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Preventive role of *spirulina* on the some biomarkers in heart aging induced by D-galactose on the male rabbits

Hiba Alameri

College of Veterinary Medicine, University of Kerbala, Karbala, Iraq
Corresponding author Email: hiba.abdlkreem@s.uokerbala.edu.iq

Wefak Albazi

College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Muna Hussain Al-Aameli

College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Abstract--The aim of this study is to investigate the preventive role of food supplementation with *Spirulina* powder in the heart aging. Twenty male rabbits randomly and equally divided into four groups, normal as control group GI, animals received 150 mg/kg BW S/C daily of D-galactos as GII, animals were received 500mg/kg BW orally of *Spirulina* as GIII while GIV group was received 150 mg/kg BW daily S/C administration of D-galactos with 500mg/kg BW orally of *Spirulina* for four weeks. The results of the study showed a significant decrease in Cardiac troponin I and Peroxynitrate with a significant increase in Nitric oxide and Glutathione in group received *Spirulina* compared with aging group GII. Conclusion, our result indicate the feed supplement of *Spirulina* powder caused preventive role against heart aging.

Keywords---*Spirulina*, D-galactos, heart aging, male rabbits.

Introduction

As the world's populace ages, the charge of age-associated sicknesses amongst older men and women has grown to be a essential fitness concern (Sharma et al ., 2021). Aging is described because the regular accumulation of organic adjustments that result in a person's practical deterioration over time (Azman and Zakaria,2019). The important frightened gadget turns into liable to oxidative strain as human beings age due to the fact their redox stability receives extra oxidized (Corzo and Silva, 2021; Lushchak, 2022; Zeng et al., 2022). In vitro improved ageing fashions consist of hydroxyurea remedy and D-galactos

induction, and in vivo studies consist of radiation induction, age-improved prone mice, Klotho mice, thymus depletion, and D-galactos-brought on in vivo investigations (Mahdi *et al.*, 2021; Wang *et al.*, 2022; Mahdi *et al.*, 2021; Wang *et al.*, 2020). D-galactos is an aldohexose, a lowering sugar located in a lot of meals consisting of milk, butter, cheese, yogurt, honey, beets, plums, Cherries, figs, and celery for example. For healthful men and women, the most each day dose is suggested. Galactose is 50 grams, with the bulk of it being digested and removed in more or less eight hours (Azman and Zakaria, 2019; Van and Veiga, 2021). However, it may be transformed to aldose and hydroperoxide catalysis with the aid of using galactose oxidase at a excessive level, ensuing withinside the era of reactive oxygen species (ROS), that could purpose oxidative strain, inflammation, mitochondrial malfunction and death (Gharban and Al-Shaeli, 2020; Prajit *et al.*, 2020; Ullah *et al.*, 2020). Cardiac ageing increases the hazard of cardiovascular contamination related to excessive stages of reactive oxygen species (ROS) and oxidative strain. Treatment with D-galactos has been verified to purpose oxidative strain in animal coronary heart tissue with the aid of using elevating MDA and nitric oxide (NO) even as lowering antioxidant enzymes like SOD (Dehghaniet *al.*, 2018; Li *et al.*, 2021; Azman and Zakaria, 2019). Myocardial fiber thickening, structural doubtful and shorter torsion, considerably enlarged septa, and myocardial interstitial congestion markedly blocking off capillaries had been all discovered in Dgalactose-dealt with animals (Rusuet *al.*, 2020; Shenet *al.*, 2020). Cardiac troponin includes cardiac troponin I (cTnI) and troponin T, which might be myocardial tissue-precise biomarkers (cTnT). Cardiovascular troponin I and T are extra selective and touchy than different cardiac troponins in myocardial infarction (Shah *et al.*, 2018; Karimiet *al.*, 2019 and Çimenet *al.*, 2020) used biomarkers together with creatine kinase and myoglobin. ET-1 stages withinside the blood or tissues are pathologically improved in quite a few illnesses, together with cardiovascular sicknesses, neurological sicknesses, malignancies, being pregnant disorders, and diabetes (Jain and Johnson, 2020) *Spirulina platensis* is a filamentous multicellular cyanobacterium. (2019, Jung *et al.*) Diverse flora with antioxidant, anti-inflammatory, antiviral, anticancer, antibacterial, anti-protozoan, and hepatoprotective activities, in addition to people who stimulate boom and reproduction, had been studied (Chowdhury *et al.*., 2009). Many research had been carried out on *Spirulina platensis*. It is located in Central African and Mexican lakes and is the maximum plentiful photosynthetic microorganism (Ouldet *al.*, 2013). Due to its excessive digestibility and protein content (60–70%) (Ouldet *al.*, 2013), fat (15–25%) (Djaghoubi, 2013), and lipid (5.6–11%), spiruline is taken into consideration a nutrient-dense meals supply (Sguera, 2008). In current years, nutritious nutritional dietary supplements have piqued hobby as a capacity supply of healing compounds because of their introduced capacity fitness benefits antioxidants, immunostimulants, anti-inflammatory, anticancer, antiviral, and antibacterial traits have all been proven to have healing efficacy in diverse investigations (Afkhamiet *al.*, 2021; Metekiaet *al.*, 2022). The purpose of this observe become to peer if *Spirulina* ought to assist keep away from a few coronary heart biomarker abnormalities because of d-gal in male rats.

Materials and Methods

The experiment protocol

Twenty healthy adult male rabbits, they were placed in the animal house of veterinary medicine college of Kerbala University . Rabbits randomly and equally divided into four groups, normal as control group GI ,animals received 150 mg/kg BW S/C daily of D-galactos according to method reported by (Bo-Htayet *al .*,2020) as GII, animals were received 500mg/kg BW orally of *Spirulina* according to method reported by (Abdel-Daimet *al .*,2020) as GIII while GIV group was 150 mg/kg BW daily S/C administration of D-galactos with 500mg/kg BW orally of *Spirulina* for four weeks,at the end of the experiment the blood collected by method of (Donovan and Brown,2006;Amin and Ahlfors, 2008). Serum cardiac troponin I (ng/ml) estimation Cardiac troponin I (cTnI) was measured using a kit manufactured in Guangzhou, People's Republic of China. company (Adams ,1994) Serum Nitric oxide (NO) M/L estimation The method of (Chang *et al.*,1998) was used to measure nitric oxide (NO). erumperoxynitrate (ONOO) m/L estimation Peroxynitrate was determined using the method of (Vanuffelen,1998). Serum reduced Glutathione (mg/dl) estimation The concentration of glutathione in serum was determined using the Ellmans reagent method, which was previously used by (Sedlak and Lindsay,1968).

Results and Discussion

The mean values of serum Cardiac troponin 1 at the end of the experiment were ($0.00 \pm 0.00C$, $1.03 \pm 0.02A$, $0.00 \pm 0.00C$, $0.33 \pm 0.03B$) for groups Control, D-gal, *Spirulina* and *Spirulina* +D-gal respectively(LSD =0.034) as shown in Figure (1), show a significant increases($p \leq 0.05$) in GII group when compared with GI, GIII and GIV groups. On the other hand the main value of serum Cardiac troponin 1 show a significant decrease($p \leq 0.05$) in GIV group when compared with GI, GIII and GIV groups. While there is no significant ($p \geq 0.05$) differences between GI and GIII groups.

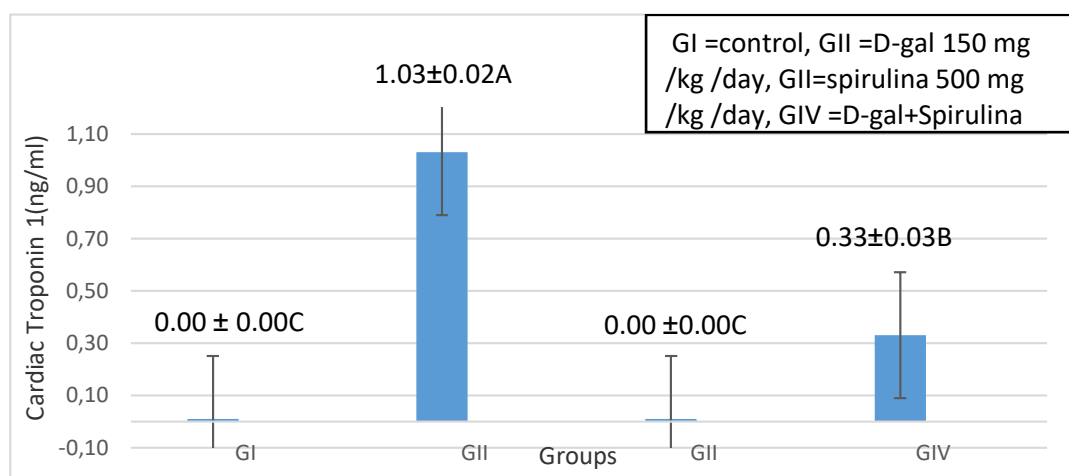


Figure (1) Effect of daily oral intubation of *Spirulina* for 4 weeks on serum Cardiac Troponin I (ng/ml) concentration of D-galactos treated male rabbits

The result in figure (1) showed a significant increase in Cardiac troponin I (cTnI) in GII received d galactose as comparing with other groups. Cardiac troponin became proposed as a marker of cardiac mobileular demise, and each proteins at the moment are robotically hired as guideline-advocated markers to resource withinside the prognosis of acute myocardial infarction. There have been no in advance investigations that documented the presence of cTnI withinside the serum of D-galactos-dealt with rabbits (Packer *et al.*,2021). The nature of cTnI, that is a low molecular weight protein member of the myofibrillary contractile equipment of coronary heart muscle, should provide an explanation for the accelerated cTnI. (Tang *et al.* ,2021). Elevated cTnI levels can predicted risk of myocardial ischemia in the stress subjected patients cardiac cell death and subsequent infarction, this is increased serum cTnI levels in the d galactose treated rabbits marked elevations in serum CK-MB activity were registered ,this might be related to concentrations of diagnostic markers of myocardial damage that is released into the extracellular fluid once myocardial cells are damaged (Chaulin, 2021; Chaulin and Duplyakov, 2021; Pouraliet *al.* ,2021). When the coronary heart's membrane is uncovered to inadequate oxygen or nutrients, it turns into permeable or ruptures, permitting cytosolic enzymes to break out into the bloodstream and lift their serum concentration (Wassieet *al.* ,2021).

Spirulina remedy led to a good sized lower in cardiac troponin I in organization GIII. The study's findings are constant with the ones of different studies (Alwaleed *et al.* ,2021). *Spirulina* blocked this effect and dramatically decreased the stages of cTnI generated via way of means of D-galactos, restoring cTnI stages to ordinary in *Spirulina* -dealt with mice. This might be due to the fact *Spirulina* includes antioxidants like -carotene and Cphycocyanin. This study's findings endorse that *Spirulina* can shield cardiac myocytes from oxidative harm due to Dgalactose (Abdul-Adel *et al.* ,2019; Jaeschkeet *al.*,2021; Abdel-Moneimet *al.*,2022). The scavenging of unfastened radicals via way of means of *Spirulina* 's non-enzymatic antioxidants and antioxidant enzymes is accountable for effectively eliminating

unfastened radical-prompted mobileular harm, improving immunological function, and stopping illnesses, in addition to improving human fitness As a result, *Spirulina* has been acclaimed as a superfood for combating viruses, slowing down the getting older process, heading off cancer, and addressing loads of fitness issues (Grosshagaueret *al.* ,2020).

The mean values of serum Nitric oxide at the end of the experiment were ($30.36 \pm 0.06B$, $24.80 \pm 0.18C$, $43.82 \pm 1.00A$, $29.79 \pm 0.12B$) for groups Control, D-gal, *Spirulina* and *Spirulina* +D-gal respectively(LSD= 2.316) as shown in Figure (2), The main value of showed a significant decrease in GII group when compared with GI, GIII and GIV groups. Beside a significantly increases in the main value of serum Nitric oxide in GIII group when compared with GII group. While there is no significant differences between GI and GIV groups.

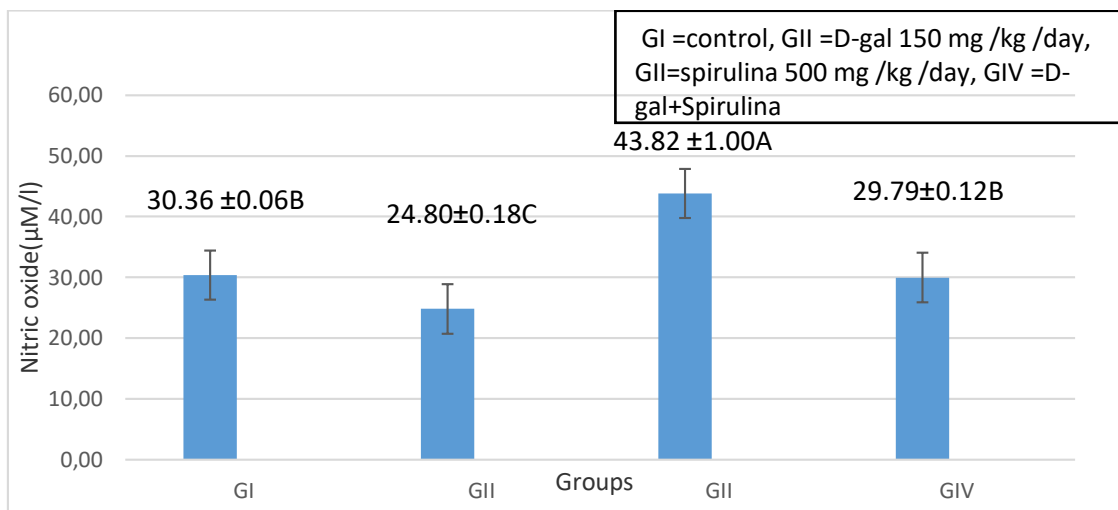


Figure (2) Effect of daily oral intubation of *Spirulina* for 4 weeks on serum Nitric oxide(µM/l) concentration of D-galactos treated male rabbits

In contrast to the manipulate group, GII who took D-gal had a sizable drop in NO. The study's findings are regular with the ones of (BoHtayet *al.*,2018). Endogenous nitric oxide (NO) is produced in vascular endothelial cells thru endothelial NO-synthase (eNOS) activation and NO launch from NO donor chemical substances withinside the manipulate of the sGC-cGMP-PKG pathway (soluble guanylyl cyclase,cyclicGMP,proteinkinaseG) and next induction of vasodilation. Endothelial cells that bind to calmodulin to spark off eNOS have a better cytosolic Ca content. It has the capacity to purpose an boom in [Ca] in endothelial cells, observed through a lower in [Ca] in vascular clean muscle cells (Bo-Htayet *al.*,2019). Because NO is an vital endothelial-derived vasodilator and cardioprotector, low NO degrees can also additionally make contributions to D-gal-brought about tissue damages. Higher oxidative pressure is usually observed through much less nitric oxide (NO) generation, which can also additionally weaken the cardioprotective effect of NO.

In assessment to the manipulate group, GIII receiving SPIRULINA had a massive upward push in NO. The study's findings are steady with the ones of different

studies (Mohitiet *al.*.,2021). In vitro, *Spirulina* extracts boosted the endothelium's basal manufacturing and launch of nitric oxide and the cyclooxygenase-structured vasoconstricting agent prostanoid. The additives in *S. platensis*, together with as phycocyanin, can raise the manufacturing of endothelial nitric oxide synthase over time, ensuing in improved nitric oxide bioavailability (Dinizet *al.*,2020).

The mean values of serum ONOO at the end of the experiment were ($4.05 \pm 0.01B$, $7.31 \pm 0.14A$, $3.51 \pm 0.15C$, $3.85 \pm 0.15B$) for groups Control, D-gal, *Spirulina* and *Spirulina* +D-gal respectively (LSD =0.583) as shown in Figure (3), show a significant increase in GII group when compared with GI, GIII and GIV groups. Beside a significantly decreases in the main value of serum Peroxynitrate in GIII group when compared with GII group. While there is no significant differences between GI and GIV groups.

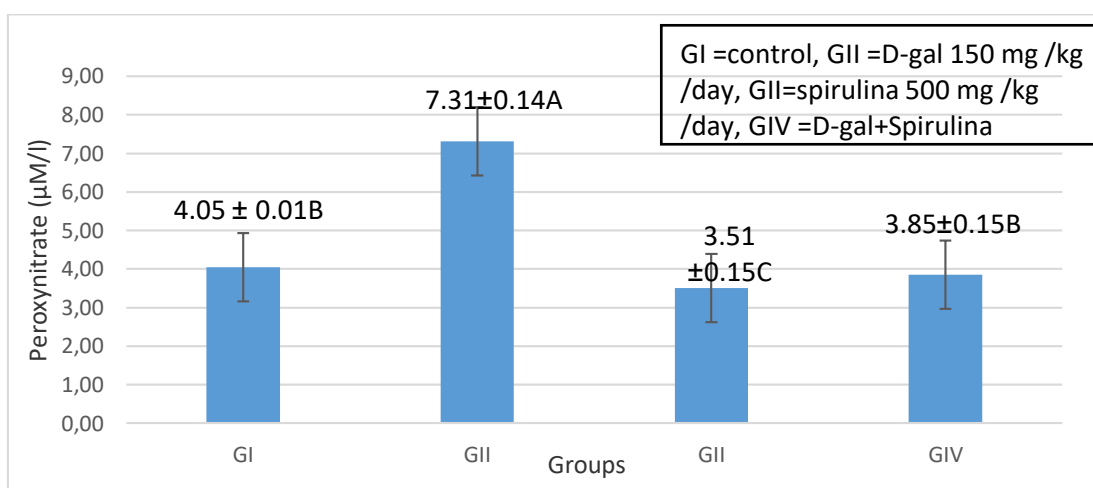


Figure (3) Effect of daily oral intubation of *Spirulina* for 4 weeks on serum Peroxynitrate (µM/l) concentration of D-galactos treated male rabbits

This end result demonstrates a large growth in serum GSH within the D-gal organization whilst in comparison to the alternative groups. D-galactos remedy has been proven to set off oxidative strain in animal coronary heart tissues via way of means of growing MDA, nitric oxide (NO) (Heilet *al.*,2020) and peroxynitrite (ONOO) and reducing antioxidant enzymes along with SOD, CAT, glutathione peroxidase (GSH-Px), NOS, and overall antioxidant capacity (Dehghani et al., 2018). D-galactos-dealt with animals had better degrees of ROS, peroxynitrite (ONOO), and MDA, decrease degrees of antioxidant enzymes along with SOD, CAT, and GSH, and decrease overall antioxidant capacity, decrease degrees of respiration chain enzymes and ATP synthesis, mitochondrial DNA mutations, and mitochondrial shape impairment (Chen *et al.*,2020; Cheng *et al.*,2022; Du *et al.*, 2012, 2015; Lei *et al.*, 2016; Zhang *et al.*, 2019).

In comparison to the other groups, this result shows a significant increase in serum ONOO- within the GIII group. *Spirulina* includes numerous lively ingredients, together with phycocyanin and -carotene, each of that have effective antioxidant and antiinflammatory homes. Phycocyanin's antioxidant and

antiinflammatory homes had been located in 1998. (Abaza *et al.*,2021, Omar *et al.*,2022). Phycocyanin can scavenge loose radicals along with alkoxyl, hydroxyl, and peroxy radicals. It additionally reduces nitrite manufacturing and inhibits the expression of inducible nitric oxide synthase (iNOS), as severa research have shown (Ibrahim *et al.*,2021, Roy and Pabbi,2022,Li *et al.*,2022).

The mean values of serum GSH at the end of the experiment were ($9.85 \pm 0.055BC$, $5.81 \pm 0.14C$, $17.44 \pm 0.13A$, $13.64 \pm 0.13AB$) for groups Control, D-gal, *Spirulina* and *Spirulina* +D-gal respectively(LSD=0.551) as shown in Figure (4), show a significant decrease in GII group when compared with GI, GIII and GIV groups. Beside a significantly increases in the main value of serum GSH in GIII group when compared with GII group.

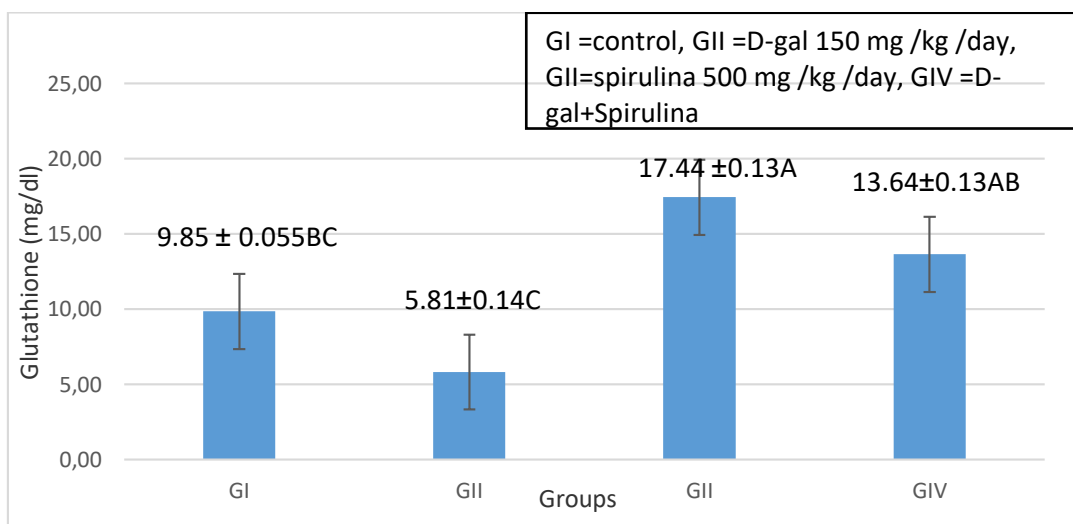


Figure (4) Effect of daily oral intubation of *Spirulina* for 4 weeks on serum Glutathione (mg/dl) concentration of D-galactos treated male rabbits

Our findings display a good sized lower in serum GSH in GII while in comparison to the alternative groups. This final results is regular with (Aquilano *et al.*,2014; Kwon *et al.*, 2019;Heilet *et al.*.,2020;Mahdi *et al.*,2021) Injection of one hundred mg/kg.bw D-gal for six weeks brought on ROS manufacturing withinside the body, which can be defined via way of means of D-gal growing oxidative pressure and ROS generation, in addition to inhibiting cycteine uptake and manufacturing, ensuing in reduced GSH stages and an boom in ROS)Dröge,2005(Glutathione is an endogen antioxidant this is decreased in oxidative popularity as a sign of oxidative pressure, reflecting the redox stability of oxidation and antioxidation. Various oxidants and antioxidants were additive that D-gal management led to a lower in serum glutathione (GSH) stages (Omidkhoda *et al.*,2020). The addition of *Spirulina* suspension appreciably restored those enzymatic sports to near-ordinary stages withinside the manipulate organization. *Spirulina* includes C-phycocyanin, which has antioxidant interest and might scavenge loose radicals like hydroxyl radicals and superoxide. C-safety phycocyanin's towards D-gal validated radical scavenging interest in addition to an inhibitory impact at the lipid peroxidation chain reaction, as referred to via way of means of (Abdel-Daimet

al., 2013). *Spirulina* remedy multiplied glutathione stages and guarded the interest of cell antioxidant enzymes which includes glutathione peroxidase (GPX), selenium-established glutathione peroxidase (GPX-Se), and oxidized glutathione reductase (GR). The findings in reality validated *Spirulina* extract's antioxidant interest. According to a latest in vitro study (Pawaret al .,2020).

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