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Anti Aging Effect of Omega-3 Supplement on Physiological Status of Heart Tissue in D- Galactose Treated Rats

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Abstract*—Background and objective**: *Ageing is a natural and inevitable phenomena. As the population ages more quickly, cardiovascular disease (CVD) becomes more common in an ageing society. Research on ageing has attracted a lot of attention worldwide due to the rising incidence and mortality rates of CVD, which represent a threat to human health. The deterioration of heart function is among the most noticeable effects of ageing in humans. Particularly vulnerable to age-related decline are post-mitotic cells, such as those found in the heart. According to the current study, d-galactose causes the heart to age by increasing oxidative stress, decreasing antioxidant levels, increasing apoptosis and altering the structure of the heart, heart fibrosis is the final result of these processes. These pathways might be the focus of future planning focused on treating anti-aging difficulties. Lower rates of heart ageing properties have been related to moderate omega-3 fatty acid intake.* ***Methods: *Twenty four male Rattus norvegicus rats ranging between 220 and 240 g were selected at random and separated equally to four groups. Normal saline was injected intraperitoneally into the animals in the control groupT1,also they were drenched with normal saline, animals in group T2 received an intraperitoneal injection of D-galactose (150 mg/kg bwt) and were drenched with 80 mg/kg bwt of Omega3 twice a day for 70 days,T3 were drenched omega 3 (80 mg/kg b.wt.) twice daily for 28 days and given an intraperitoneal injection of D-galactose (150 mg/kg b.wt.) for 70 days. D-galactose (150 mg/kg b.wt) was given intraperitoneally (I/P) into T4 rats for 70 days. Twenty-four hours following the last treatment, all animals were killed, and samples of heart tissue were taken in order to examine the histological changes. To check the indicators of oxidative stress and cardiac muscle injury, serum samples were also collected.* ***Conclusion***: *After taking an omega-3 supplement for 70 days, oxidative stress marker catalase and the aging-damaging effects of D-galactose on heart muscle were significantly reduced (CPk, LDH) were estimated and showed improvement for serum results, tissue heart sections stained with a special masson trichrome stain demonstrate the improvement in heart muscle architecture after omega-3 supplementation*.

***Keywords*—** *D-galactose, Omega 3, Rats, heart, masson trichrome, oxidative stress*.

I. INTRODUCTION

The pathological effects of cardiomyocyte ageing are demonstrated by the fact that ageing is a major risk factor for cardiovascular disease and is associated with a number of changes in cardiac structure and function, such as left-sided hypertrophy and raised cardiomyocyte size, as well as a decrease in the total number of cardiomyocytes and ventricular dysfunction (Shimizu and Minamino, 2019). As the heart ages, mitochondrial malfunction may occur, leading to the generation of reactive oxygen species and age-related cardiac failure. The expression of the ageing proteins p53, p21, and p16 rises when ROS are produced in heart tissue (Shimizu and Minamino, 2019). When D-galactose is administered, P53, P21 and P16 expression is increased (Wang *et al.*, 2022). D-galactose-induced ageing models is one of the artificially manufactured ageing models that have been chosen for numerous research, in which animals are continuously injected with the reducing monosaccharide D-galactose over a predetermined period of time, which is guaranteed to result in abnormalities of glucose metabolism in vital organs like the heart. Aldose reductase catalyses the reduction of galactose to galactitol as its concentration rises in cells. The latter builds up inside the cell, causing enlargement, malfunction, and ultimately ageing. On the other hand, D-galactose speeds up the ageing process by producing advanced products of glycation (AGEs) and reactive oxygen species (ROS) through a different metabolic pathway. Thus, Research on cardiac ageing can be conducted using models of D-galactose-induced ageing (Lu *et al.*, 2010). Age-related illnesses are closely associated with telomere attrition. Telomeres are located at the tips of eukaryotic cell chromosomes and are made up of a tract of brief DNA repeats that repeat in unison with related protecting proteins (Bei *et al.*, 2018). Evidence has shown that oxidative stress rises with age, and ageing mechanisms have been extensively studied using d-galactose (d-gal)-induced ageing mouse models. After receiving d-gal injections for 8–10 weeks, rats exhibit ageing characteristics, such as cardiac ageing and elevated levels of advanced glycation end products (AGE) and reactive oxygen species (Bei *et al.*, 2018). In mice administered 100–150 mg /kg/day of the d-gal for 70 days , the use of protective therapeutic measures reversed the emergence of significantly reduced telomere length and TERT expressions in heart tissue (Hong *et al.*, 2021). D galactose administered intraperitoneally to mice for six weeks at a dose of 120 mg/kg/day causes oxidative harm, ageing, tissue degeneration, and eventually a drop in cardiac index (Liu *et al.*, 2019). The cardiac index increased and cardiac fibrosis developed when D galactose was administered subcutaneously in mice for a period of four weeks with a dosage of 500 mg/kg/day or intraperitoneally in Wistar rats for eight weeks in dose of 150 mg /kg/ day (Cheng

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et al., 2021). When D-gal. is taken in excess, more ROS are created, which leads to oxidative stress and cardiac muscle injury. In several investigations evaluating the suitability of providing D gal. with dosages of 50, 500 mg/kg/day in 6-10 weeks to establish a D-galactose-induced heart ageing model, it was observed that the initial age of the experimental animals with dose, and treatment period were closely associated. There is a negative relation between the age of lab animals and the time of therapy within 150 , 200 mg/ kg/in day D-gal. (Chang *et al.*, 2017). SOD, the antioxidant enzyme system includes catalase, glutathione peroxidase and other enzymes. Following a month of subcut injected 500 mg/kg in a day of D-gal., the animal's total antioxidant capacity value sharply decreased (Ge *et al.*, 2021). D-galactose promotes the production of inflammatory factors by stimulating the NF-κB inflammation signalling pathway, which speeds up the ageing process and the development of an inflammatory state (Azman *et al.*, 2021). Resveratrol administration markedly raised superoxide dismutase (SOD) activity and reduced MDA levels (Al-Tamemi and Al-Okaily, 2023). Collagen deposits and extracellular matrix remodelling are hallmarks of organ fibrosis. Age makes cardiac fibrosis more probable (Murtha *et al.*, 2019).Moderate intake of fatty fish and marine omega-3 fatty acids was associated with lower rates of HF(Levitan, Wolk and Mittleman, 2009). Long-chain omega 3 of poly unsaturated fat acids, which can be found as a supplement or in seafood, are thought to be significant cardiovascular health regulators. According to observational research, LC n-3 PUFAs may lower the incidence of coronary heart disease, particularly sudden cardiac death (Zheng *et al.*, 2022). During the GISSI-HF trial, patients with chronic heart failure (CHF) showed a slight survival benefit when supplemented with 1 gram/d omega 3 poly unsaturated fat acid (Moertl *et al.*, 2011). In prediabetic ageing rats given D-gal and sucrose, fish and flaxseed oils reduced the incidence of depressive-like behaviour and had a protective effect on cognitive deterioration. When poly unsaturated fat acid which rich oil diets, particularly the fishes diet, reduced plasma concentrations of non esterified fatty acids, TNFα, brain dopamine, but not glycaemic status, Latency period for escape and time spent in the quadrant in the Morris Water Maze test improved (Guo *et al.*, 2018). SOD and CAT are the two primary parts of antioxidant enzymatic mechanisms of defence. The task of catalysing the transformation of harmful hydrogen peroxide into safe water belongs to CAT (Zhao *et al.*, 2019).

II. Materials and Methods

Animal Ethical approval: Ethical approval was granted by the local committee of animal care and use (P.G. 584 date 3-12-2024), and the study's animal care and treatment were conducted at the college of vet. medicine within Al-qasim green university in strict compliance with the code of ethics for animal experiments.

Animals and experimental design

Twenty four male rats (*Rattus norvegicus)* weighing (220–240 g) were utilized in the present investigation. Animals were kept in cages with a 12-hours in light/dark sequence at 22–25 °C. Rats were kept for 2 weeks at the animal house inside the college of veterinary medicine Al-qasim green university for accilimatization had full access to water and pellets throughout the study period. Rats were randomly assigned into four equal groups and treated daily for 70 days. Animals in the control group T1 were administered an intraperitoneal injection of normal saline also they were drenched with normal saline (NS) , while those in group T2 were injected (I/P) with D-galactose (150 mg/kg bwt) according to (Chang *et al.*, 2017). and drenched with 80 mg/kg bwt of Omega3 twice a day for 70 days. T3 were injected I/P with D-galactose (150 mg/kg b.wt) for 70 days and drenched with omega 3 (80 mg/kg b.wt) twice daily for 28 days. T4 rats were I/p injected with D- galactose (150 mg/kg b.wt) for 70 days. (D-galactose $C_6H_{12}O_6$ >99%, Lot 28899-62205, MW 180.2 soluble in water, CAS no. 59-23-4 USA, Omega-3 – acid ethyl esters high purity, 90 %, Uk, concentration 840 mg for each 1000 mg Omacor dissolved in 28ml of soy bean oil).

Tissue samples collection

Animals were subjected to regular weighing both prior to and throughout the duration of the experiment. This was carried out to precisely administer D-galactose and Omega 3 doses based on the weights of the rats. The rats were anesthetized with ketamine and xylazine in accordance with (Veilleux-Lemieux et al., 2013). Heart tissues of the sacrificed specimens were preserved in a solution of natural buffer formalin (10%) in order to facilitate further histological examinations. Blood samples were collected at the end of the experiment to quantify serum catalase enzyme using colorimetric method assay kit was obtained from Elabscience, USA. Operation Equipment: Spectrophotometer. Serum samples were separated for measurement of Creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), immediately after serum samples were collected, and estimated by using the clinical chemistry analyzer Cobas c311an open reagent system, Roche diagnostic cobas c311 automated, software-controlled analyzer.

III. Results

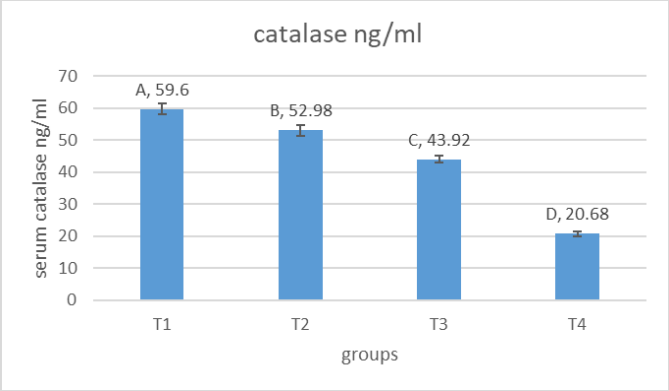

Figure (A-1)

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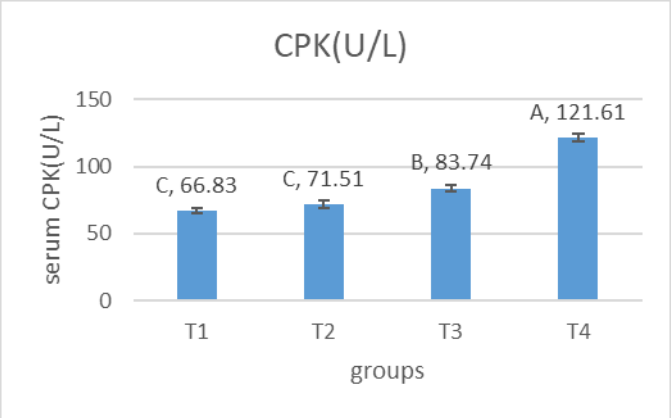


Figure(A-2)

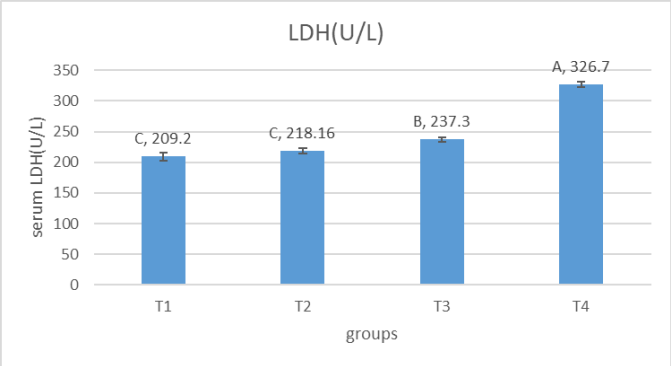


Figure (A-3)

Figure (A). Effect of omega -3 and D-galactose on serum catalase (1) ng/ml , CPK U/L (2) , LDH U/L (3) of adult males rats injected (I/P) with D-galactose (150 mg/kg bwt) and drenched with 80mg/kg bwt of Omega3 twice a day for 70 days.

Values expressed as mean ± SE. N= 5 rats. T1= Control group rats were injected with normal saline. T2=were injected (I/P) with D-galactose (150 mg/kg bwt) and drenched with80 mg/kg bwt of Omega3 for 70 days rat T3= rats were injected I/P with D- galactose (150 mg/kg b.wt) for 70 days and drenched with omega 3 (80 mg/kg b.wt)for 28 days T4= rats were I/p injected with D- galactose (150 mg/kg b.wt) for 70 days. Different letters denote significant differences between groups, ($p \leq 0.05$).

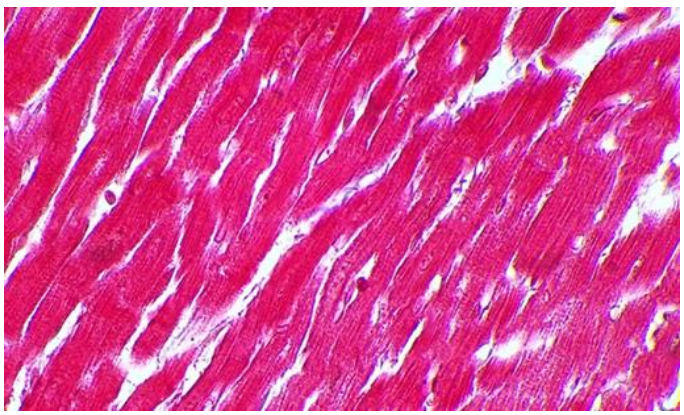
Serum catalase ng/ml

The results of serum catalase analysis, which are shown in Figure (A-1), show that the T4 group's serum CAT level was statistically significantly ($p \leq 0.05$) lower than the other groups. T2 group showed a significant decrease as compared to the T1 and a significant increase as compared with T3 and T4, results showed a statistically significant increase ($p \leq 0.05$) in T1 in a comparison to all other groups.

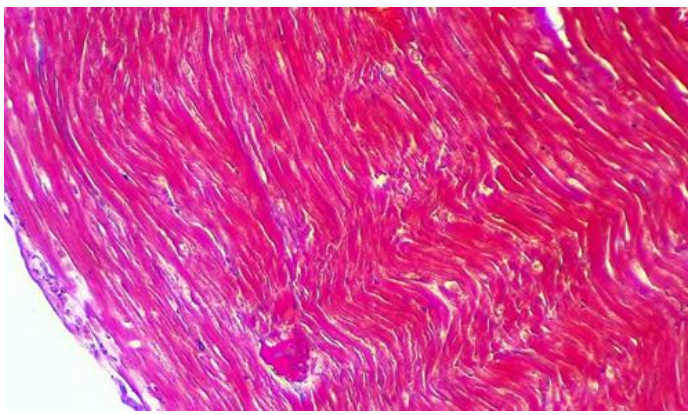
Serum creatinine phosphokinase CPK U/L: Figure (A-2) shows that Serum CPK levels were significantly higher in the T4 group than in the other groups ($p \leq 0.05$). A significant reduction ($p \leq 0.05$) was observed in the T1 group when compared to the T3 and T4 groups. Comparing T2 to the T1 control group, not a noticeable rise was seen.

Serum lactate dehydrogenase (LDH) IU /L: Figure (A-3) shows that The T4 group had significantly greater serum LDH levels than the other groups ($p \leq 0.05$). A statistically significant decrease ($p \leq 0.05$) was observed in the T1 group in comparison to the T3 and T4 groups. Likewise, there was no noticeable increase in T2 in comparison to the T1 (control group).

According to the results of the current study we can notice the harmful effect of 70 days of IP injection of D-galactose in T4 rats for the above parameters ,catalase , CPK and LDH and we can notice the improvement effect of Omega-3 for the same parameters for group T2 which has been drenched for 70 days as compared with those who received omega 3 for only 28 days with IP D- galactose injection for 70 days for (T3).



Figure(B-1). Histopathological section of rat heart for control group T1 showing negative rection (no deposition of collagen fibers). (Masson trichome, stain, 400x).



Figure(B-2). Histopathological section of rat heart for T2 showing negative blue reaction indicate no deposition of the collagen fiber among the myocardial fibers (Masson trichrome, stain, 200x).

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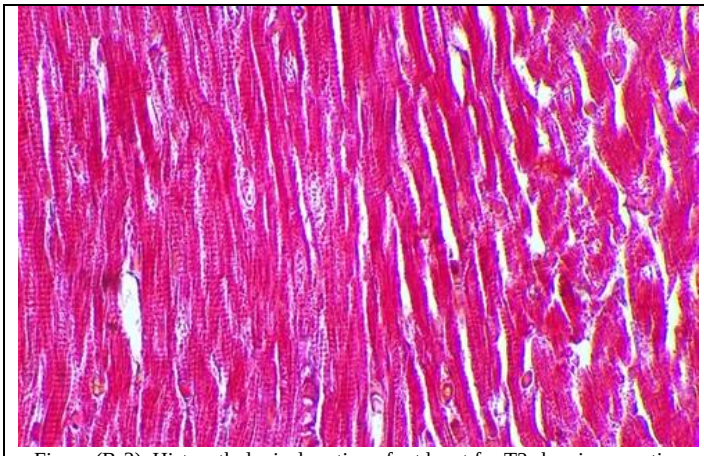

Figure (B-3). Histopathological section of rat heart for T2 showing negative blue reaction indicate no deposition of the collagen fiber among the myocardial fibers (Masson trichome, stain, 400x).

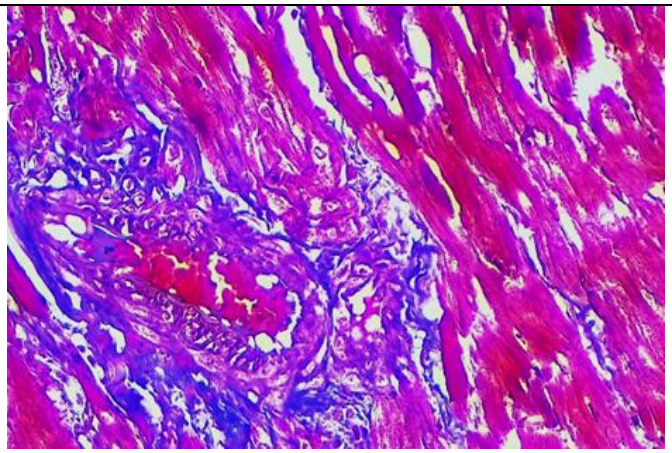

Figure (B-4). Histopathological section of rat heart for T3 showing the blue colour indicate severe deposition of the collagen fiber around the blood vessels (Masson trichome, stain, 400x).

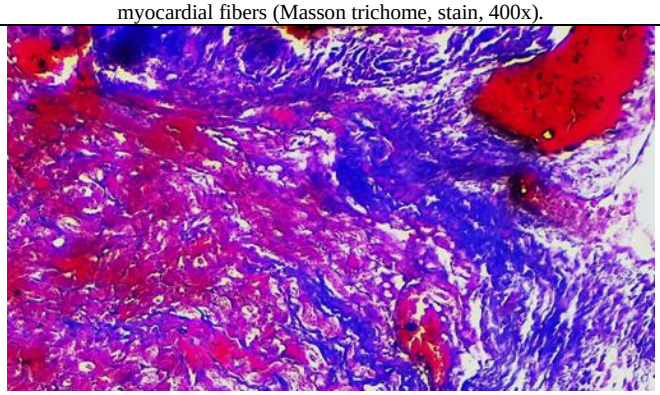

Figure (B-5). Histopathological section of rat heart for T3 showing the blue color indicate severe deposition of the collagen fiber among the myocardial fibers (Masson trichrome, stain, 200x).

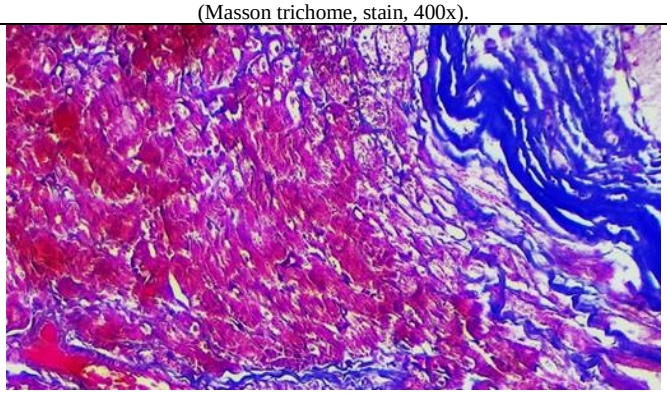

Figure (B-6). Histopathological section of rat heart for T4 showing the blue color indicate severe deposition of the collagen fiber among the myocardial fibers (Masson trichrome, stain, 200x).

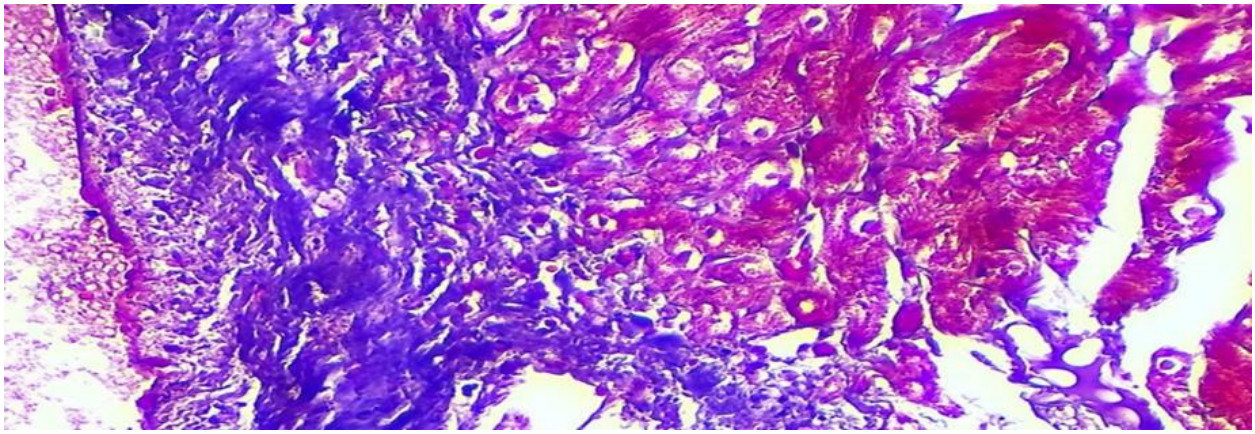

Figure (B-7). Histopathological section of rat heart for T4 showing the blue color indicate severe deposition of the collagen fiber among the myocardial fibers (Masson trichrome, stain, 200x).

IV. Discussion

The steady accumulation of cellular damage that occurs with ageing causes the organs to gradually malfunction. Left ventricles enlargement, heart dysfunction and elevated collagen deposition, are all signs of aging-related changes in the heart. Age is a major factor in the development of age-related cardiovascular disorders, which is why older people have a noticeably higher incidence of these conditions. Thus, the development of more effective treatment methods to treat age-related cardiac insufficiency is urgently needed (Yan *et al.*, 2021). Both of CPK and LDH levels which are crucial for cell energy activities, were employed to measure injury-induced heart muscle tissue damage by D-galactose. Aging induced group given D-galactose intraperitoneally had lower serum catalase levels and higher serum lactate dehydrogenase (LDH) and creatinine phosphokinase CPK than other experimental groups This work supports by (El-Akabawy *et*

al., 2024). who revealed that intraperitoneal injection with D-galactose, similar results approved by (Shimizu and Minamino, 2019) who said that age-related cardiac dysfunction may result from mitochondrial dysfunction that occurs with the ageing heart and the generation of reactive oxygen and nitrogen species as a result. Ageing indicators are expressed in heart tissue as a result of ROS generation , the protein p53 p21, and p16 to increase (Shimizu and Minamino, 2019). An imbalance between oxidant and antioxidant levels causes oxidative stress, which can disrupt control and even harm molecules and cell (Al-Okaily, 2024). There is evidence that administering d-galactose reduced the expression of antioxidants and raised the expression of oxidative stress (Wu *et al.*, 2017), which explain the similar results we have got for the present study regarding the significant decrease in serum catalase results for T4 group which were IP injected with D-galactose for 70 days. Galactose reductase is capable of converting excess d-galactose to galactitol, because galactitol cannot be further metabolised, it elevate the accumulation in the cells, which was obvious make changes in the normal oncotic pressure and weaken the antioxidant defensive mechanism, allow more free radicals to accumulate (Yanar *et al.*, 2011). One of the main causes of the accelerated processes that lead to ageing is oxidative damage caused by hydrogen peroxide (H2O2) and a superoxide anion (O2−), which are produced by an excessive metabolism of d-galactose (Aydın *et al.*, 2012). One of the main characteristics of the complex ageing process is generally acknowledged to be impaired redox homeostasis brought on by elevating the synthesis of ROS (Uzun *et al.*, 2013). Despite the fact that ROS are necessary for preserving regular cell function, too many of them generated by the metabolism of d-gal. can harm and destroy proteins with lipids and DNA, increasing the peroxidation of these substances (Li *et al.*, 2016). Another study which were discussed the mechanism of role of D-galactose in aging and oxidative stress markers was declare that proteolysis regularly breaks down oxidized proteins, but excess can evade this process, form huge molecular weight, and hasten the age process of the heart. While important antioxidant found in the heart, like total thiol group and no protein thiol group , were dramatically fall, advanced oxidation protein products, such as protein carbonyl group , protein-bound di tyrosine, kynurenine, and N-formyl kynurenine, increased (Cebe *et al.*, 2014). This suggests that d-Galactose inducing heart ageing is largely caused by impaired cellular redox homeostasis. Another study supporting the present study results revealed that because D-gal. treatment reduced GPx1 and CAT1 expression, it hindered antioxidant capability. Furthermore, they hypothesized that D-galactose might simply change antioxidant status. Regarding results we have got concerned with LDH also CPK for Rats treated with D-galactose exhibited a significant increase in both serum biomarkers, suggesting that D-galactose caused the rats to age. In this senescence induction model, after nine weeks of D-galactose induction, the rats' LDH and CPK activity significantly increased. This increase in biomarker activity is associated with hepatocellular damage and is a risk factor for the development of chronic liver disease. It is a sensitive marker of hepatitis, biliary cirrhosis, and conditions characterised by inflammation, regeneration, intrahepatic and extrahepatic bile blockage, and cardiovascular damage (Sofy *et al*., 2014). Regarding the gradual significant increase occur in T3, T2,T1for serum catalase results comparing with T4 rats , indicating the improvement in Oxidative status for these rats groups drenched with omega3 and we can notice the significant differences among these groups showing that groups obtained omega 3 for the whole 70 days had a better results and better improvement for oxidative stress status , the present results showed a similar results stated by (Morato *et al.*, 2015) who stated that Consuming Omega-3 boosted the activity of endogenous enzymes (catalase, superoxide dismutase, and glutathione peroxidase) and reduced muscle damage (creatine kinase and lactate dehydrogenase). Another study supports our results which has been proved that when rats exposed to continuous long-term exercise, omega-3 supplementation resulted in an increase in GSH, CAT activity, and a decrease in MDA levels (Sarikaya *et al.*, 2023). In order to ascertain whether ageing effects or omega-3 ameliorating properties related to differences in myocyte space were in fact related to changes in structural proteins like collagen, we used Masson's trichrome stain in order to observe the fibrillar collagen which might be located in the extra myocyte space. This stain has been used in relation to histopathological changes that occur in the cardiac muscles of rats. When compared to remarkably, omega-3-drenched rats for 70 days, we found that the accumulation of blue collagen fibers, a sign of ageing and fibrosis, and increased positive stain for the whole collagen (blue) in figures B-4, 5, 6, 7, which include T3 and T4 samples treated with D-galactose inducing ageing, reduced or mitigated age-related elevation in collagen-positive staining as in figures B-2,3, which clearly protect against age-related changes in collagen fiber meshwork structure. Histopathological changes obtained from current study were similar to a study which revealed that in a d-galactose-induced ageing rat model, cordycepin may prevent heart dysfunction, indicating the possibility for therapeutic cardio protection in aging ,cordycepin alleviated the decrease in the antioxidant factors superoxide dismutase (SOD) and catalase (CAT) levels and activity brought on by d-galactose treatment (Feng and Huang, 2022). Current results were comparably agree with (Liu *et al.*, 2024) ,who studied samples of Masson's trichrome and HE staining pictures In the aging-induced group administered D-galactose, representative mouse heart images from several groups were seen, along with the heart index, analysis of cardiomyocyte cross-sectional area, and fibrotic regions were seen. Another study has been done by (Feng *et al.*, 2021) who revealed that the cardiac architecture of D-gal-induced ageing animals was abnormal, mostly due to the disordered structure of cardiomyocytes and elevated spaces between cells. Regarding the improvement that occur in Omega –3 drenched groups for 70 days showed in figures (B-2, B-3), studied were support the present results which were stated that Masson's trichrome staining showed that PUFA therapy significantly reduced interstitial fibrosis, indicating that PUFA might partially repair cardiac fibrosis in the Sod. mice, additionally, compared to untreated animals,

the cardiomyocyte diameter was considerably reduced in Sod. 2 mice treated with n-3 PUFA, suggesting that n-3 PUFA treatment reduced cardiac hypertrophy (Li *et al.*, 2017). Also (Saravi *et al.*, 2023) who found a similar results to ours which proved that analysis of cardiac interstitial fibrosis (IF) in aged mice showed that a degree of cardiac fibrosis that extends to left ventricular stiffness occurs with ageing. The expression of transcriptional indicators of fibrosis, such as collagen α-1(I) chain, was markedly elevated in age-related interstitial fibrosis, and showed that long-term n3 fatty acid ALA attenuated the age-associated increases of fibrotic transcriptional markers observed in aged mice and significantly improved the physiological cardiac phenotype by reducing the interstitial fibrotic area in the ageing heart when compared to aged mice.

V. CONCLUSION

A recent study found that Omega-3 enhanced the biomarkers for cardiac muscle injury (LDH and CPK) and oxidative stress (CAT) that were affected by the harmful effects of D-galactose treatment over 70 days. Because of omega-3 supplements have antioxidant, antiaging, and antiapoptotic qualities, they decrease the buildup of collagen fibers and histopathological alterations (fibrosis) caused by D-galactose when diagnosed by using Masson Trichrome stain for heart muscle.

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Authors' contribution: The completed manuscript was reviewed and approved by all authors.

Ethical consideration: authors has examined the manuscripts for ethical issues, including redundancy, multiple publishing and/or submission, misconduct, data fabrication and/or falsification, plagiarism and permission to publish.

Conflicts of Interest

No conflict of interests.

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