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Histological study on the beneficial effect of *Spirulina* for aorta from damage induced by D-galactose

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Abstract*---Spirulinal*** powder supplementation is being studied to see if it can help slow the progression of aging in the heart. As a control group, twenty male rabbits were randomly and equally divided into four groups, each receiving 150 mg/kg BW of d-galactose S/C daily as Group II; 500 mg/kg BW of *Spirulinal* orally as Group III; and 150 mg/kg BW daily S/C administration of d-galactose with 500 mg/kg BW orally as Group IV for four weeks. Using the specific stain Masson's Trichrome, stained sections of the aorta from the D-gal group (GII) and the combination group (CII) exhibit histological changes in the tissue (GIV). Tissue slices stained with Masson's Trichrome stain reveal the location of collagen and muscle fiber accumulation, which appears as a red keratin and muscle fiber accumulation area. Our findings show that *Spirulinal* powder, a feed supplement, has a protective effect against heart aging.

***Keywords*---**D-galactose, *Spirulinal*, Aorta, Masson's Trichrome

Introduction

Cardiovascular diseases have many causes and are one of the main causes of death. global The World Health Organization (WHO) says that the physiopathology of cardiovascular diseases, especially those caused by atherosclerosis, is a change in the blood vessels that can lead to changes in blood flow that affect the heart and nervous system. (Borcea *et al.*, 2021). Animal models of aging made with D-galactose (D-gal) have been used a lot to study how aging works and how anti-aging drugs work (Azman and Zakaria, 2019). When a lot of D-gal is injected

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regularly, cell metabolism slows down and cell damage speeds up the aging process. This causes structural and functional changes in the heart and circulatory system (Ahmad *et al* ., 2021). *Spirulinal* is a cyanobacteria (microalgae) spiral blue-green algae. *Spirulinal* platensis has many biological uses, such as preventing or treating hypercholesterolemia, hyperglycemia, cardiovascular diseases, diabetes, other metabolic diseases, certain inflammatory diseases, allergies, cancer, environmental toxins, drug-induced toxicity, and viral infections. (Abdel-Daim*et al* .,2020; Ibrahim *et al.*,2021(

Materials and Methods

The protocol for the experiment

The Kerbala University veterinary medicine college's animal house was home to twenty mature male rabbits. There were four groups: normal (GI), 150 mg/kg BW S/C daily of d-galactose (Bo-Htay*et al.*, 2020), 500 mg/kg BW *Spirulinal* orally (Abdel-Daim*et al.*, 2020), and 150 mg/kg BW daily of *Spirulinal* (Bo-Htay*et al*) (GIII), *Spirulinal* and d-galactose S/C were administered orally to the GIV group every day for four weeks. At the end of the experiment , blood samples were taken from each experimental group(Donovan and Brown, 2006;Amin and Ahlfors, 2008), also aorta prepared for histological evaluation using the method Using a light microscope and Masson's Trichrome stain (MTC), the paraffin-embedded blocks were cut into 5 micron-thick sections and examined for histology. Estimation of serum Endothein-1 (ng/l) Endothelin 1 (ET-1) was measured by method of (Goldie ,2000)

Results

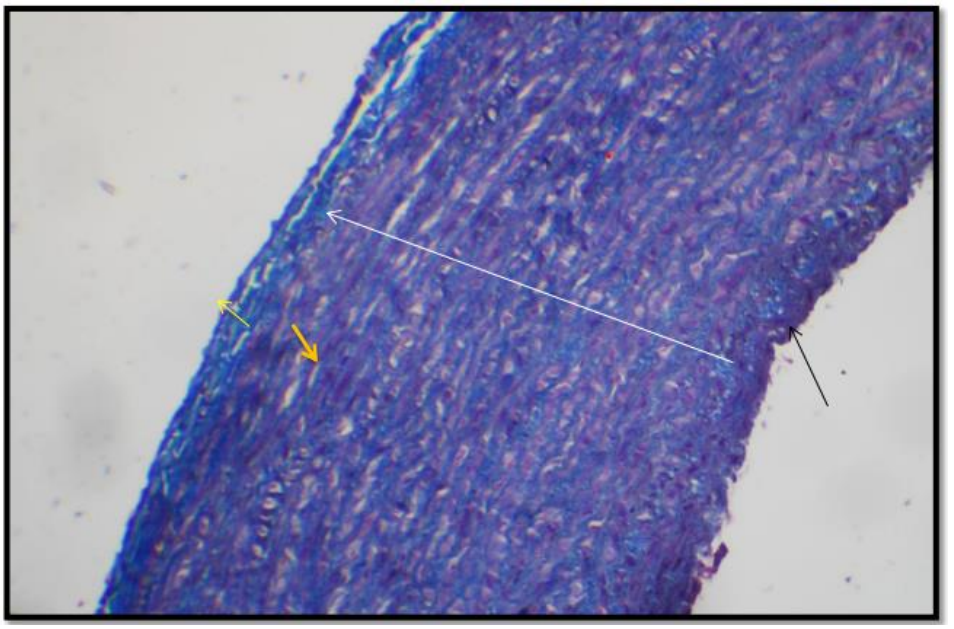
Histological study
Histomicrometry examination

In the control group of our study, we used all of the histology stains. The elastic artery seems to have three layers: the tunic intima, which is made up of endothelium, basal lamina, subendothelial connective tissue, smooth muscle cells, and the first layer, the internal elastic lamina. The tunic medium had a second layer of collagen fibers that were thicker and had more fenestrated elastic laminae and less smooth muscle. The third layer of connective tissue is called the tunic adventitia. It is made up of collagen, elastic fibers, and vasovasorum Figure (1,2)

Aortic artery histomorphology in rabbits treated with d-galactos GII was examined in comparison to those of controls and other groups. showed irregular nuclei of endothelial cells in the intimal surface, loss of squamous cells with the presence of foam cells, increased aortic medial thickness, deterioration of elastic fiber architecture in the tunica media, and a higher number of adipocytes penetrating connective tissue.Figure(3,4)

On the other hand, we found that the intima, media, and serosa layers of the aorta in *Spirulinal*-treated rabbit groups looked like normal microscopic structures and that the thickness of the aortic media was less than in treated

rabbit groups. Figure(5,6) A microscopic look at the rabbits that were given d-galactose and *Spirulinal* shows that their bodies are built in the same way as the control groups. Compared to the d-galactose-treated rabbits group, the endothelial cells of the intimal layer had a squamous appearance. This caused the aortic media layer thickness to go down when image analysis was done, and the structure of the lamina fibers to look more regular and organized, and the smooth muscles in the tunica media to grow less. Our results showed that the three layers of the aorta (the intima, media, and serosa) were normal in the *Spirulinal*-treated rabbit groups, but the aortic media was thicker than in the d-galactose-treated rabbit groups .Figure(7,8)



Figure(1) Photomicrograph of Aorta section from a control group animal showed , normal histological architecture of aortic wall, normal tunica intima(black arrow) endothelia , significant regular wide tunica media(white arrow)with arranged elastic fibers (orange arrow), and narrow ,outer area of tunica adventitia (yellow arrow). (Masson's Trichome, 10X).

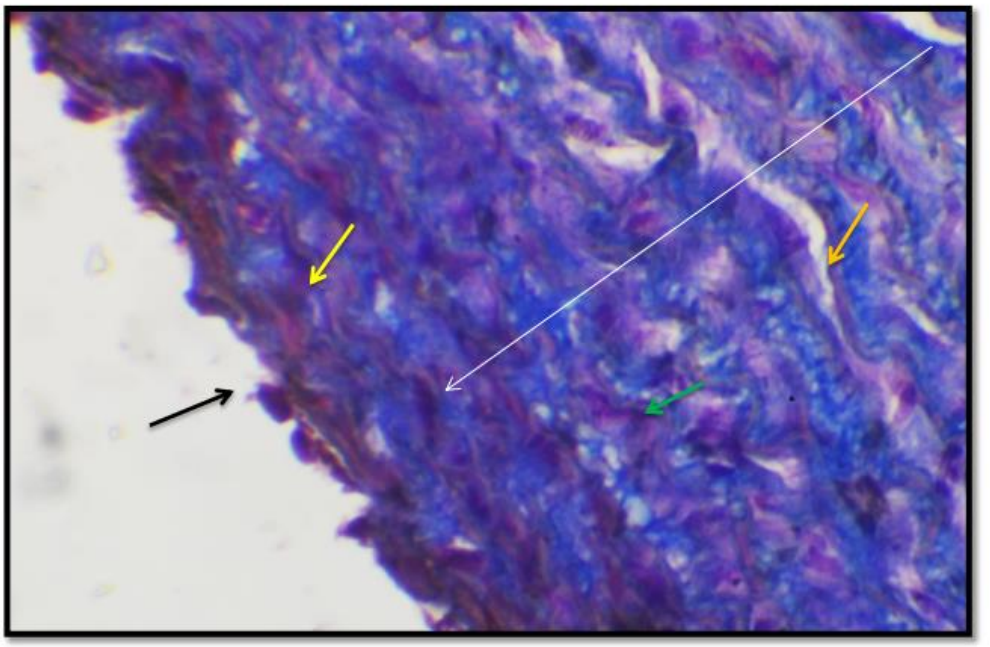
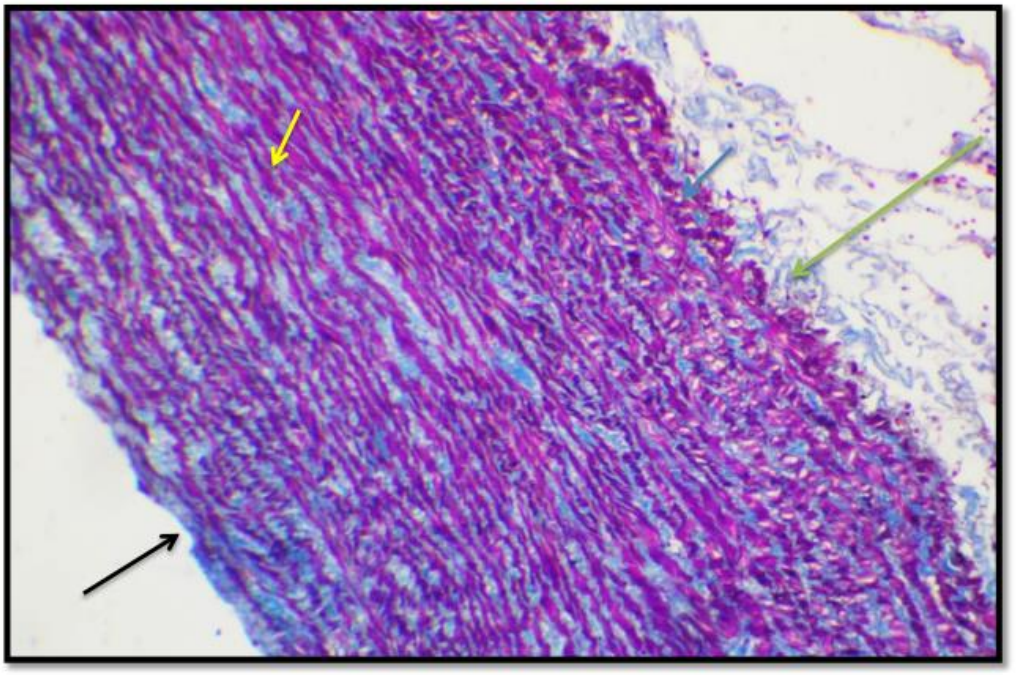
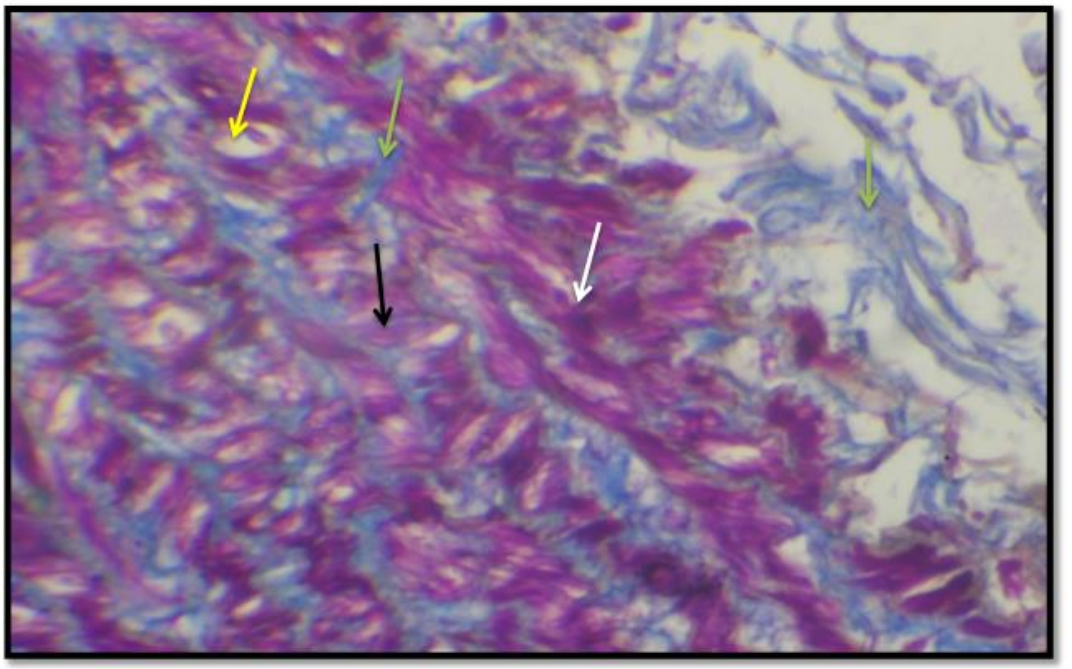


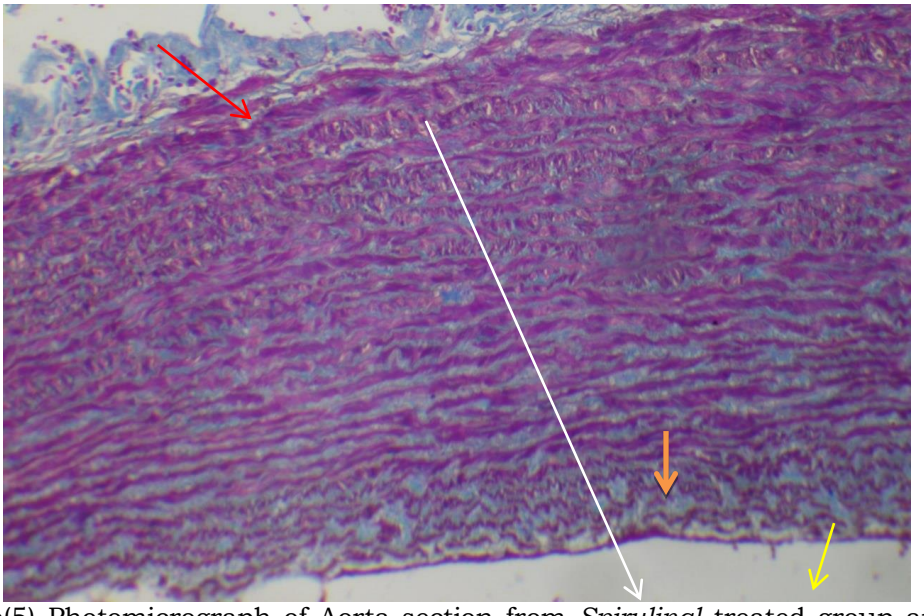
Figure (2) Photomicrograph of Aorta section from a control group animal showed, normal histological architecture of aortic wall, normal tunica intima(black arrow) endothelia , significant regular tunica media(white arrow)with arranged elastic fibers (orange arrow) remarkable smooth muscle fibers which revealed in deep red to purple color (green arrow), and significant arranged elastic laminae (yellow arrow) parallel with blue collagen fibers . (Masson's Trichome ,40X).



Figure(3) Photomicrograph of Aorta section from D galactose treated group animal showed, necrosis and sluophing of tunica intima endothelia or no cells in some areas (black arrow) , regular tunica media(white arrow), moderate to sever necrosis of smooth muscle fibers(yellow arrow) , sever Mononuclear inflammatory cells infiltration(blue arrow),and relatively thickness on the tunica adventitia (green arrow). (Masson's Trichome, 10X).

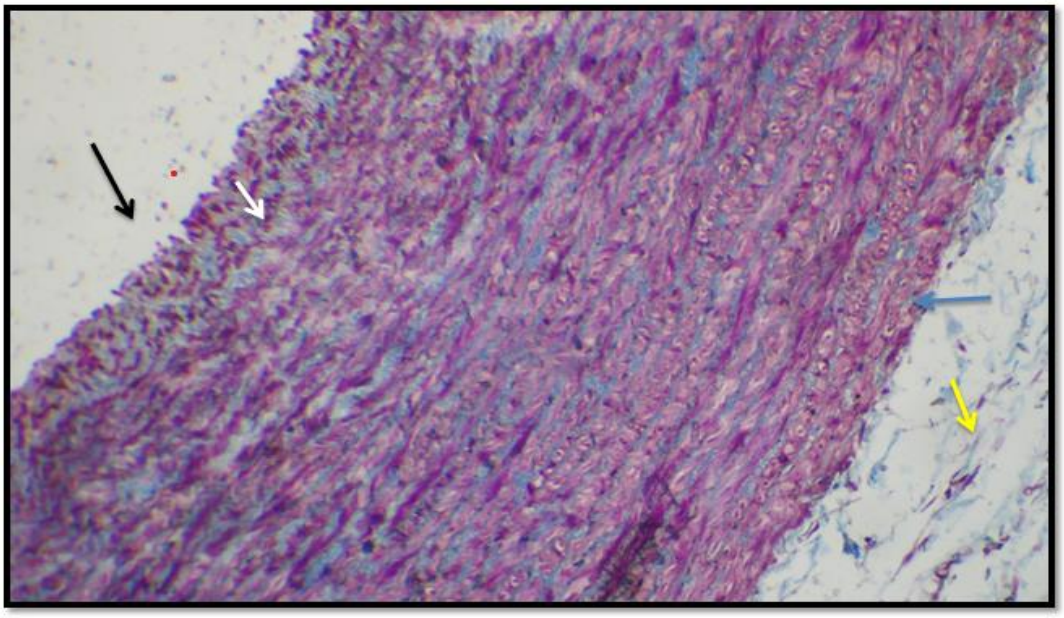


Figure(4) Photomicrograph of Aorta section from D galactose treated group animal showed, remarkable de arranged smooth muscle fibers (black arrow), with vacuolation (yellow arrow), sever Mononuclear inflammatory cells infiltration(white arrow), with deep stained collagen fibers (green arrow). (Masson's Trichome, 40X).



Figure(5) Photomicrograph of Aorta section from *Spirulinal* treated group animal look likenormal histological architecture of aortic wall, normal tunica intima(black arrow) endothelia , significant regular wide tunica media(white arrow)with arranged elastic fibers (orange arrow), and narrow ,outer area of tunica adventitia (yellow arrow). (Masson's Trichome, 10X).

Figure(6) Photomicrograph of Aorta section from *Spirulinal* treated group animal revealed near to normalhistological structure of aortic wall, seminormal tunica intima(black arrow) endothelia , significant regular tunica media(white arrow) and significant arranged elastic laminae (yellow arrow) parallel with blue collagen fibers (Masson's Trichome, 40X).



Figure(7) Photomicrograph of Aorta section from d-galactose and *Spirulinal* group revealed ,focal necrosis (desquamation)of tunica intima endothelia (black arrow) , decreased collagen fibers in tunica adventitia (yellow arrow) , less inflammatory cells infiltration(blue arrow), dearrangedelstic fibers (white arrow). (Masson's Trichome, 10X).

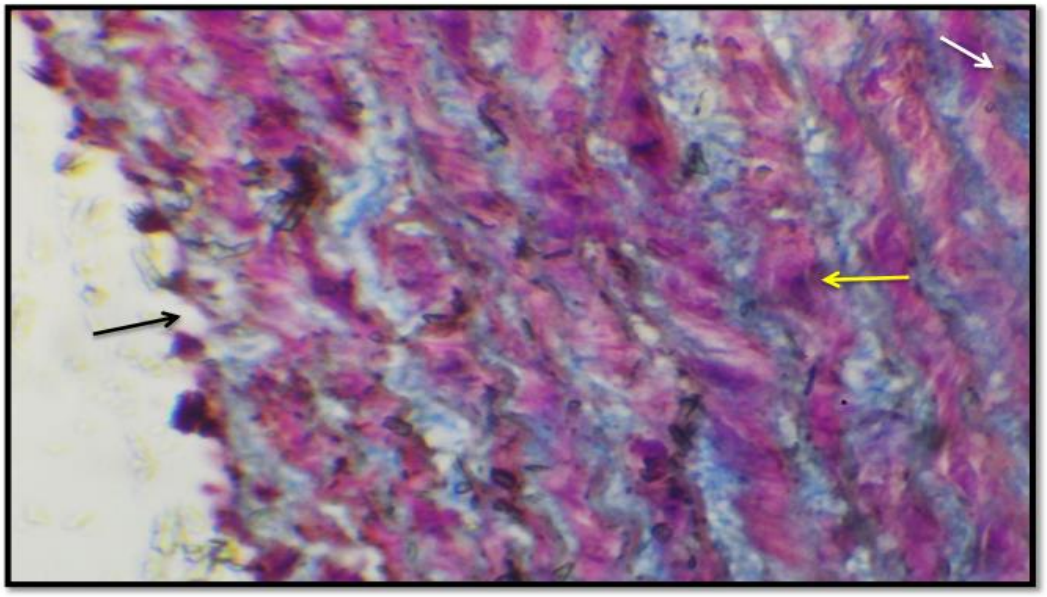


Figure (8) Photomicrograph of Aorta section from d-galactose and *Spirulinal* group revealed ,focal necrosis desquamation)of tunica intima endothelia (black arrow), mild necrosis of smooth muscle fibers (yellow arrow) more muscle fibers (reverse to normal , less or no inflammatory cells infiltration(white arrow). (Masson's Trichome, 10X).

Endothelin-1

Serious differences in Endothelin-1 serum levels ($p \leq 0.05$) have been found between groups GIII, GIV, and GII. Additionally, the GIII group showed a substantial drop in serum endotheline ($p \geq 0.05$) when compared to the control and other groups. No significant differencesexist between the GIV and GI groups. The (LSD=0.693)

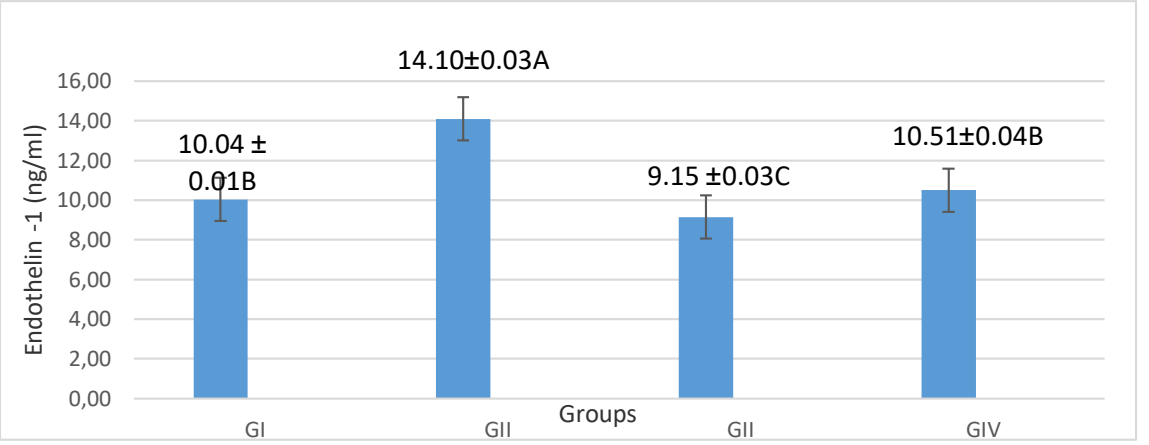


Figure (9) Effect of daily oral intubation of *Spirulinal* for 4 weeks on serum Endothelin -1 (ng/ml) concentration of D-galactose treated male rabbits

Discussion

More than a dozen studies have shown that the aortic tunica medial thickening and microscopic structures found in the control rabbit groups are consistent with findings published by other researchers (Heil*et al* .,2020; Wang *et al* .,2021). Intrinsic cardiac aging may eventually make the heart more sensitive to different stressors and more likely to get heart diseases. 33 models of premature aging caused by D-gal show changes to the heart that are similar to what happens when rodents age normally,D-gal-induced ageing animals showed aberrant cardiac architecture and increased collagen accumulation in the interstitial and perivascular regions of the heart, as was demonstrated(Lakatta, 2001; Gharban and Al-Shaeli, 2021).Since D-galactose activates ROS, which has been linked to aging, prior research have shown that aberrant ROS accumulation leads to heart inflammation and fibrosis, which can be reversed by supplementation. Necrotic and apoptotic pathways in the aging heart contribute to the increasing loss of myocytes, culminating in myocyte hypertrophy and proliferation of cardiac fibroblasts,according to the results of Masson's trichrome staining, aging causes collagen buildup and fibrotic disarray, which leads to fibrosis and cardiac remodeling. Most injuries result in fibrosis, which is caused by an imbalance between the creation and breakdown of extracellular matrix components (Gharban, 2021; Guo *et al.*, 2022).

Antioxidant chemicals such as carotenes, phenolic compounds, and phycobiliproteins found in *Spirulinal*) have been shown to inhibit the signaling pathways of heart remodeling (Anvar and Nowruzi, 2021). *Spirulinal*

supplementation has also been shown to inhibit the synthesis of TNF-, IL-1, and IL-6, all of which are involved in the modeling process (Sharp III and Lefer,2021; Memije*et al.*,2018). In addition to C-phycocyanin, there are several other metabolites that play a role in this process. The endothelium of major vessels like the aorta expresses more eNOS when this protein is present, which benefits the cardiovascular system (Blas *et al* .,2022). Also, it has been observed that phycocyanobilin (the prosthetic chromophore group of C-phycocyanin) upregulates the endothelium stress-response enzyme heme oxygenase 1 (HMOX-1) (HMOX-1). Reduced oxidative stress, apoptosis, and inflammation are all reduced when HMOX-1 is overexpressed in the heart.(McCarty, 2021; Gligorijević*et al* .,2021).But the other parts of *Spirulinal*, like carotenoids, chlorophylls, and vasoactive peptides, work together to keep cardiovascular problems from happening. If we take more *Spirulinal*, we can probably stop all heart problems from happening. found that the C-phycocyanin is linked to vascular endothelial modulation. (Atallah*et al* ., 2021).

Endothelin-1 levels in the GII group that received d-galactose were significantly higher than those in the control group, as shown in figure (9). The findings of this study are in agreement with those of (Frank *et al* .,2022). Endothelin-1 (ET-1), which is released by endothelial cells, has a two to threefold increase in plasma levels in heart failure regardless of the etiology (Biasucci*et al* .,2021). Endothelins are a group of peptides (ET-1, ET-2, and ET-3) that are found in both vascular and non-vascular tissues and have several biological functions. Vascular endothelial cells produce Endothelin-1, a 21-amino acid polypeptide that acts as a potent smooth muscles vasoconstrictor and mitogen (Rathod*et al* .,2022). In time-dependent ways, ET-1 may cause oxidative stressors that reduce the antioxidant GSH/GSSG ratio and increase lipid peroxidation. Increased water content in the heart muscle and increased vascular permeability. This study showed that acute inflammations and interstitial edema in the rabbit heart cause cardiac damage (Liu *et al* .,2020; Garcia-Herreros el al .,2020).

Endothelin-1 was found to be significantly lower in *Spirulinal* group GIII than in *Spirulinal* group GII, as shown in the same table. The study's findings are in agreement with my own findings (Trotta *et al* .,2022). These goods, including *Spirulinal*, are anti-oxidant/anti-inflammatory/antitumor, and they also improve immunity and protect organs from harm. Autotrophic *Spirulinal* is the sole source of GLA that is as high as 500 times that of human milk (Han *et al.*,2021). In the therapy of cardiovascular disease, linoleic acid and gamma-linolenic acid (GLA) play an important role since they are critical components of mitochondrial phospholipids (Majewski*et al* .,2022; Machowiec*et al*. .,2021).

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