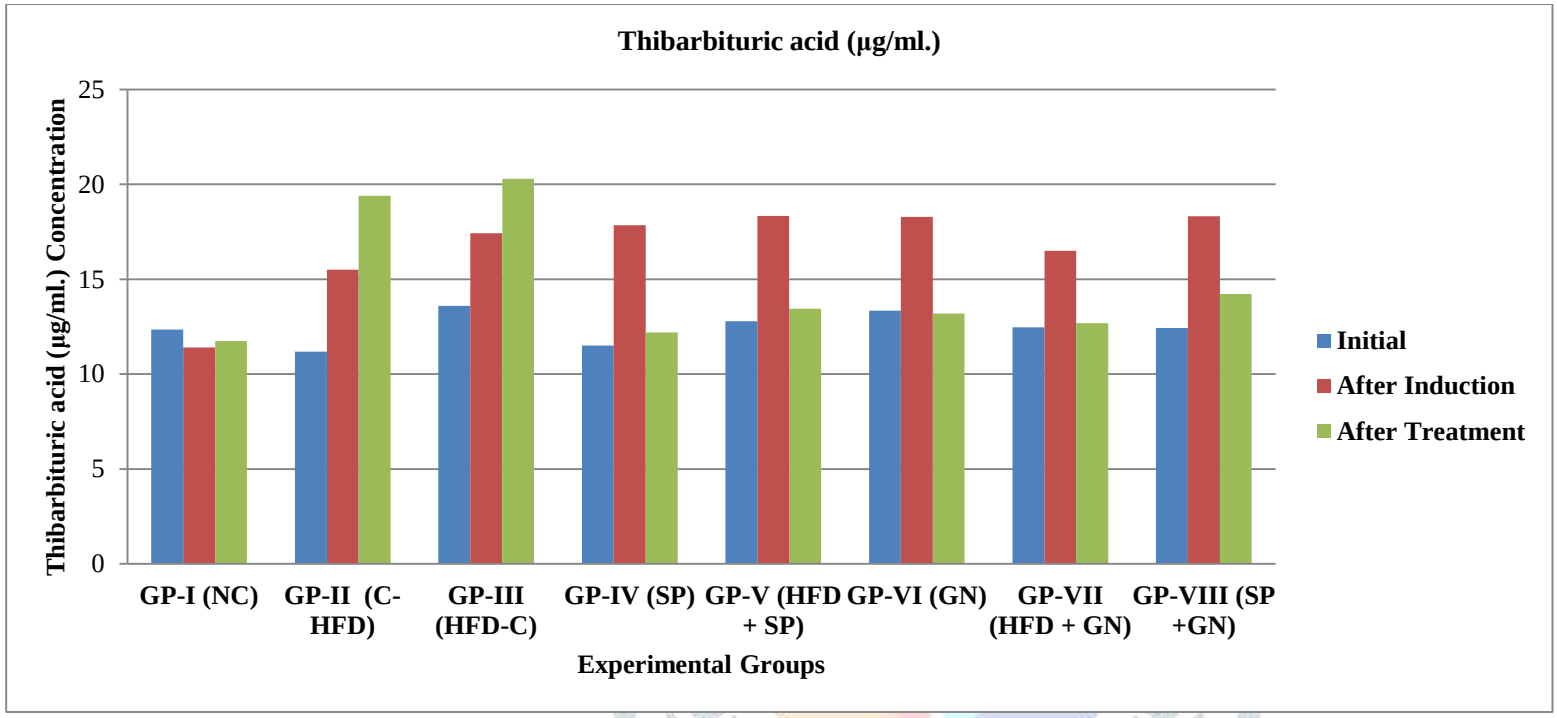
Free radicals are known to be generated during period of hypercholesterolemia. Changes in status of antioxidants in hypercholesterolemic state and their response provide a new insight about the pathogenesis. Antioxidant enzymes TBARS, GSH, SOD and CAT were studied before and after the therapy. A significant increase in TBARS levels were observed in all experimental subjects. Independently *Spirulina*, and combined treatment SP+GN significantly brought down the TBARS levels in same experimental treated groups. TBARS levels increased 57% from base line with continue consumption of HFD, which was decreased by about 33.8% via administration of *Spirulina*, 27.82% through *Spirulina* along with HFD.

Spirulina contain active ingredients, notably phycocyanin and β-carotene that have potent antioxidant and anti-inflammatory activities [23]. Phycocyanin has the ability to scavenge free radicals, including alkayl hydroxyl and perxyl radicals. It also decreases nitric production, suppresses inducible nitric oxide syntheses expression, and inhibits liver microsomal lipid per-oxidation [24]. Treatments along with HFD showed only marginal changes in TBARS activity as compared to individual treatment GN and *Spirulina.* The therapy of *Ginger* and *Spirulina* and their impact on TBARS level was also shown decreasing impressive results which was slightly differentiated. Administration of *Ginger* showed 27.78% reduction; while *Ginger* therapy with HFD gave 23.15% result whereas 22.3% decreased level of TBARS was observed by treatment of SP+GN (Table-1).

A marked decrease in lipid per-oxidation accompanied with an increase in endogenous TBARS levels [25]. In 250mg/kg dose of individual plants and combination showed a significant decrease in the level of TBARS, and it showed better recovery profiles in all experimental treated groups.

Table 1. Effect of *Spirulina, Ginger* and combination on TBARS activity in HFD induced hypercholesterolemic rats.

Groups	Thibarbituric acid (µg/ml.)		
	Initial	After Induction	After Treatment
GP-I (NC)	12.35 ± 0.48^{ab}	11.40 ± 0.44^{a} (-7.69%)	11.75 ± 0.72^{a} (+2.95%)
GP-II (C-HFD)	11.18 ± 0.43^{a}	15.50 ± 0.62^{b} (+38.64%)	19.40 ± 0.16^{c} (+25.16%)
GP-III (HFD-C)	13.60 ± 0.17^{b}	17.42 ± 0.57^{b} (+28.08%)	20.30 ± 0.12^{c} (+16.53%)
GP-IV (SP)	11.50 ± 016^{ab}	17.84 ± 0.25^{b} (+55.13%)	12.20 ± 0.40^{a} (-31.6%)
GP-V (HFD + SP)	12.78 ± 0.62^{ab}	18.33 ± 1.09^{b} (+43.42%)	13.44 ± 0.35^{a} (+26.67%)
GP-VI (GN)	13.34 ± 1.05^{a}	18.28 ± 0.56^{b} (+37.03%)	13.20 ± 0.81^{a} (-27.78%)
GP-VII (HFD + GN)	12.46 ± 0.67^{a}	16.50 ± 1.34^{b} (+32.42%)	12.68 ± 0.37^{a} (-.23.15%)
GP-VIII (SP + GN)	12.44 ± 1.05^{a}	18.32 ± 0.23^{b} (+47.26%)	14.22 ± 1.35^{a} (-22.37%)



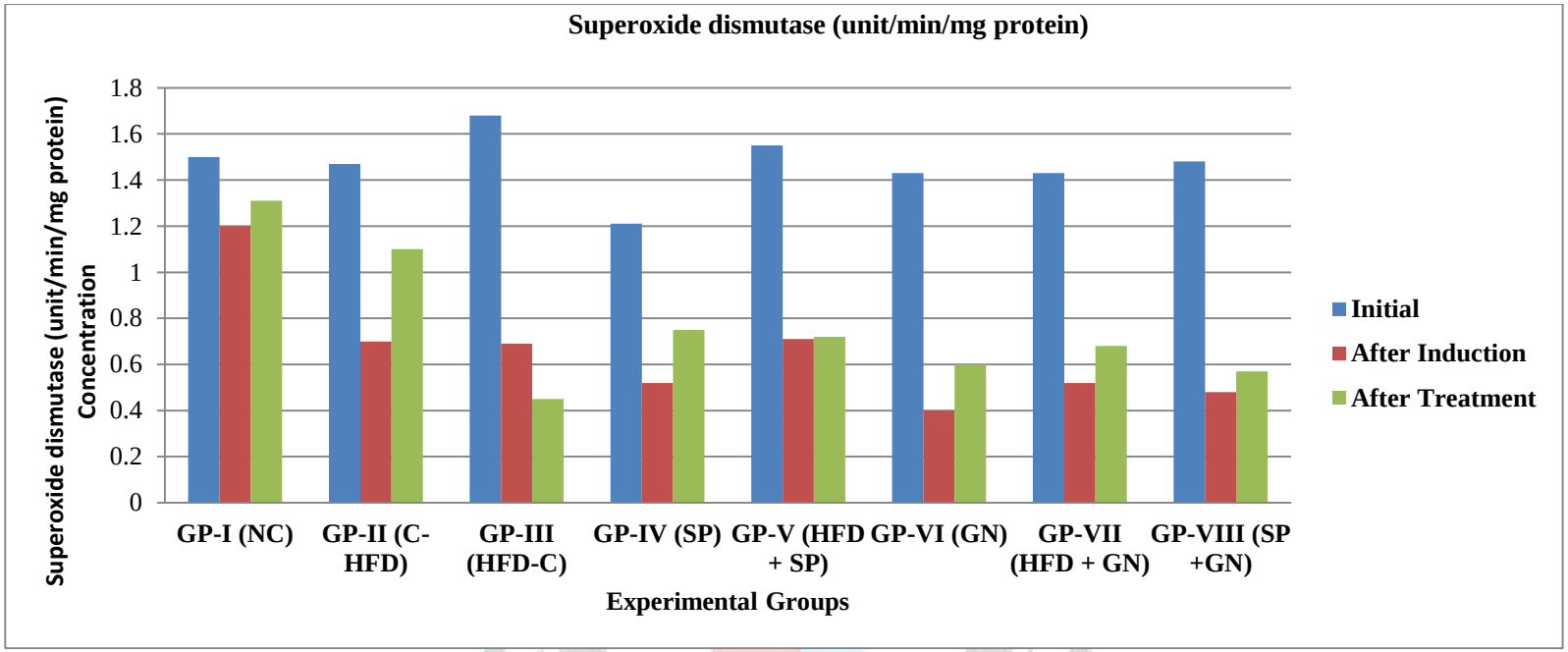
Combination impact of SP+GN on SOD activity of HFD induced hypercholesterolemia

The SOD activities were considerably decreased in HFD induced rats. The significant decrease in SOD activity due to HFD indicates inefficient scavenging of reactive oxygen species which might be implicated to oxidative inactivation of enzymes [26] SOD activities were increased significantly ($p<0.05$) about 44.23% by *Spirulina*. Results indicated that treatment of individual *Spirulina* provided better comeback as compared to individual *Ginger*. β-carotene, vitamin E and selenium worked as the membrane antioxidants and protected against single oxygen mediated lipid per-oxidation [27,28]. The *Ginger* treated hypercholesterolemic subject was shown 50% increasement, 30% enhancement was observed by *Ginger* along with HFD, whereas 18.7% increase SOD activity was recorded with SP+GN action. SOD is believed as a front of protection alongside the possible free radicals that grounds oxidative stress. Present learning highlighted that rats which accepted regular HFD till one month be evidence for a significant diminish in the SOD activity; the results validate with evidences [29].

Ginger alone may exhibit activity of blocking oxidative damage through hydroxyl lipid per-oxidation and protein oxidation which must have avoided the loss of membrane permeability and dysfunction of cellular protein and in turn a decline in the endogenous stage of hydroxyl radical [30, 31]. SOD detoxifies superoxide radicals and converts them to H_2O_2 which is additional converted to H_2O as a result of action of CAT or GSH peroxides. The observed results indicated, the occurrence of potential comprising constituents may be responsible to scavenge the super oxide anion radicals and thereby maintain the high activity of SOD (Table 2).

Table 2: Effect of *Spirulina, Ginger* and combination on SOD activity in HFD induced hypercholesterolemic rats.

Groups	Superoxide dismutase (unit/min/mg protein)		
	Initial	After Induction	After Treatment
GP-I (NC)	1.50 ± 0.24^{a}	1.20 ± 0.07^{b} (-20%)	1.31 ± 0.19^{a} (+9.16%)
GP-II (C-HFD)	1.47 ± 0.12^{a}	0.70 ± 0.03^{a} (-52.3%)	1.10 ± 0.21^{a} (+57.14%)
GP-III (HFD-C)	1.68 ± 0.18^{a}	0.69 ± 0.04^{a} (-58.92%)	0.45 ± 0.03^{a} (-34.78%)
GP-IV (SP)	1.21 ± 0.11^{a}	0.52 ± 0.04^{a} (-57.02%)	0.75 ± 0.45^{a} (+44.23%)
GP-V (HFD + SP)	1.55 ± 0.28^{a}	0.71 ± 0.06^{a} (-54.19%)	0.72 ± 0.17^{a} (+1.40%)
GP-VI (GN)	1.43 ± 0.21^{a}	0.40 ± 0.12^{a} (-772.02%)	0.60 ± 0.05^{a} (+50%)
GP-VII (HFD + GN)	1.43 ± 0.21^{a}	0.52 ± 0.08^{a} (-63.63%)	0.68 ± 0.02^{b} (+30.76%)
GP-VIII (SP + GN)	1.48 ± 0.15^{a}	0.48 ± 0.10^{a} (-67.56%)	0.57 ± 0.03^{a} (+18.75%)



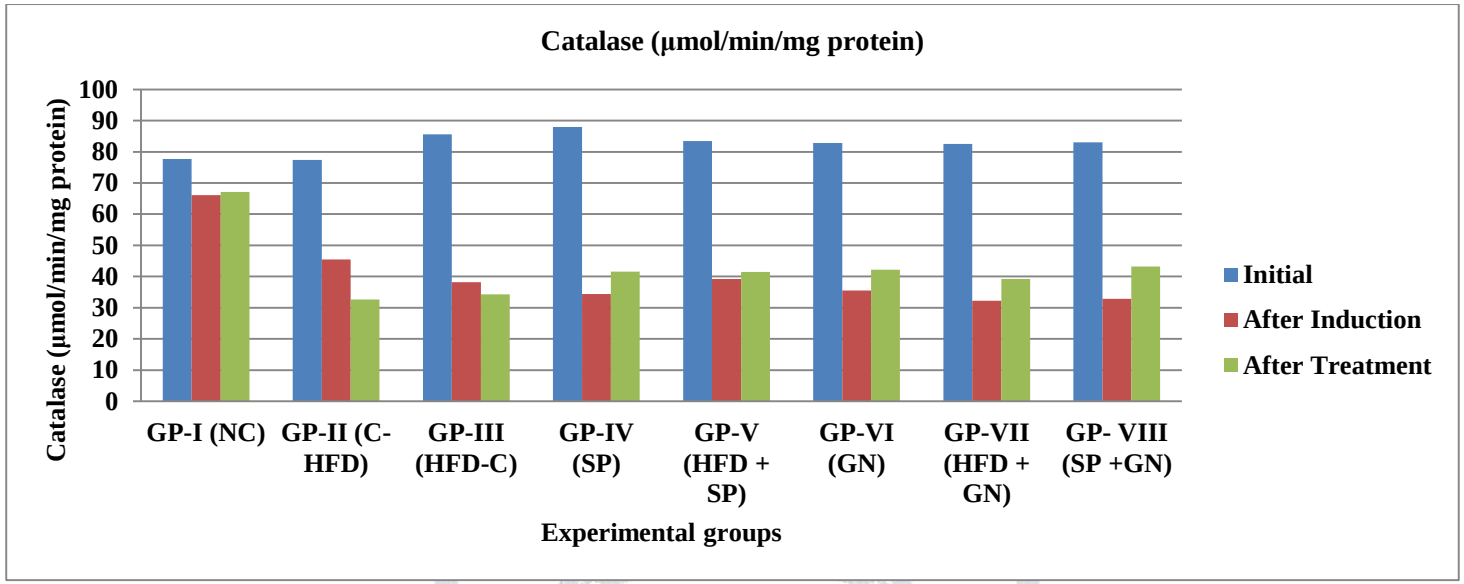
Combination impact of SP+GN on CAT activity of HFD induced hypercholesterolemia

The CAT activity was diminished by means of the consumption of HFD in experimental hypercholesterolemia. CAT activity was elevated in all hypercholesterolemic groups, except normal control following different therapy. The levels were increased significantly to 21% by *Spirulina*, 5.9% via *Spirulina* along with HFD. *Ginger* treated group was shown 23% increase in CAT activity, 31% raised level of CAT was establish via *Ginger* by way of HFD while 16% raised level was observed by the action of GN+SP (Table-3). Catalase is vital enzyme that functions together with super oxide dismutase and glutathione per-oxidase from side to side enzymatic antioxidant defense management, catalyses the disintegration or decomposition of hydrogen peroxides to water and oxygen to defend cells against O_2 toxicity and lipid per-oxidation [32, 33]. These compounds supported in the reduction of spontaneous lipid per-oxidation. Hypercholesterolemia impairs systemic vascular reactivity in response to endothelium-dependent vasodilators, which may be mediated partly through increased formation of lipid peroxides [34]. Decreased activities of Catalase in vital organs have been observed in rats, and this activity may result in a number of deleterious effects due to accumulation of superoxide radicals (O_2-) and hydrogen peroxide [35]. Administration of *Ginger* and combination with Spirulina increases the activities of Catalase in rats. The results of increased Catalase activity of *Ginger* clearly shows that a free radicals scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of O_2- and OH^+ [36]. The scavenging components from natural sources are promising candidates for drugs of hypercholesterol, depending on their reactivity toward free radicals, localization, mobility in lipoprotein and fate of its radicals. Groups of rats which received *Ginger* or *Spirulina* for a period of 5 weeks showed

significant elevation in CAT activity which indicates the antioxidant properties of *Ginger* as well as *Spirulina* with due to their potential free radicals scavenging activity.

Table 3: Effect of *Spirulina, Ginger* and combination on Catalase activity in HFD induced hypercholesterolemic rats.

Groups	Catalase (µmol/min/mg protein)		
	Initial	After Induction	After Treatment
GP-I (NC)	77.70 ± 1.35^{a}	66.10 ± 0.5^{e} (-14.92%)	67.16 ± 0.37^{e} (+9.61%)
GP-II (C-HFD)	77.44 ± 7.58^{a}	45.50 ± 1.07^{d} (-41.2%)	32.60 ± 0.16^{a} (-28.35%)
GP-III (HFD-C)	85.58 ± 2.51^{a}	38.16 ± 0.43^{c} (-55.4%)	34.30 ± 0.35^{a} (-10.11%)
GP-IV (SP)	87.94 ± 0.63^{a}	34.35 ± 0.31^{a} (-62.1%)	41.55 ± 0.80^{c} (+21.95%)
GP-V (HFD + SP)	83.42 ± 0.26^{a}	39.17 ± 0.25^{c} (-53.0%)	41.51 ± 0.84^{c} (+6%)
GP-VI (GN)	82.86 ± 0.50^{b}	35.51 ± 1.27^{b} (-58.65%)	$42.22 \pm .0.30^{b}$ (+23.23%)
GP-VII (HFD + GN)	82.50 ± 0.24^{b}	32.28 ± 0.30^{a} (-60.64%)	39.20 ± 0.22^{a} (+21.43%)
GP- VIII (SP + GN)	83.03 ± 0.51^{b}	32.88 ± 0.11^{a} (-60.3%)	43.20 ± 0.46^{b} (+31.38%)



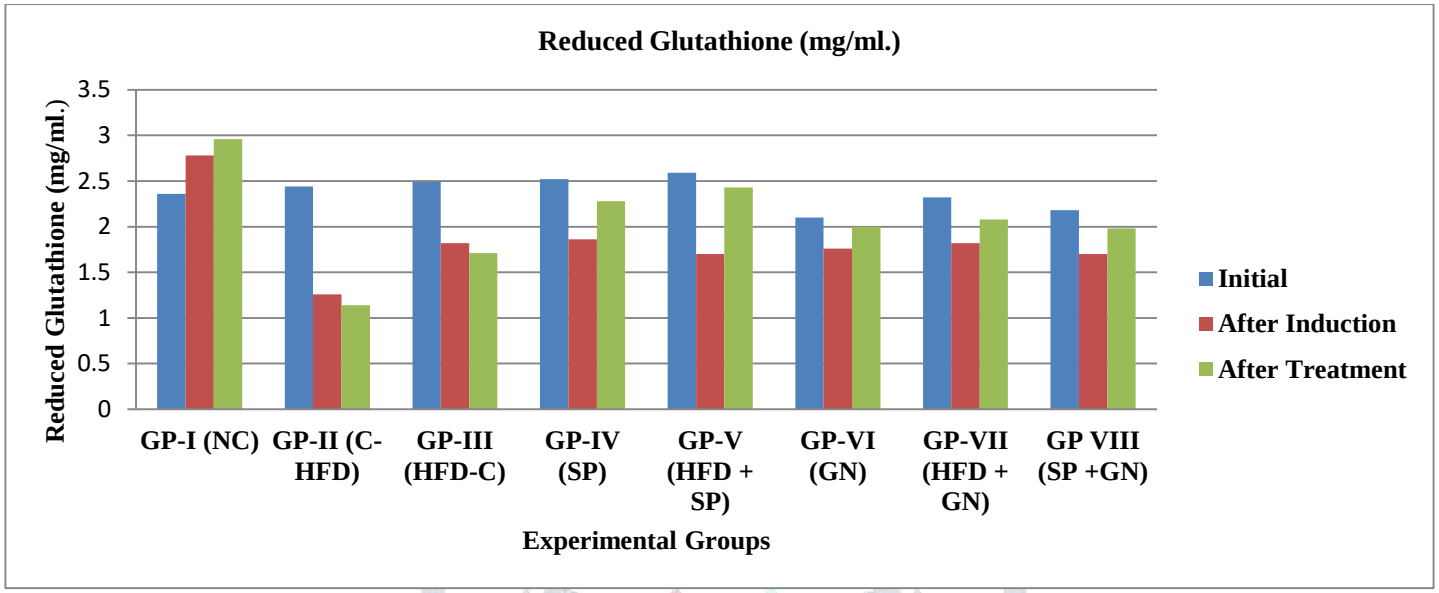
Impact of Combination of SP+GN on GSH activity of HFD induced hypercholesterolemia

The GSH activity is noteworthy affected in all experimental HFD induced hypercholesterolemic rats. Enzymatic antioxidants are inactivated via free radicals. Vitamin C, reduced glutathione and other thiols are water soluble antioxidants that eliminate free radicals from cytosol via reacting with them. Glutathione acts as a substrate for Glutathione per-oxidase and Glutathione-s-transferase and Glutathione oxidase during the breakdown of H_2O_2 and lipid per-oxidation. Attention of glutathione is regulated through Glutathione synthetase, Glutathione per-oxidase and Glutathione reductase [37, 38]. GSH protects the cell against oxidative stress by reacting with peroxides and hydro-peroxides. The GSH activity was elevated in all groups following different therapy. The levels were increased significantly by about 21% by *Spirulina,* 22.5% by *Spirulina* along with HFD. Selenium of *Spirulina* provided glutathione per-oxidase activity and causes reduction of hydrogen peroxide to toxic elements. Observations also reported in *Spirulina* and other related algae [39,40]. Hypercholesterol increases the oxidative stress and decreased the level of GSH in rats may increase their susceptibility to oxidative injury. *Ginger* treated hypercholesterolemic rats were shown 13.6% increase GSH activity, 14% raised level of GSH was observed by *Ginger* along with HFD whereas 16% raised level was observed with *Spirulina* with *Ginger* action (Table-4). *Ginger* showed strong in-vitro and in-vivo antioxidant properties performed a major possible mechanism for the protective actions against toxicity of free radicals [41]. The reduction of oxidized form of glutathione requires NADPH, as a cofactor and enzyme glutathione reductase. The reduced availability of NADPH, which could be either due to reduced synthesis or increased metabolism of NADPH through some other pathway, could be also responsible for less range of GSH in HFD induction. Selenium is a main component of glutathione per-oxidase present in *Spirulina* (GPx) causes reduction of H_2O_2 to nontoxic elements. *Spirulina* carotenoids and phycocyanin are well known antioxidants and performed mechanisms for hepatoprotective activity [42]. *Spirulina* phycocyanins express potent peroxyl radical scavenging response in case of both in vitro and in vivo observation [43,44]. Reduction in TBARS level In this study the results was showed the activity of GSH was significantly decreased with HFD consumption, the reduction in GSH activity may be implicated to either free radical dependant inactivation of enzyme or depletion of its co-substrate i.e. GSH and NADPH. Antioxidant also supported to exogenous cholesterol inhibition activity and the up regulating expression of 3-hydroxy-3-methyl glutaryl coenzymes A reductase activity [45,46].

Table 4. Effect of *Spirulina, Ginger* and combination on GSH (Reduced Glutathione) level in HFD induced hypercholesterolemic rats.

Groups	Reduced Glutathione (mg/ml.)		
	Initial	**After Induction**	**After Treatment**
GP-I (NC)	2.36 ± 0.22^{a}	2.78 ± 0.72^{a} (+17.7%)	2.96± 0.72^{a} (+6.47%)
GP-II (C-HFD)	2.44 ± 0.39^{a}	1.26 ± 0.10^{a} (-48.36.%)	1.14 ± 0.10^{a} (-7.93%)
GP-III (HFD-C)	2.49 ± 0.32^{a}	1.82 ± 0.64^{a} (26.90%)	1.71 ± 0.64^{a} (-6.04%)
GP-IV (SP)	2.52 ± 0.33^{a}	1.86 ± 0.23^{a} (-26%)	2.28± 0.23^{a} (+21.05%)
GP-V (HFD + SP)	2.59 ± 0.16^{a}	1.70 ± 0.28^{a} (-35.76%)	2.43± 0.14^{ab} (+22.54%)
GP-VI (GN)	2.10 ± 0.18^{a}	1.76 ± 0.09^{a} (-16.19%)	2.0 ± 0.09^{ab} (+13.63%)
GP-VII (HFD + GN)	2.32 ± 0.12^{a}	1.82± 0.07^{a} (-21.55%)	2.08 ± 0.31^{ab} (+14.28%)
GP VIII (SP + GN)	2.18 ±. 0.14^{a}	1.70 ± 0.11^{a} (-22%)	1.98 ± 0.31^{ab} (+16.47%)

Abbreviations: (GP) - Group, (NC) - Normal Control, (C-HFD) - Continuous High Fat Diet, (HFD-C) - Control High Fat Diet, (SP) - *Spirulina*, (HFD + SP) *Spirulina* along with High Fat Diet, (GN) - *Ginger*, (HFD + GN) *Ginger* along with High Fat Diet, (SP +GN) Spirulina with combination of Ginger. (Unit: mg/ml) Values are expressed as mean ± SE, n=4. The same letters are not significantly different (P≤0.05) by Tukey test.



The activity of GSH was significantly increased with *Spirulina*, and *Ginger* alone and with combination. In this respect, used of medicinal plants and *Spirulina* may be a particularly useful agent, as it could enhance endogenous antioxidants without producing any cytotoxic effects. *Spirulina* have a synergistic effect, so their antioxidant effects increase. As a result, we can say that, combined therapy decreases oxidative or free radicals damages with the balancing of enzymatic and non-enzymatic antioxidant status.

(4). Conclusion

Spirulina exhibited maximum decline of lipid per-oxidation among individual therapies and in combinations, In case of Reduced Glutathione (mg/ml.) individually action of *Ginger* and *Spirulina* was showed (13.63%) and (21.05%) recovery action whereas in combination therapy was shown 16.74% increasing response. *Ginger* (27.78%), *Spirulina* (31.6%) and combination of both were showed (22.37%) decreasing beneficial Thibarbituric acid (µg/ml.) response. Likewise the level of Superoxide dismutase (unit/min/mg protein) increase with *Ginger* (50%) and *Spirulina* (44.23%) and in combination therapy (18.75%). Catalase (µmol/min/mg protein) increasing recovery response of *Ginger* (23.23%), *Spirulina* (21.95%) and in combination was shown (31.38%). The above facts concludes that even every constituents have their own antioxidant values when tested individually, also shows potential free radical scavenging properties in combination too. *Spirulina* found to be effective formulating with medicinal plants like Ginger and boosting them to maintain such property in a synergistic way. *Spirulina* may increase safety and efficacy in combinational therapies which can't be gained through individual treatment. Further studies are needed to elucidate the exact cooperative action of various constituents present in combinations acting for the antioxidant status. Present study reveals that combination shows potential antioxidant activity.

Funding: This research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sectors.

Acknowledgement: Nil

Conflicts of interest: The authors have no conflict of interest to declare

References

[1]. Zhang, F. Man, Y.B. Mo, W.Y. 2020. Application of *Spirulina* in aquaculture: a review on wastewater treatment and fish growth, *Review Aquaculture*. 12 (2), 582-599,

[2]. Bhattacharya, S. and Shivaprakash, M.K. 2005. Evaluation of three Spirulina species grown under similar conditions for their growth and biochemicals, *J of Science and Food Agriculture.* 85 (2), 333-336,

[3]. E. Koru, Earth food *Spirulina* (Arthrospira): Production and quality standards, In Tech (2012).
[4]. Colla LM, Muccillo-Baisch AL, Costa JAV. 2008. *Spirulina platensis* Effects on the levels of total Cholesterol, HDL and triacylglycerols in Rabbits fed with a hypercholesterolemic diet. *Brazilian Archive Biology and Technolgy*, 51:405-411.

[5]. Nakaya N, Homa Y, Goto Y. 1988. Cholesterol lowering effect of *Spirulina*. *Nutr Rep Int.* 37:1329-1337.

[6]. Torres-Duran PV, Ferreira-Hermosillo A, Juarez-Oropeza MA. 2007. Antihyperlipemic and antihypertensive effects of *Spirulina maxima* in an open sample of mexican population: a preliminary report. *Lipids Health Disease.* 6(33):1-8.

[7]. Ramamoorthy A, Premakumari S. 1996. Effect of supplementation of *Spirulina* on hypercholesterolemic patients. *Journal of Food Science and Technology*, 33:124-128.

[8]. Mani UV, Desai S, Iyer U. 2000. Studies on the long-term effect of *Spirulina* supplementation on serum lipid profile and glycated proteins in NIDDM patients. *Journal of Nutraceutical Functional & Medical foods*. 2:25-32.

[9]. Ahmed, R. and Sharma, S. 1997. Biochemical studies on combined effect of garlic *Allium sativum* Linn and ginger *Zingiber officinale* Rosc in albino rats, *Indian Journal of Experimental Biology,* 35, 841-843.

[10]. Bhandari, U., Kanojia, R. and Pillai, K.K. 2005. Effect of ethanolic extract of *Zingiber officinale* on dyslipidaemia in diabetic rats. *Journal of Ethnopharmacology*, 972, 227-230.

[11]. Kaushik, P. and Goyal, P. 2011. Evaluation of Various Crude Extracts of *Zingiber officinale* Rhizome for Potential antibacterial activity: A study *in Vitro, Advances in Microbiology*, 1,7-12.

[12]. Al-Amin, Z.M., Thomson, M., Al-Qattan, K.K., Peltonen-Shalaby, R. and Ali, M. 2006. Anti-diabetic and hypolipidaemic properties of ginger *Zingiberofficinale* in streptozotocin-induced diabetic rats, *British Journal of Nutrition* 964, 660-666.

[13]. Aruoma, O.I., Spencer, J.P., Warren, D., Jenner, P., Butler, J. and Halliwell, B.1997. Characterization of food antioxidants illustration using commercial garlic and ginger preparation, *Food chemistry*, 60, 49-156.

[14]. ICMR, 2003. Ginger: its role in xenobiotics metabolism. *ICMR Bulletin*, 33 6, 57-63.

[15]. Thomson, M., Al Qattan, K.K. and Al Sawan, S.M. 2002. The use of ginger *Zingiber officinale* Roscoe as a potential antiflammatory and antithrombotic agent, *Prostaglandins Leukot Essential Fatty Acids*, 676, 475-478.

[16]. Samuels R, Mani UV, Iyer UM, Nayak US. 2002, Hypocholesterolemic effect of *Spirulina* in patients with hyperlipidemic nephrotic syndrome. *Joutnal Medicine Food.* 5:91-96

[17]. Olubunmi, B.A., Seun, F.A. and Funmilayo, T.A. 2013. Food value of two varieties of ginger *zingiberofficinale* commonly consumed in Nigeria, *Hindawi publishing corporation. ISRN Nutrtition.*

[18]. Kaushik, P. and Goyal, P. 2011. Evaluation of Various Crude Extracts of *Zingiber officinale* Rhizome for Potential Antibacterial Activity: A Study *in Vitro,Advances in Microbiology*, 1,7-12.

[19] Ellman, G.L. (1959) Tissue Sulfhydryl Groups. *Archives of Biochemistry and Biophysics*, 82, 70-77.

[20]. H Ohkawa, N Ohishi, K Yagi Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction *Anal Biochem* 1979, 95(2):351-8.

[21]. Asru K. Sinha 1972. Analytical Biochemistry, 47 2,389-394.

[22]. Winterbourn CC. The estimation of red cell superoxide dismutase activity. *J Lab Clin Med. 1975*, 85(2):337-341.

[23]. C Romay 1998 Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. *Inflamm Res.* 47(1):36-41.

[24]. Tomas Moreira, Maria Manconi, Jehzabel Pendas. Nurys Ledon (2009) Phycocyanin liposomes for topical anti-inflammatory activity: In vitro in-vivo studies *J.Ppharancy and Pharmacology* 61(4):423-30.

[25]. Karadeniz A, Yildirim A, Simsek, N, Kalkan Y, Celebi F (2008). *Spirulina platensis* protects against gentamicin-induced nephrotoxicity in rats. *Phytotherapy Res*., 22: 1506-1510

[26]. Sharma MK, Sharma A, Kumar A, Kumar M (2007). *Spirulina fusiforms* provides protection against mercuric chloride induced oxidative stress in Swiss albino mice. *Food. Chem. Toxicol*., 45: 2412-2419.

[27]. Lian Liu, Anva Miron, Blanka Klimova, Dan wan and Kamil Kuca (2016). The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: an overview Archives of Toxicology volume 90, 1817-1840. (2016)

[28]. Mendi, S.D. 2011. Developpement of bambirad a ginger - spiced cheese as a functional food. PhD thesis, ENSAI – The University of Ngaoundere, Cameroon.

[29]. Watson AD, Berliner JA, Hama SY, et al. Protective effect of high density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest.*1995; *96*:2882–2891.

[30]. Goldberg A, Alagona P, Capuzzi DM, et al. Multiple-dose efficacy and safety of an extended-release form of niacin in the management of hyperlipidemia. *Am J Cardiol.* 2000; 85:1100-1105.

[31]. Peter C (2008). Antioxidant potential of *Spirulina platensis* preparations. *Phytotherapy Res.*, 22: 627-633

[32]. Haksar A, Sharma A, Chawla R, Kumar R, Lahiri SS, Islam F, Arora MP, Sharma RK, Tripathi RP, Arora R. Study of the hypoglycemic activity of Lepidium sativum L. aqueous extract in normal and diabetic rats *Phytother Res*. 2009, 23(2):293-6.

[33]. Ruitang Deng and Te-Jin Chow Hypolipidemic, Antioxidant and Antiinflammatory Activities of Microalgae *Spirulina* Cardiovascular Therapy 2010 28(4): 33-45.

[34]. Seo JS, Lee KS, Jang JH, Quan Z, Yang KM, Burri BJ (2004). The effect of dietary supplementation of β-carotene on lipid metabolism in streptozotocin-induced diabetic rats. *Nutr. Res.*, 24: 1011-1021.

[35]. Pari L, Amarnath Satheesh M.J Ethnopharmacol. 2004 Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes Mar;91(1):109-13.

[36]. Kumar M, Sharma MK, Kumar A (2005). *Spirulina fusiformis.* A food supplement against mercury induced hepatic toxicity. J. Health. Sci., 51: 424-430.

[37]. Nagaoka S, Shimizu K, Kaneko H, Shibayama F, Morikawa K, Kanamaru Y, Otsuka A, Hirahashi T, Kato T. A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of Spirulina platensis concentrate in rats. *J Nutr.* 2005;135:2425–2430.

[38]. Parikh P, Mani U, Iyer U. Role of *Spirulina* in the Control of Glycemia and Lipidemia in Type 2 Diabetes Mellitus. *J Med Food.* 2001;4:193-199.

[39]. Devi MA. and Venkataraman LV. Hypocholesterolemic effect of blue-green algae *Spirulina platensis* in albino rats. Ann Nutr Reports Int. 1983; 28:519-530.

[40]. Iwata K, Inayama T. and Kato T. Effects of Spirulina platensis on plasma lipoprotein Effects of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. *J Nutr Sci Vitaminol*. 1990;36 (2):165-171.

[41]. Ganesh Chandra Jagetia, Annie Shriwaikar, Shaval K Rao, Prasad M Bhilegaonkar J. Mint oil offers behavioral radioprotection: a radiation induced conditional taste aversion study. *Pharm Pharmacol.* 2003, 55(8):1151-8.

[42]. Eddouks M, Maghrani M, Zeggwagh NA, Michel JB.*J Ethnopharmacol*. 2005, 28;97(2):391-5.

[43]. Bhat VB, Madyastha KM. Scavenging of Peroxynitrite by Phycocyanin and Phycocyanobilin from Spirulina platensis : Protection against Oxidative Damage to DNA. *Biochem Biophys Res Comm.* 2001; 285:262-266.

[44]. Huuskonen J, Ehnholm C. Phospholipid transfer protein in lipid metabolism. *Curr Opin Lipidol*.2000; 11:285-289.

[45]. Antonio M. Gotto JR. 2001 Low High-Density Lipoprotein Cholesterol as a Risk Factor in Coronary Heart Disease, *Circulation. 103*:2213-2218

[46]. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens.* 2000;18:655-673.