RESEARCH ARTICLE

Comorbid Cholesterol (2%) Diet-Induced Atherosclerosis and Isoproterenol Hydrochloride Induced Myocardial Infarction

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ABSTRACT

Cardiovascular diseases remain a primary cause of morbidity and mortality globally, with atherosclerosis and myocardial infarction playing significant roles. Using a rabbit model, this experimental investigation aimed to explore the combined effects of coexisting conditions, specifically a 2% cholesterol diet-induced atherosclerosis and isoproterenol hydrochloride-induced myocardial infarction. New Zealand white rabbits were utilized for this study, leveraging their metabolic similarity to humans, which has been pivotal in lipid-lowering drug research. Hypercholesterolemic rabbits have notably contributed to the discovery of statins, the primary treatment for hyperlipidemic patients worldwide. After 12 weeks of treatment, rabbits subjected to a high-fat diet (HFD) and Isoproterenol hydrochloride (ISO) exhibited significant increases in cholesterol, triglyceride (TG), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and other atherogenic markers compared to normal rabbits. In contrast, *Spirulina* treatment (33 mg/kg B.wt/66 mg/kg b.wt.) markedly reduced cholesterol, TG, VLDL, LDL, and improved high-density lipoprotein (HDL) levels while enhancing cardiac antioxidant activity. Our findings underscore *Spirulina'*s potential in alleviating diet and ISO-induced atherosclerosis and cardiac toxicity, suggesting its promise for preventing atherosclerosis-associated cardiac injury in patients.

Keywords: 2% Cholesterol diet, Atherosclerosis, Isoproterenol hydrochloride, Myocardial infarction, *Spirulina*.

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INTRODUCTION

Health outcomes and medical expenses are negatively affected by comorbidity in clinical management. The term's definition is ambiguous, as are related terms like multimorbidity, morbidity burden, and patient complexity. This discourse aims to clarify the definitions of comorbidity and explore how they are interconnected with other concepts. As clinicians, epidemiologists, and health services planners and financiers, it is crucial to evaluate the effectiveness of a construct in analyzing specific phenomena. Several factors contribute to the occurrence of two or more conditions in the same patient simultaneously, including direct causation, shared risk factors, heterogeneity, and independence. The article also discusses the implications of therapeutic management. We propose that constructs can be used to improve clinical care, epidemiology, and health care delivery by facilitating a more precise application of constructs.[1] Cholesterol elevates the risk of cardiovascular disease, leading to atherosclerosis development.[2] It appears that reactive oxygen species (ROS) play a role in hypercholesterolemic atherosclerosis, even though the exact mechanism is unknown.[3] The reduction of cholesterol and oxidative stress has, therefore, been promoted as a method of preventing atherosclerosis.[4,5]

Myocardial infarction is the leading cause of death worldwide, making it an important global health concern. Globally, heart disease and stroke are anticipated to become the leading causes of death and disability by 2020. The anticipated number of fatalities is expected to surpass 20 million annually, escalating further to over 24 million by 2030.[6] This concerning trend is particularly challenging for developing nations such as India, where the simultaneous impact of infectious diseases and the rising burden of non-communicable diseases, including myocardial infarction, places strain on societal and healthcare systems. It is estimated that myocardial infarction manifests 10 to 15 years earlier in India than it does in Western countries and that a growing number of young Indians succumb to this condition.[7] Notable abnormalities, including lipidemia, peroxidation, and compromised plasma membrane integrity, mark the aftermath of myocardial infarction. This highlights the urgency for comprehensive strategies to address the increasing prevalence of myocardial infarction and its associated complications in diverse populations, particularly

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in regions grappling with a dual burden of infectious and non-communicable diseases.[8]

Natural products have long been a cornerstone in the discovery of many modern drugs, driving extensive chemical, pharmacological, and biological screening efforts worldwide. Cyanobacteria *Spirulina* thrives in highly alkaline and saline environments. As a food source with significant nutritional value, *Spirulina* enjoyed considerable popularity in the Americas, Asia, and Central Africa.[9] *Spirulina* is characterized by its multicellular cylindrical trichomes that have an open helix pattern. A microscope usually reveals segmented trichomes with discernible cross walls, typically with a relatively large diameter and occasionally tapering at the ends. It has also been used as a nutritional supplement and dietary staple for centuries.[10] This genus' dry weight is approximately 70% protein, and it contains 4000 mg/kg of carotenoids, omega-3 and omega-6 polyunsaturated fatty acids, gamma-linolenic acid (GLA), sulfolipids, glycolipids, polysaccharides, vitamins, and minerals.[11,12]

Spirulina is non-toxic and can be consumed as a food without any side effects, according to WHO.[13,14] Various animal species have shown protection from drug and pesticide toxicity by consuming *Spirulina*, with antioxidants and anti-inflammatory properties.[15-21] This study examines the effect of *Spirulina platensis* alkaloid extract on hypercholesterolemic male rabbit lipid profiles.

In space missions, NASA successfully used *Arthrospira*, also known as *Spirulina*, as a dietary supplement for astronauts. The producer of *Arthrospira platensis* is known as *Arthrospira platensis*. The toxins produced by mast cells are effectively reduced by this blue-green alga, which is immune-modulating and anti-inflammatory. Various studies, including randomized controlled trials and systematic reviews, have investigated *Spirulina*'s efficacy in addressing diverse diseases, indicating potential symptom improvements and possible anticancer, antiviral, and antiallergic effects.[22,23] *Spirulina* is a minute filamentous cyanobacterium characterized by its spiral or helical structure. Its historical usage as a food source traces back to the Aztec civilization. With a protein content of 70%, *Spirulina* is impressively nutritious, with vitamins B12 and provitamin A, minerals (particularly iron), phenolic acids, and tocopherols. Since *Spirulina*'s cell walls do not contain cellulose, digestion is easier.[24-26] *Spirulina* thrives in alkaline lakes or controlled outdoor ponds with high pH levels. *Spirulina* is a dietary supplement currently marketed as a health drink or tablet and is primarily found in health food stores.[27] In spite of its hype as "the food of the future" that contributes to increased energy levels, *Spirulina*'s effectiveness has been questioned. A polysaccharide and an essential fat may aid in energy release. In a placebo-controlled randomized trial, *Spirulina* and placebo did not significantly differ in fatigue scores. When administered at a dose of 3 g/day, *Spirulina* failed to significantly reduce chronic fatigue compared to a placebo, suggesting a limited impact.[28]

In a number of cardiovascular diseases, oxidative stress plays a significant role.[29,30] When excessive amounts of reactive oxygen species (ROS) are produced, the body's internal antioxidant defenses cannot kee*p* up, leading to cell death.[31,32] Nitric oxide (NO), a vasodilator, is diminished by heightened ROS levels, making heart function more difficult both physiologically and pathologically.[33] By modulating the vascular tone and protecting the heart from ischemic injury, endothelial nitric oxide synthase (eNOS) produces NO under controlled conditions.[34] Conversely, excessive NO levels, which are facilitated by induced nitric oxide synthase (iNOS), allow myocardial contractility to be diminished and ROS production to be exacerbated, ultimately leading to cardiomyocyte apoptosis.[35,36]

Its antithrombotic, anti-inflammatory, antioxidant, and anti-inflammatory effects make rosuvastatin an effective medication for reducing lipid levels and lipoprotein cholesterol.[37] Studies have shown that rosuvastatin can prevent experimentally induced myocardial infarction (MI) by left coronary artery occlusion in rats.[38] Strawberry, pomegranate, and grape polyphenols elagic acid exhibit a number of biological effects, such as antioxidants, antihyperlipidemic, anti-inflammatory, and hepatoprotective properties.[39,40] It has been observed that experimentally induced MI may be prevented by it.[41]

It is crucial for the normal function of the heart to have adequate levels of catecholamines. As a synthetic catecholamine, isoproterenol, at toxic doses, can cause myocardial necrosis resembling MI in humans by producing ROS.[42]

Relationships between comorbid diseases

An individual with comorbid conditions has at least two medical conditions at the same time. The presence of comorbid diseases can significantly impact disease progression, treatment outcomes, and overall health. Here are some relationships between comorbid diseases

Diabetes mellitus and cardiovascular disease

Heart-related disease are associated with diabetes mellitus. Cardiovascular complications are exacerbated by diabetes and worsen outcomes for individuals with existing and cardiovascular disease (CVD).[43,44]

COPD and CVD

Smoking, inflammation, and aging are all common risk factors for COPD and cardiovascular diseases. As a consequence of COPD, cardiovascular events and mortality are more likely to occur, while cardiovascular diseases worsen COPD's symptoms and prognosis.[45,46]

Depression and cardiovascular disease

In many cases, depression is associated with cardiovascular diseases, as well as the reverse. Individuals with cardiovascular diseases who suffer from depression are less likely to adhere to treatment, are more likely to develo*p* complications, and are more likely to die. The development or exacerbation of depression can be caused by cardiovascular disease inversely.[47,48]

CVD and chronic kidney disease

Cardiovascular events are the leading cause of mortality in individuals with chronic kidney disease (CKD), and CKD is a significant risk factor for developing cardiovascular disease. Cardiovascular diseases worsen outcomes in the presence of CKD, and kidney dysfunction can exacerbate cardiovascular diseases.[49,50]

Rheumatoid arthritis and cardiovascular disease

A number of diseases and conditions increase cardiovascular risk, including coronary artery disease and heart failure. Chronic inflammation shared risk factors, and potential effects of rheumatoid arthritis (RA) treatments contribute to the higher prevalence of cardiovascular events in individuals with RA.[51,52]

Pathways to Comorbidity

Various factors contribute to the overall health status of both populations and individuals, encompassing genetic and biological traits at the individual level and the broader political and policy landscape. These factors collectively influence the development of specific diseases and are thus anticipated to also influence the occurrence of coexisting diseases. It is reasonable to assume that diseases may cluster within an individual if they share common underlying influences or if the individual's resilience or vulnerability is altered. However, there are additional explanations for such clustering.

Chance, selection bias, and causal association are all possible mechanisms that may explain the presence of different diseases within the same individual. Comorbidity arising from chance or selection bias lacks a direct causal link but remains significant as it can lead to misconceptions about causality. For instance, the co-occurrence of two diseases may simply be a matter of statistical probability. For instance, in a population where 4% have type 2 diabetes and 5% have eczema, approximately 0.2% of the population (0.04 * 0.05 = 0.002) would be expected to have both conditions purely by chance. Therefore, any meaningful association would need to deviate significantly from this expected estimate.

Alternatively, selection bias presents another explanation. Berkson noted that disease clusters were more common among patients seeking healthcare compared to the general population in his early observations of selection bias. This observation likely stemmed from individuals seeking medical care being more likely to receive a diagnosis, regardless of the condition. To mitigate this bias, utilizing community samples rather than solely relying on patients attending healthcare services can provide more accurate insights.

MATERIALS AND METHODS

Chemicals

Spirulina was purchased from Urban Platter, Rosuvastatin from Yarrowchem, Mumbai, and all other analytical-grade chemicals were purchased from the Sisco Research Laboratory.

Animals Used

For this study, 24 male laboratory rabbits were procured from Vyas Labs, Hyderabad, and their weights ranged from 1500 to 1600 grams. A 10-day acclimatization period was conducted with the rabbits living in specially constructed cages and eating bread and vegetables. As part of the environmental control, the temperature and humidity were maintained at 25°C and 60%, respectively.

Hypercholesterolemia Inducing

An experiment was conducted in which 18 male rabbits were randomly divided into two groups, while six rabbits were divided into two groups as well. Two weeks of oral injection of soluble cholesterol (BDH product) were administered to the first grou*p* to induce hypercholesterolemia. Moreover, a 0.9% concentration of normal saline was administered as a negative control grou*p* to the second group.[53]

Experimental Procedure

This study used male rabbits aged 8 to 10 weeks from an inbred strain, such as white New Zealand. Within acceptable limits, there is no restriction on body weight variation. Blood samples are taken from the marginal ear vein to determine total cholesterol, glycerides, and HDL levels, as shown in Figure 1. After this transition, all groups except the control grou*p* and positive control I were maintained on a diet enriched with 2% cholesterol for a period of 12 weeks. All other groups, except grou*p* I, receive two consecutive subcutaneous injections of Isoproterenol hydrochloride at the end of their treatment periods. Isoproterenol hydrochloride was administered in two doses. All animals are euthanized 24 hours after the second dose. Induction of anesthesia is achieved by intraperitoneal administration of xylazine (6 mg/kg) and ketamine (30 mg/kg). The process involves collecting blood samples, centrifuging them, and separating the serum. The serum is subjected to biochemical measurements as soon as it is obtained.

Grouping of Animals (n = 6)

- Grou*p* I: Control diet
- Grou*p* II: Control diet + 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 milligrams per kg based on body weight)
- Grou*p* III: Control diet + 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 milligrams per kg based on body weight) + Rosuvastatin (2.5 milligrams per kg based on body weight)
- Grou*p* IV: Control diet+ 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 milligrams per kg based on body weight) + *Spirulina* (33 milligrams per kilogram based on body weight)
- Grou*p* V: Control diet+ 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 milligrams per kg based on body weight) + *Spirulina* (66 milligrams per kilogram based on body weight)

Blood Biochemical Parameters Estimation

After 12 weeks of cholesterol feeding, blood was collected from rabbit marginal ear vein for estimation of lipid profiles such as cholesterol, triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) then estimated myocardial infarction parameter CkMB after ISO injection (Figure 1). Based on lipid

Figure 1: Experimental procedure

profile, atherogenic risk is calculated using the atherogenic index of plasma (AIP).

Cardiac Tissue Parameters

A saline solution was immediately injected into the dissected heart after it was carefully dissected. Following homogenization, the homogenate was centrifuged for 5 minutes at 3000 revolutions per minute (rpm) after being homogenized. The supernatant was used to assess various biochemical parameters, including glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). For histology purposes, frontal heart segments were embedded as well.

Analysis Statistics

SPSS version 19 was used for the statistical analysis. Kolmogorov-Smirnov was used to test the normality of the data. With a significance level of $p \leq 0.05$, a one-way analysis of variance (ANOVA) was conducted to determine if significant differences existed between treatments.

RESULTS AND DISCUSSION

Given their physiological similarities with human lipid metabolism, rabbits are valuable in lipid-lowering drug research. Rabbits with hypercholesterolemia have contributed greatly to the development of statin, a lipid-lowering drug widely used by hyperlipidemic patients worldwide.[54] After 12 weeks of treatment, rabbits treated with a high-fat diet (HFD) and isoproterenol hydrochloride (ISO) exhibited significant increases in cholesterol levels (136.7 ± 0.42 mg/dl, $p < 0.001$), TG levels (146.3 ± 0.38 mg/dl, $p < 0.001$), VLDL levels (29.3 ± 0.17 mg/dl, $p < 0.001$), LDL levels (93.7 ± 0.6 mg/dl, $p < 0.001$), atherogenic index of plasma (1.0 ± 0.07, $p < 0.001$), cholesterol risk ratio (CRR) (10.8 ± 0.48, $p < 0.001$), atherogenic coefficient (AC) (9.8 ± 0.51, $p < 0.001$), Ck-MB levels (22.2 ± 0.42, $p < 0.001$), LDH levels (172.5 ± 0.31, p<0.001), AST levels (183.5 ± 0.38, $p < 0.001$), ALT levels (192.5 ± 0.63, $p < 0.001$), and decreased high-density lipoprotein levels (13.7 ± 0.59, $p < 0.001$) compared to normal rabbits (Table 1 and 2). In contrast, treatment with *Spirulina* (33 mg/kg B.wt/66 mg/kg b.wt.) significantly decreased cholesterol levels to 111.2 ± 0.39 ($p < 0.001$), 90.5 ± 0.28 ($p < 0.001$), respectively, TG levels to 125.0 ± 0.25 ($p < 0.01$), 96.2 ± 0.31 ($p < 0.001$), respectively, VLDL levels to 25.0 ± 0.11 ($p < 0.01$), 19.2 ± 0.14 ($p < 0.001$), respectively, LDL levels to 56.3 ± 0.54 ($p < 0.01$), 27.8 ± 0.65 ($p < 0.01$), respectively, AI*P* to 0.6 ± 0.03 ($p < 0.01$), 0.3 ± 0.008 ($p < 0.01$), respectively, CRR to 3.7 ± 0.08 ($p < 0.01$), 2.1 ± 0.11 ($p < 0.01$), respectively, AC to 2.7 ± 0.009 ($p < 0.001$), 1.1 ± 0.15 ($p < 0.01$), respectively, Ck-MB levels to 16.2 ± 0.47 ($p < 0.01$), 12.8 ± 0.33 ($p < 0.01$), respectively, significantly increased HDL levels to 29.8 ± 0.22 ($p < 0.01$), 43.5 ± 0.59 ($p < 0.01$), respectively, significantly decreased LDH levels to 153.8 ± 0.27 ($p < 0.01$), 132.8 ± 0.28 ($p < 0.001$); AST levels to 164.2 ± 0.21 ($p < 0.001$), 140.7 ± 0.24 ($p < 0.001$) and ALT levels to 159.2 ± 0.51 ($p < 0.001$), 139.0 ± 0.42 ($p < 0.001$), respectively (Figure 2 and 3), At the end of HFD + ISO treatment, cardiac superoxide dismutase (SOD) decreased to 8.7 ± 0.31 ($p < 0.001$), reduced glutathione (GSH) to 10.3 ± 0.26 ($p < 0.001$), catalase to 0.2 ± 0.04 ($p < 0.001$), and glutathione peroxidase (GPx) to 5.2 ± 0.34 ($p < 0.001$) compared to the normal control (Table 3). In contrast, *Spirulina* treatment (33/66 mg/kg b.wt.) significantly increased SOD activity to 14.8 ± 0.15 ($p < 0.01$), 22.0 ± 0.28

Table 1: Lipid profile, total glycerol, HDL, VLDL, LDL and atherogenic indices (AIP, CRR, AC) in the cardiac tissue of rabbit Fed with normal diet, cholesterol-rich diet - with & without isoproterenol (ISO) induced myocardial infarction

Groups	*Total cholesterol*	*Total glycerol*	*HDL*	*VLDL*	*LDL*	*Atherogenic indices*		
						AIP	*CRR*	*AC*
Group I	83.0 ± 0.52	73.7 ± 0.35	44.7 ± 0.67	14.7 ± 0.16	23.6 ± 1.32	0.2 ± 0.02	1.9 ± 0.06	0.9 ± 0.06
Group II	136.7 ± 0.42	146.3 ± 0.38	13.7 ± 0.59	29.3 ± 0.17	93.7 ± 0.60	1.0 ± 0.07	10.8 ± 0.48	9.8 ± 0.51
Group III	90.5 ± 0.28	96.2 ± 0.31	43.5 ± 0.51	19.2 ± 0.14	27.8 ± 0.65	0.3 ± 0.08	2.1 ± 0.11	1.1 ± 0.15
Group IV	111.2 ± 0.39	125.0 ± 0.25	29.8 ± 0.22	25.0 ± 0.11	56.3 ± 0.54	0.6 ± 0.03	3.7 ± 0.08	2.7 ± 0.09
Group V	90.5 ± 0.28	96.2 ± 0.31	43.5 ± 0.51	19.2 ± 0.14	27.8 ± 0.65	0.3 ± 0.08	2.1 ± 0.11	1.1 ± 0.15

Table 2: Serum cardiac markers CKMB, LDH, AST, ALT in the cardiac tissue of rabbit fed with normal diet, cholesterol rich diets - with & without Isoproterenol (ISO) induced myocardial infarction

S. No	*Group*	*CKMB*	*LDH*	*AST(SGOT)*	*ALT*
1	Group I	9.8 ± 0.30	85.3 ± 0.43	108.5 ± 0.36	98.0 ± 0.25
2	Group II	22.2 ± 0.42	172.5 ± 0.31	183.5 ± 0.38	192.5 ± 0.63
3	Group III	12.0 ± 0.37	110.2 ± 0.31	112.8 ± 0.36	115.0 ± 0.45
4	Group IV	16.2 ± 0.47	153.8 ± 0.27	164.2 ± 0.21	159.2 ± 0.51
5	Group V	12.8 ± 0.33	132.8 ± 0.28	140.7 ± 0.24	139.0 ± 0.42

Table 3: Isoproterenol (ISO) induced myocardial infarction in rabbits fed with a normal diet (Control), a cholesterol-rich diet (Chol) and an ISO-induced diet - showed changes in the parameters of cardiac tissue SOD, GSH, catalase and GPx

S. No	*Group*	*SOD*	*GSH*	*Catalase*	*GPx*
1	Group I	27.2 ± 0.35	21.8 ± 0.19	0.7 ± 0.07	16.3 ± 0.26
2	Group II	8.7 ± 0.31	10.3 ± 0.26	0.2 ± 0.04	5.2 ± 0.34
3	Group III	23.5 ± 0.39	12.2 ± 1.51	0.5 ± 0.04	11.5 ± 0.34
4	Group IV	14.8 ± 0.15	14.8 ± 0.15	0.4 ± 0.03	8.3 ± 0.27
5	Group V	22.0 ± 0.28	18.8 ± 0.27	0.6 ± 0.06	12.5 ± 0.27

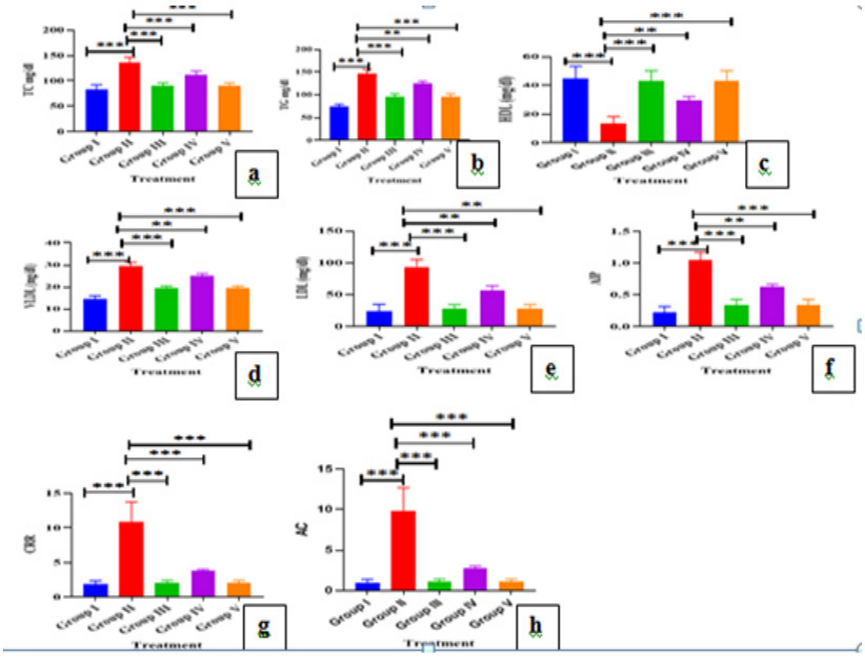

Figure 2: Effect of *Spirulina* on in high-fat diet + Isoproterenol induced comorbid rabbit (Lipid Profile) a) total cholesterol; b) total glycerol; c) HDL; d) VLDL; e) LDL; f) AIP; g) CRR; h) AC

($p < 0.001$) respectively, increased GSH to 14.8 ± 0.15 ($p < 0.05$), 18.8 ± 0.27 ($p < 0.01$), respectively, catalase activity to 0.4 ± 0.03 ($p < 0.05$), 0.6 ± 0.06 ($p < 0.01$), respectively, and increased GPx activity to 8.3 ± 0.27 ($p < 0.05$), 12.5 ± 0.27 ($p < 0.05$), respectively (Figure 4). Researchers compared the study results with rosuvastatin (2.5 mg/kg based on body weight), the standard HMG-CoA reductase inhibitor. The drug rosuvastatin inhibits cholesterol biosynthesis by inhibiting the enzyme that limits the rate of cholesterol synthesis and thus lowers cholesterol levels.[55] The liver exports cholesterol-ester-rich lipoproteins into the circulation more readily when mammals are subjected to atherosclerosis caused by dietary cholesterol, resulting in decreased antioxidant activity.[56-59] In our study, HFD and ISO-treated rabbits significantly increased total cholesterol, TG, LDL, and VLDL and decreased HDL, increasing atherogenic risk, consistent with prior research. Our treatment with *Spirulina* reduced total cholesterol, LDL-cholesterol, and VLDL-cholesterol, as well as AIP, and increased cardiac antioxidant activity, indicating the potential of *Spirulina* in ameliorating diet-induced and ISO-induced atherosclerosis and cardiac toxicity. Histological examination revealed that HFD+ISO treated rabbits exhibited

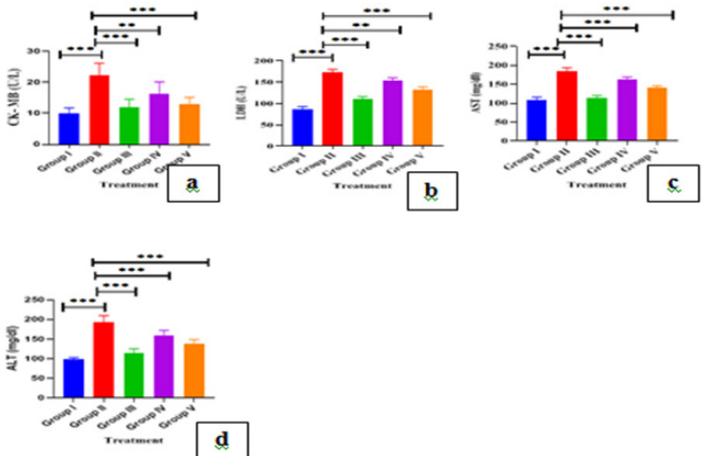

Figure 3: Effect of *Spirulina* on A high-fat diet + Isoproterenol-induced co-morbid rabbit (Cardiac markers) a. CKMB; b. LDH; c. AST; d. ALT

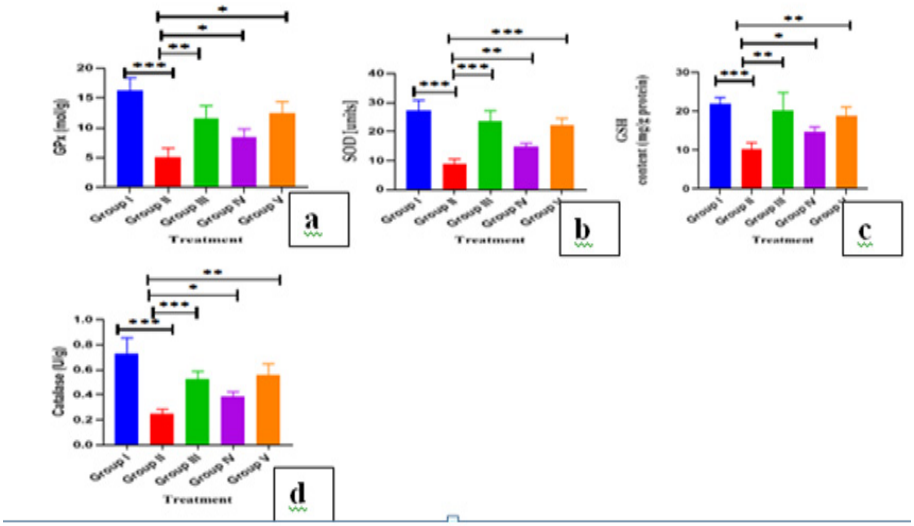

Figure 4: Effect of *Spirulina* on in high-fat diet + Isoproterenol induced comorbid rabbit (Cardiac tissue parameters) a) GP_x; b) SOD; c) GSH; d) Catalase

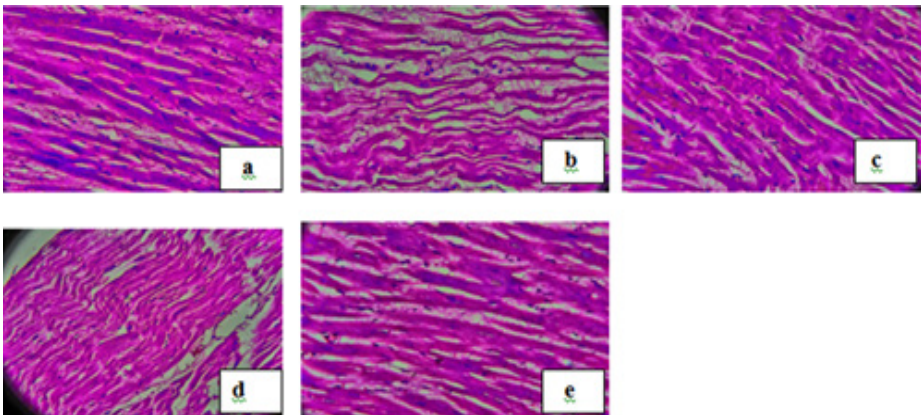

Figure 5: Sections of rabbit cardiac apexes photographed under a microscope. Isoproterenol (ISO) treated rabbit heart tissue exhibits an increase in edematous intramuscular space and intensive cardiomyocyte necrosis. a) Control Diet; b) Control Diet+ 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 mg/kg B.wt); c) Control Diet+ 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 mg/kg B.wt)+ Rosuvastatin (2.5 mg/kg B.wt.); d) Control Diet+ 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 mg/kg B.wt)+ *Spirulina* (33 mg/kg B.wt.); e) Control Diet+ 2% Cholesterol (After 12 weeks of feeding) + ISO (5.25 and 8.5 mg/kg B.wt)+ *Spirulina* (66 milligrams per kilogram B.wt.)

necrotic cardiomyocytes associated with macrophages, wavy cardiomyocyte appearance, and inflammatory cell infiltration, while *Spirulina* treatment reduced necrotic cardiomyocytes, inflammatory cells, and restored near-normal morphology of cardiac myocytes (Figure 5).

CONCLUSION

In conclusion, the potent free radical scavenger *Spirulina* demonstrates robust cardioprotective effects against HFD+ISO-induced cardiovascular impairment in rabbits. These findings suggest the potential safe use of *Spirulina* as a preventive measure against atherosclerosis-associated cardiac injury in patients.

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