Effectiveness of omega-3 on some physiological aspects in aged rats' kidneys

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Abstract

Background: The kidneys are particularly susceptible to the effects of aging. A major disruption of the body's homeostasis may occur from unfortunate events. It has been suggested that omega-3 can help reduce the effects of aging caused by D-galactose (D-gal). This study aimed to investigate the potential benefits of omega-3 in reducing dysregulation in kidney functions, redox homeostasis, and inflammatory markers in D-gal- treated rats. **Methodology:** Sixty adult male rats were randomly divided into four groups as follows: Control group (T1) were injected with normal saline(i/p) and given orally soya bean oil for 90 days, group T2 were injected with D-galactose at 150 mg/kg BW i/p and given omega-3 (75 mg/ kg BW) for 90 days while group T3A were injected with same dose of D-gal for 60 days then given orally omega-3 (75 mg/kg BW) for 30 days and finally group T3B injected with D-gal at (150 mg/kg BW (i/p)) for 90 days. Blood samples were collected from the anesthetized rats to estimate the levels of blood urea nitrogen, cystatin C, protein carbonyl, catalase, tumor necrosis factor-alfa (TNF-α) and interleukin-1β (IL-1β) concentrations in serum. Furthermore, kidney tissue specimens have been collected for a histopathological investigation and to measure the expression of the kidney IL-10 gene. **Results:** The findings indicated a notable decrease in catalase, along with a significant elevation in blood urea nitrogen, cystatin C, albumin, protein carbonyl, TNF-α, and IL-1β levels in the T3B group. In addition, omega-3 displayed enhanced kidney barrier integrity; additionally, and T2 group showed reduced kidney inflammatory lesions and improved IL-10 gene expression. The findings showed that giving omega-3 to the T2 and T3A groups significantly enhanced kidney functions, inflammatory criteria, kidney barrier integrity, oxidative stress-related parameters, IL-10 gene expression, and histopathological abnormalities. **Conclusions:** Omega-3 exhibits preventive action against kidney dysfunctions induced by D-galactose in treated rats, as evidenced by modulation of kidney function tests and histopathological alteration through its antioxidant and anti-inflammatory capacities.

Keywords: Aging, D-gal, kidney functions, oxidative biomarker, omega-3

Introduction

A variety of molecular and cellular damage is linked to the aging process in humans. After the age of 30, the first physical changes associated with aging start to appear. These include changes in hormone levels, a rise in body fat, loss of bone, muscle mass, and cartilage as well as a decline in kidney function (Partridge *et al*., 2018). The kidneys' primary functions include eliminating waste from the blood and controlling the concentrations of several vital substances. One of the main causes of mortality and disability in the globe is chronic renal disease (Kovesdy, 2022). The kidney is highly susceptible to senescence; therefore, it is critical to diagnose early any kidney failure to maintain life (Fang *et al*., 2020).

Endogenous and exogenous antioxidants inactivate free radicals, preventing them from causing harm in the context of systemic homeostasis (Pawluk *et al*., 2022). Oxygen free radicals and antioxidant defense systems are in equilibrium under normal physiological conditions. In pathological events, oxidative stress is caused by an imbalance between pro-oxidants and antioxidants, which damages cells (Jomova *et al*., 2023). The physiological level of reactive oxygen species (ROS), fibrotic disorders, inflammation, and high urine protein levels (proteinuria) are all essential for good kidney function; any alteration in the redox balance in the renal cortex and medulla may be detrimental changes in renal blood flow due to the retention of Na+ (Liu *et al*., 2024). Essential fatty acids that are required to maintain the structure and function of an organism are omega-3 polyunsaturated fatty acids (PUFAs). These consist of docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), α-linolenic acid (α-ALA), and eicosapentaenoic acid (EPA). The evidence reported that omega-3 (PUFAs), especially EPA and DHA, have anti-inflammatory and anti-oxidative stress properties that decrease mitochondrial dysfunction, improve autophagy flux, control metabolic pathways, and may have protective benefits (Sala-Vila *et al*., 2022). Therefore, this study was designed to investigate the potential role of omega-3 supplementation in attenuating D-galactose (D-gal)–induced age-related kidney dysfunction in rats.

Materials and Methods

Ethics approval

In complete adherence to the code of ethics for animal research, the animals used in this study were cared for and treated at the University of Baghdad's College of Veterinary Medicine. The municipal committee of animal care and use granted ethical permission (P.G./552, date 10-3-2024) to perform this research.

Experimental design

For this study, sixty Wistar male adult rats weighing between 250 and 300 grams and aged 4-4.5 months were used. The rats were kept at temperatures between 22 and 25°C in cages with a 12-hour light/dark cycle. Water and pellets were freely available to the animals during the trial. Prior to the trial, the rats were acclimated for 15 days. Control group (T1) was injected with normal saline(i/p) and given orally soya bean oil for 90 days, group T2 was injected with D-gal at 150 mg/kg BW i/p and given omega-3 (75 mg/ kg BW) for 90 days while group T3A was injected with the same dose of D-gal for 60 days then given orally omega-3 (75 mg/kg BW) for 30 days and finally group T3B was injected with D-gal (150 mg/kg BW(i/p)) for 90 days (Table 1).

Table 1: Study design

Group	Number of Animals	Saline Control (ml)a + Soybean Oil (ml)b	Concentration of D-Gal (mg/kg) a/ Days of Treatment	Concentration of Omega-3 (mg/kg) b/ Days of Treatment
T1	15	1+0.75	90/0	90/0
T2	15	1 +0.50	90/150	90/75
T3A	15	1 + 0.50	60/150	30/75
T3B	15	1	150/90	0/0

a: Delivered intraperitoneally, b: Delivered orally

Blood and tissue samples collection

After 90 days of the experiment, blood samples were collected by heart puncture technique. All animals were anesthetized at the end of the treatment, then sacrificed, and the blood samples were collected and placed in gel tubes.

Spectrophotometer technique

It was used to estimate blood urea nitrogen.

ELISA technique

The concentrations of cystatin c, catalase, protein carbonyl, TNF-α, and IL-1beta were measured using enzyme-linked immunosorbent test (ELISA) (BTLAB company, China).

Real-time PCR

To evaluate the expression of the IL-10 gene, total RNA was extracted from kidney tissue specimens of all the study groups using the RNA extraction kit (genaid, Korea). The extracted RNA concentration was measured by Quantus™ Fluorometer (Promega, USA). The total RNA was then reversed transcribed to cDNA using the kit from ADDBio (Korea) as in Table 2.

Table 2: Conversion of RNA to cDNA reaction components

Substance	Amount(μl)
ddH$_2$O	6.0
Reverse transcriptase (RT) 2× add script cDNA	20
dNTPs	4.0
Random oligos hexamer	2.0
RNA	8.0
Total volume	40

Then, AddScript RT-qPCR SYBR master kit (AddBio, Korea) was used for the qPCR reaction. The IL-10 gene-specific primers, including the forward (F) primer (5′- CCTTACTGCAGGACTTTAAGGGTT-3′) and the reverse (R) primer (5'-CTGGGGCATCACTTCTACCAG-3'), also F primer for the housing keeping gene (Gapdh) (5-'CACCCACTCCTCCACCTTTG-3') and the R primer (5'-CCACCACCCTGTTGCTGTAG-3') were used to evaluate gene expression (Hortobagyi *et al*., 2024). The thermal cycling conditions (Table 3) were carried out using BioRAD (USA). According to Schmittgen and Livak (2008) recommendations, the RT-qPCR results were evaluated.

Table 3: The qPCR program used for studying gene expression

Temperature (ºC)		Time	Repeat
Initial denaturation	95	5 min	1
Denaturation	95	20 sec	40x
Annealing	55	30 sec	40x
Extension	72	30 sec	40x
Melting analysis	95	15 sec	1
Melting analysis	60	60 sec	1
Melting analysis	+0.3 of 95	15 sec	With machine read

Histopathological examination

Another portion of each specimen was preserved by fixing it in a 10% formal saline solution. The tissue sections were stained with hematoxylin and eosin (H and E) for histological examination (Suvarna *et al*., 2018).

Measurement of kidney vascular leakage

An intravenous injection of 100 microliters of Evans blue dye (Sigma-Aldrich, USA) was given to the experimental animals (five rats per group) two hours before their sacrifices, then reported the whole kidney were extracted and stored in formamide (Fisher Scientific, USA) at 37ºC for 48 h (Saeed *et al*., 2012).

Statistical analysis

SPSS version 24 was the computer application used to analyze the data. The data were expressed as mean ± standard deviation (SD). The groups were compared at the level of significance of P < 0.05 using a one-way analysis of variance (ANOVA) and the least significant difference (LSD) test.

Results and Discussion

Effects of omega-3 on kidney function

Figure 1-A shows a significant elevation (p<0.05) of serum blood urea nitrogen in the T3B group compared to other experimental groups. Additionally, the D-gal and omega-3 treated groups (T2 and T3A) indicated a significant (P < 0.05) decrease in this parameter compared to the T3B group, which showed a significant (P ≤ 0.05) decrease in this parameter for the T2 group compared to the T3A and T3B groups. Furthermore, non-significant (P >0.05) differences were noted between control (T1) and T2 groups. The results revealed a significant (P ≤0.05) increase in cystatin C across all treated groups as compared with the control group. The current results also showed a significant (P ≤ 0.05) decrease in this parameter for T2 group compared to T3A and T3B groups, also the results showed a statistically significant (P <0.05) increase in serum cystatin c as observed in the value of T3B group compared to T2, T3A and control groups (Figure 1-B) .
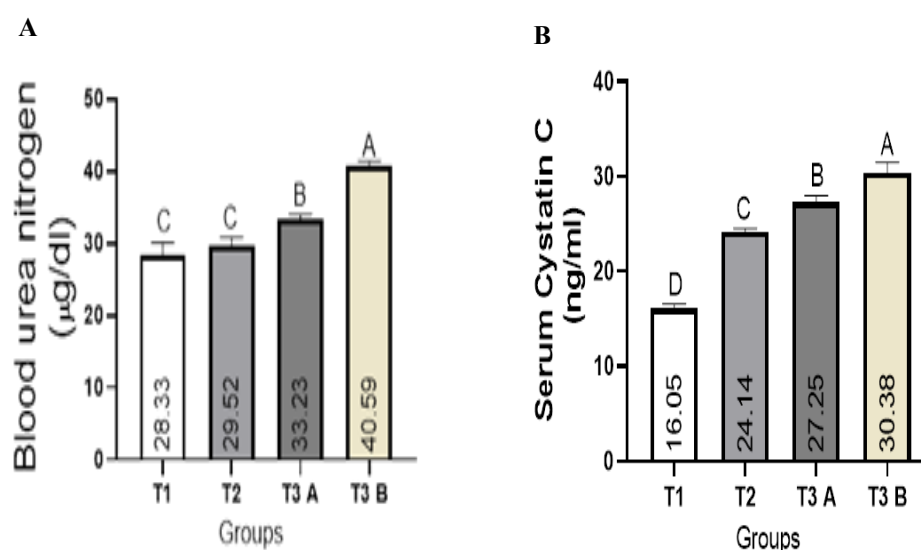

Figure 1: Effects of treating adult male rats with D-gal, omega-3, or both for 60 and 90 days on the concentration of: (A) serum blood urea nitrogen, (B) cystatin C. Values are expressed as mean ± SD, n= 5. Different capital letters denote significant differences between groups (P < 0.05)

The imbalance between ROS and antioxidant defenses develops with aging and causes kidney function to gradually decline (Gibson-Corley *et al*., 2013). Cystatin C, and blood urea nitrogen are important biomarkers for assessing renal health. When renal function is impaired, these metabolites continue to accumulate in the blood, resulting in elevating their amounts (Peng *et al*., 2021). In the current study, the elevated blood urea nitrogen and Cystatin C due to D-gal administration indicated that the kidney function was affected and associated with renal decline, that is coinciding with histopathological changes of the kidney in the T3B group. These findings are consistent with

Martinovic *et al.* (2025) who demonstrate that D-gal increases ROS generation, which in turn causes tissue oxidative damage, impacts cell metabolism, and leads to a reduction of kidney functions. In addition to producing ROS, the kidney is an essential organ that contains several mitochondria that produce adenosine 5'-triphosphate (ATP), which powers cellular processes. The pathophysiology of renal impairment is significantly influenced by oxidative status, which is caused by excessive ROS levels and results in DNA damage (Ho & Shirakawa, 2022).

Furthermore, omega-3 supplementation alleviated the harmful effects of D-gal (group T2A and T3B) on kidney functions. Omega-3 supplements enhance renal function by controlling oxidative stress and producing anti-renal aging benefits. Omega-3 has antioxidant properties by inhibiting lipid peroxidation, which helps to stabilize reactive radicals and limit the severity of D-gal (Li *et al*., 2025). Asemi *et al*. (2016) have shown that consuming omega-3 supplements increases antioxidant capacity and nitric oxide (NO) levels, which enhances vasodilation.

These results are in line with Buoite Stella *et al*. (2018), who noticed a reduction in vascular oxidative stress after omega-3 supplementation in rats. Omega-3 has anti-inflammatory and protective effects on kidney function (de Lima *et al*., 2023). Urea, cystatin C concentrations significantly increased in the T3B group as a result of a reduction in the glomerular filtration rate index. The glomerular filtration rate may decrease as a result of decreased renal blood flow or backflow of the glomerular filtration rate, all of which are signs of renal damage and can result in reducing kidney function (Ashtiyani *et al*., 2012). According to Farhana and Lappin (2023), omega-3 fatty acids improve glomerular filtration rate and renal clearance. Increased blood flow to the kidneys, decreased proteinuria, improved vascular function, and reduced inflammation and oxidative stress are all benefits of omega-3 fatty acids, which also aid in lowering blood urea nitrogen levels by enhancing renal function and lowering urea generation (Fazelian *et al*., 2021).

The anti-inflammatory and antioxidant properties of omega-3

The results generally indicate a significant ($p<0.05$) decrease in the serum concentration of catalase with a substantial ($p<0.05$) increase in serum protein carbonyl in the D-gal treated group compared to the control group (Figure 2 A and B). On the contrary, the results showed that consuming omega-3 led to a significant decrease in serum protein carbonyl and a considerable increase in catalase concentrations compared to D-gal treated groups.
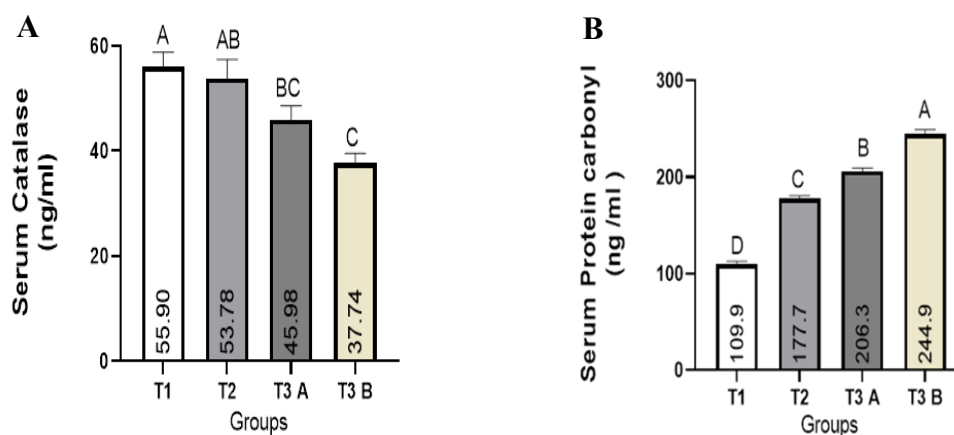

Figure 2: Effects of treating adult male rats with D-gal, omega-3, or both for 60 and 90 days on: (A) serum catalase concentration and (B) serum protein carbonyl. Values are expressed as mean ± SD, n= 5. Different capital letters denote significant differences between groups (P < 0.05)

In the current study, D-gal caused oxidative damage by increasing protein carbonyl and decreasing catalase levels. These results are consistent with several laboratory studies (Al-Kurdy, 2020). An imbalance between the generation and removal of ROS in the kidney can lead to macromolecular damage, accelerating the aging process by decreasing the expression of Nrf2, a crucial transcription factor that controls intracellular antioxidants. Oxidative stress accumulation contributes significantly to kidney aging (Al-Okaily, 2024). Supplementation of omega-3 to the group T2 for a prolonged time effectively reduced aging-induced oxidative stress by markedly alleviating the oxidative status, both of which had clear recovery effects.

The depletion of the body's antioxidant system due to increased use of CAT may be the cause of the marked drop in catalase concentration in D-gal-treated rats (T3B) (Pan *et al*., 2021). According to Li and his colleagues (2024), D-gal causes oxidative stress in mice's kidneys, and omega-3 therapy dramatically increases Nrf2 expression, indicating improved activation of the cellular antioxidant defense mechanism. Furthermore, the oxidative stress causes Nrf2 to release its cytoplasmic inhibitor protein, which enters the nucleus and activates a number of genes linked to antioxidant defense, such as catalase and superoxide dismutase (El-Emam *et al*., 2020). Omega-3 dramatically increases the expression of antioxidant genes, showing its effectiveness in lowering inflammation and oxidative stress, both of which are critical for kidney protection (Lee *et al*., 2023).

Figure 3-A indicates that serum TNF-α level was significantly ($P<0.05$) higher in the T2, T3A and T3B groups compared to the T1 group. In addition, the results revealed non-significant ($P >0.05$) differences between the T2 and T3A treated groups.
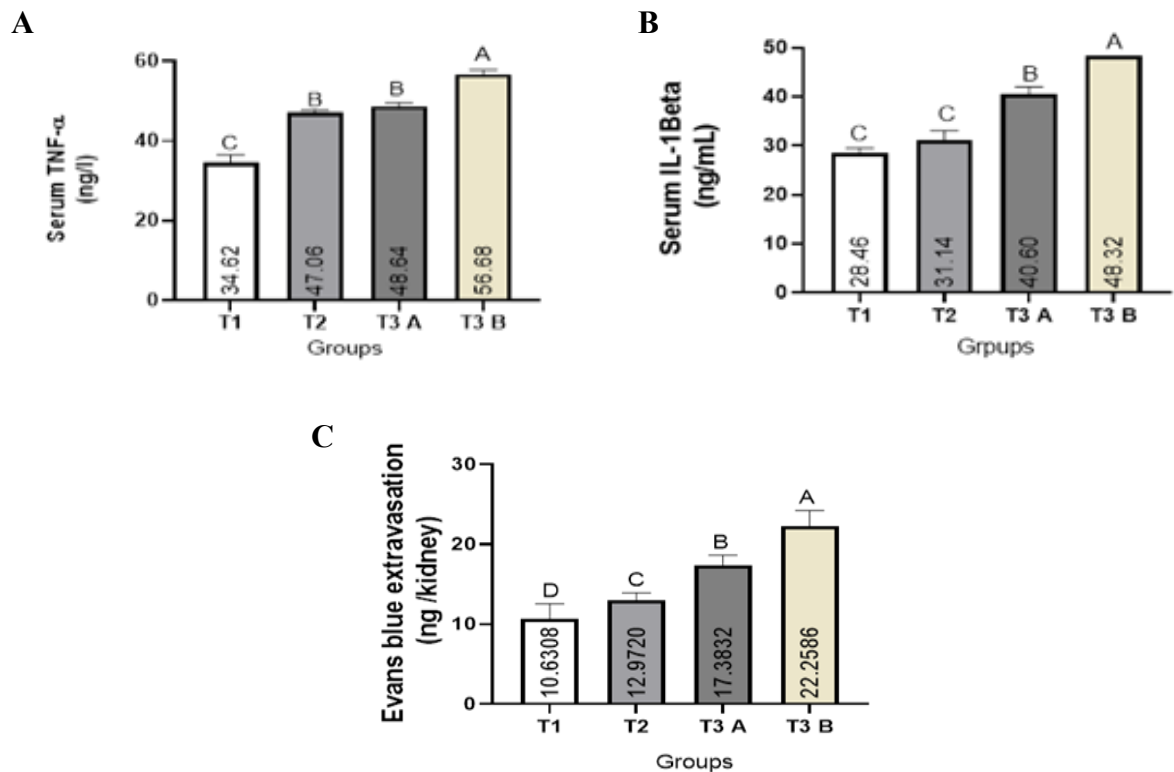

Figure 3: Effects of treating adult male rats with D-gal, omega-3, or both for 60 and 90 days on: (A) serum TNF-α, (B) serum IL-1β and (C) kidney-blood barrier. Values are expressed as mean ± SD, n= 5. Different capital letters denote significant differences between groups ($P < 0.05$)

The results of IL-1β cytokines in serum were dramatically raised (P<0.05) in the T3B group compared to the other experimental groups (Figure 3-B). However, non-significant (P >0.05) differences in this cytokine between T1 and T2 groups were reported. In addition, IL-1B cytokines in serum dramatically raised (P<0.05) in the T3B group compared to T2 and T3A groups. Concerning the kidney blood-barrier, the results of Evans blue dye level in the kidney (Figure 3-C), showed a highly significant elevation in this criterion in the T3B-treated groups compared to other groups. While the administration of omega-3 showed a significant (P ≤ 0.05) decrease of Evans blue dye level in the kidney in T2 and T3A compared with the T3B group.

Pro-inflammatory cytokines may be seen in the blood and are linked to age-related kidney disease (Sarkar and Fisher, 2006). D-gal causes an accumulation of advanced glycation end products (AGEs), which are linked to aging and kidney problems. It operates this by binding to cell surface receptors, including the receptor for AGE (RAGE), which starts signaling cascades that lead to inflammation (Wang *et al.*, 2024), also they activate intracellular signaling molecules including nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), which include p38 MAPK and ERK1/2. These signaling pathways contribute to inflammation and cellular damage by increasing the production of pro-inflammatory cytokines and activating inflammatory pathways (Zhou *et al.*, 2024). These findings are consistent with the study of Wati *et al.* (2025), which demonstrates that the activated NADPH oxidase enzyme caused an excessive accumulation of ROS during the aging process and the release of inflammatory cytokines by activating the TNF-α signaling pathway.

In comparison to the D-gal-injected group (T3B), the levels of these inflammatory cytokines were much lower in the model rats receiving omega-3 in the T2 group. This suggests that omega-3 may also have a strong anti-inflammatory effect and can influence the expression and methylation levels of genes associated with age-related processes or diseases (Xie *et al.*, 2021). Thereby, it has been suggested that omega-3 may delay aging through a variety of complex mechanisms such as increasing ATP production, inhibiting the generation of pro-inflammatory cytokines, and suppressing the IκB phosphorylation (Colussi *et al.*, 2023).

 The integrity of the barrier depends on tight connections. Damage to these junctions can weaken endothelium and epithelial barriers, which can lead to the onset and progression of several age-related illnesses (Dun *et al.*, 2018). The fundamental processes via aging that cause modifications in tight junctions are yet unknown, though following D-gal-induced senescence, the paracellular pathway's permeability was reduced, tight junction molecules Cldn3 and Ocln were expressed less often, and Cldn1, 3, and 7 were distributed irregularly throughout the cell membrane (Costea *et al.*, 2019).

It is possible that oxidative stress, which breaks down the kidney-blood barrier and accelerates age-related kidney disease, is the cause of the significant increase in Evans blue extravasation concentration in rats given D-gal (T3B) group. This result is in agreement with Ding *et al.* (2022). The results showed that omega-3 protected the blood-kidney barrier by reducing its permeability, as indicated by a decrease in Evans blue extravasation concentration. The current results are in line with Hamady & Al-Okaily (2022). EPA and DHA anti-inflammatory regulatory effects on cytokine dynamics and cellular mediator pathways may help to maintain the kidney's cellular barrier by enhancing its integrity and reducing its permeability (Chen *et al.*, 2025). Therefore, improved tight junction of protein expression, epithelial proliferation, mucus layer enrichment, immunomodulation, and inhibition of inflammatory cell infiltration are the primary molecular mechanisms of omega-3 fatty acids for preservation of barrier homeostasis under stressful conditions (Durkin *et al.*, 2021).

The IL-10 gene expression in the kidney

The results in Figure 4 show a substantial (p≤0.05) decline in the fold change of gene expression of the IL-10 gene in the kidney of the T3B group compared with T2 and T3A groups. Additionally, a notable rise in the fold change of the IL-10 gene expression was noted in rats receiving omega-3 with D-gal for 90 days (T2 group) compared to the other experimental groups.
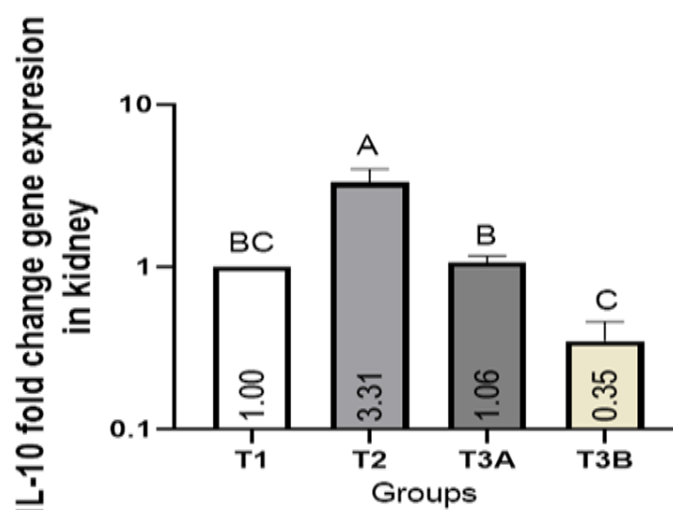
Figure 4: Effects of D-gal, omega-3, or both on the IL-10 gene expression in adult male rats' kidney tissue after 90 days of treatment. Values are expressed as mean ± SD, n=3. Different capital letters denote significant differences between groups (P < 0.05)

The pathogenesis of renal dysfunction may be attributed to a significant deficiency of IL-10; thus, it could play a role in the progression of acute kidney dysfunction. The exact mechanism is still unclear and requires further studies; however, IL-10 deficiency may be strongly linked with increased TGF-β production and pro- and anti-inflammatory diseases (Zhang *et al*., 2021). Through blocking toll-like receptor signaling, generating anti-inflammatory chemicals, reducing NF-κB activity in mice, and changing gene expression to decrease the production of pro-inflammatory cytokines, omega-3 fatty acids inhibited Th1 responses (Sundaram *et al*., 2022). In addition, omega-3 reduces the synthesis of pro-inflammatory mediators and alters the composition of cell membranes to have an anti-inflammatory impact (Sun *et al*., 2016). Moreover, omega-3 fatty acids promote the synthesis of resolvins and protectins linked to inflammation, reduce the proliferation of lymphocytes, and prevent the release of inflammatory cytokines, including IL-2, TNF-α, and elevated IL-10 (da Silva Batista *et al*., 2024). Moreover, omega-3 fatty acids can promote the growth and proliferation of Treg cells and improve their differentiation, probably through enhancing the release of IL-10 from regulatory T cells (Tregs') (Lobato *et al*., 2024; Bodur *et al*., 2025).

Histopathological examination

Compared to the control group (Figure 5 a), histopathological alterations were observed in D-gal. treated group (T3B). These alterations were characterized by atrophy, necrosis, and inflammatory cells in kidney tissues (Figure 5 d). Whereas tissue sections of rats' kidney that received omega-3 revealed mild histopathological changes (Figure 5 b,c) compared to group T3B.

The development and incidence of renal dysfunction, as well as the increase in interstitial tissue in the aged kidney, are closely associated with D-gal-induced aging (Qiu *et al.,* 2024). The present results are in line with the study of El-Horany *et al.* (2020), which showed that D-gal therapy caused significant tubular damage by causing necrotic tubular epithelial cells. This change may be due to the increased generation of ROS and lipid peroxidation, which contribute to inflammation and damage of renal tissues (Khudair & Al-Okaily, 2022). Supplementation with omega-3 fatty acids enhances kidney function by improving the antioxidant defense system, reducing oxidative stress, inflammation, and fibrosis, and upregulating Nrf2 expression (Al-Dabbagh *et al.,* 2024., Bodur *et al.,* 2025).
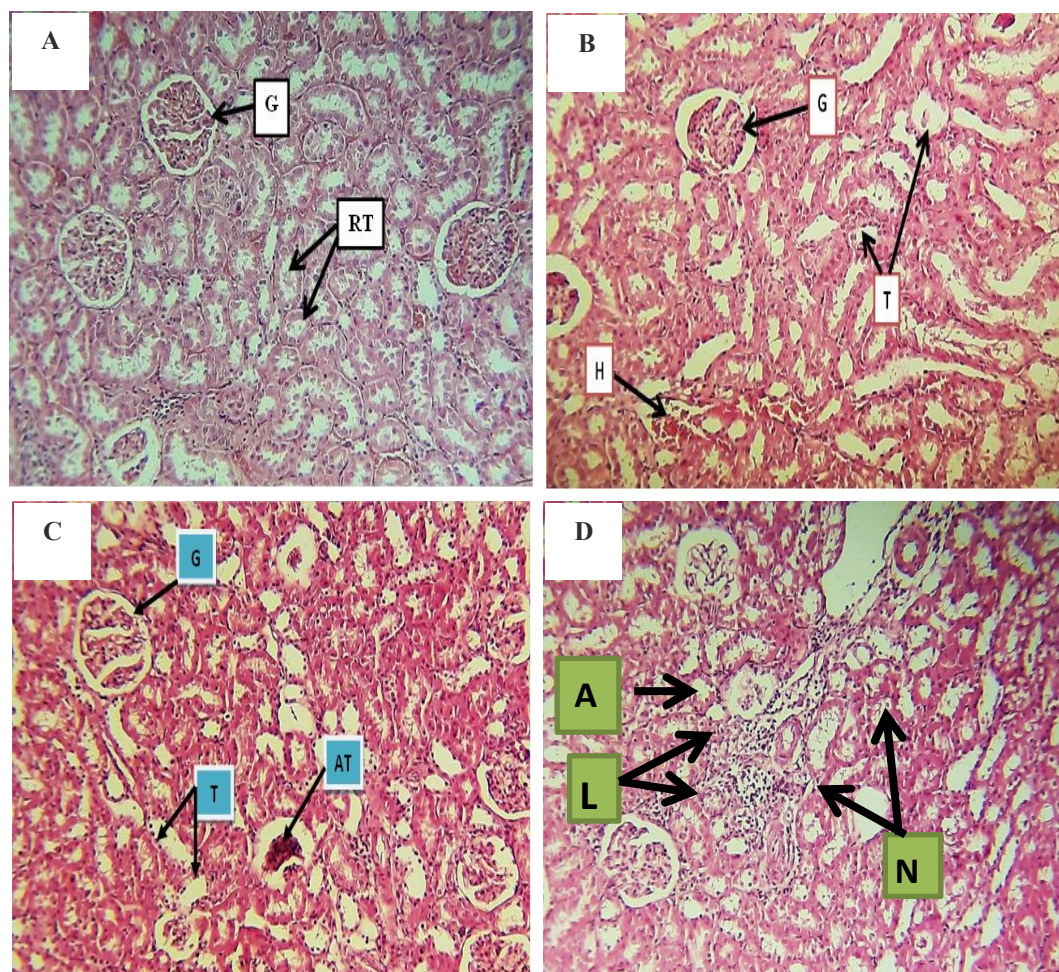
Figure 5: Histopathological changes in a rat's tissue. (a) Control group: (G) Glomerulus, (RT) renal convoluted tubules. (b) T2 group: (G) Glomerulus, (T) Tubular dilated, (T) Mild hemorrhage. (c) T3A group: (G) Glomerulus, (GA) Glomerular atrophy, (T) Tubular dilation. (d) T3B group: (A) swollen, (N) Necrosis, (L) focal interstitial Inflammatory cells infiltration. H & E stain 100x

Conclusions

According to this study, administering omega-3 fatty acids to rats treated with D-gal enhanced kidney function and characterized by alleviation of oxidative stress and attenuation of inflammation. These findings were supported by reduced histopathological alterations, indicating that omega-3 has notable anti-aging properties.

References

Al-Dabbagh, A. S. M., Mshemish, B. A. R., & Al-Mugdadi, S. F. H. (2024). Investigating the potential protective effects of omega-3 against doxorubicin-induced renal injury in rats through modification of antioxidant markers. *Immunopathologia Persa*. https://doi.org/10.34172/ipp.2024.41707

Al-Khaqani, B., & Mohammed, A. (2024). Ovalbumin-induced asthma in rats is alleviated by resveratrol treatment. *Journal of Animal Health & Production, 12,* 121-127. https://doi.org/10.17582/journal.jahp/2024/12.2.121.127

Al-Kurdy, M. J. J. (2020). The effect of black currant selenium nanoparticles on dyslipidemia and oxidant-antioxidant status in D-galactose treated rats. *Kufa Journal for Veterinary Medical Sciences, 11*(1). https://doi.org/10.36326/kjvs/2020/v11i13300

Al-Okaily, B.N.(2024).Unveiling the Mysteries of Oxidative Stress: An Insightful Review of Recent Studies, *Journal of Animal Health & Production. 12* (3), 395-412. https://doi.org/10.17582/journal.jahp/2024/12.3.395.412

Asemi, Z., Soleimani, A., Shakeri, H., Mazroii, N., & Esmaillzadeh, A. (2016). Effects of omega-3 fatty acid plus alpha-tocopherol supplementation on malnutrition–inflammation score, biomarkers of inflammation and oxidative stress in chronic hemodialysis patients. *International Urology & Nephrology, 48*, 1887-1895. https://doi.org/10.1007/s11255-016-1399-4

Ashtiyani, S. C., Najafi, H., Kabirinia, K., Vahedi, E., & Jamebozorky, L. (2012). Oral omega-3 fatty acid for reduction of kidney dysfunction induced by reperfusion injury in rats. *Iranian Journal of Kidney Diseases, 6*(4), 275.

Bodur, M., Yilmaz, B., Ağagündüz, D., & Ozogul, Y. (2025). Immunomodulatory Effects of Omega-3 Fatty Acids: Mechanistic Insights and Health Implications. *Molecular Nutrition & Food Research, 69*(10), e202400752. https://doi.org/10.1002/mnfr.202400752

Buoite Stella, A., Gortan Cappellari, G., Barazzoni, R., & Zanetti, M. (2018). Update on the impact of omega 3 fatty acids on inflammation, insulin resistance and sarcopenia: a review. *International Journal of Molecular Sciences, 19(*1), 218. https://doi.org/10.3390/ijms19010218

Calder P. C. (2010). Omega-3 fatty acids and inflammatory processes. *Nutrients, 2*(3), 355–374. https://doi.org/10.3390/nu2030355

Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Calina, D., & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry, 11*, 1158198. https://doi.org/10.3389/fchem.2023.1158198

Chen, Z., Mao, Q. Y., Zhang, J. Y., Wu, Y. X., Shan, X. F., Geng, Y., & Xiang, R. L. (2025). Cellular Senescence Contributes to the Dysfunction of Tight Junctions in Submandibular Glands of Aging Mice. *Aging Cell,* e14470. https://doi.org/10.1111/acel.14470

Colussi, N. A., Todaro, J. S., Rodríguez, J. P., Olea, G. B., Ferrini, L. A., Stoyanoff, T. R., & Aguirre, M. V. (2023). Dietary supplementation with integral chia and flax flours ameliorates systemic inflammation. *Medicina, 84*(2), 206–220. https://pubmed.ncbi.nlm.nih.gov/38683505

Costea, L., Mészáros, Á., Bauer, H., Bauer, H.-C., Traweger, A., Wilhelm, I., Farkas, A. E., & Krizbai, I. A. (2019). The Blood–Brain Barrier and Its Intercellular Junctions in Age-Related Brain Disorders. *International Journal of Molecular Sciences, 20*(21), 5472. https://doi.org/10.3390/ijms20215472.

da Silva Batista, E., Nakandakari, S. C. B. R., Ramos da Silva, A. S., Pauli, J. R., Pereira de Moura, L., Ropelle, E. R., & Cintra, D. E. (2024). Omega-3 pleiad: The multipoint anti-inflammatory strategy. *Critical Reviews in Food Science & Nutrition, 64*(14), 4817-4832. https://doi.org/10.1080/10408398.2022.2146044

de Lima, K., Mazur, C. E., Vicente Cavagnari, M. A., Castilho, A. J., & Schiessel, D. L. (2023). Omega-3 supplementation effects on cardiovascular risk and inflammatory profile in chronic kidney disease patients in hemodialysis treatment: An intervention study. *Clinical Nutrition ESPEN, 58*, 144–151. https://doi.org/10.1016/j.clnesp.2023.09.914

Ding, G., Shao, Q., Yu, H., Liu, J., Li, Y., Wang, B., Sang, H., Li, D., Bing, A., Hou, Y., & Xiao, Y. (2022). Tight Junctions, the Key Factor in Virus-Related Disease. *Pathogens, 11*(10), 1200. https://doi.org/10.3390/pathogens11101200

Dun, Y., M. Liu, J. Chen, et al. (2018). Regulatory Effects of Saponins From Panax japonicus on Colonic Epithelial Tight Junctions in Aging Rats. *Journal of Ginseng Research 42*(1), 50–56. https://doi.org/10.1016/j.jgr.2016.12.011

Durkin, L. A., Childs, C. E., & Calder, P. C. (2021). Omega-3 polyunsaturated fatty acids and the intestinal epithelium—a review. *Foods, 10*(1), 199. https://doi.org/10.3390/foods10010199

El-Emam, S. Z., Soubh, A. A., Al-Mokaddem, A. K., & Abo El-Ella, D. M. (2020). Geraniol activates Nrf-2/HO-1 signaling pathway mediating protection against oxidative stress-induced apoptosis in hepatic ischemia-reperfusion injury. *Naunyn-Schmiedeberg's Archives of Pharmacology, 393*(10), 1849-1858. https://doi.org/10.1007/s00210-020-01887-1

El-Horany, H. E., Gaballah, H. H., & Helal, D. S. (2020). Berberine ameliorates renal injury in a rat model of D-galactose-induced aging through a PTEN/Akt-dependent mechanism. *Archives of Physiology & Biochemistry, 126*(2), 157–165. https://doi.org/10.1080/13813455.2018.1499117

Fang, Y., Gong, A. Y., Haller, S. T., Dworkin, L. D., Liu, Z., & Gong, R. (2020). The ageing kidney: Molecular mechanisms and clinical implications. *Ageing Research Reviews, 63*, 101151. https://doi.org/10.1016/j.arr.2020.101151

Farhana, A., & Lappin, S. L. (2023). Biochemistry, lactate dehydrogenase. *StatPearls - NCBI Bookshelf.* https://www.ncbi.nlm.nih.gov/books/NBK557536

Fazelian, S., Moradi, F., Agah, S., Hoseini, A., Heydari, H., Morvaridzadeh, M., & Heshmati, J. (2021). Effect of omega-3 fatty acids supplementation on cardio-metabolic and oxidative stress parameters in patients with chronic kidney disease: A systematic review and meta-analysis. *BMC Nephrology, 22*(1), 157-165. https://doi.org/10.1186/s12882-021-02351-9

Gibson-Corley, K. N., Olivier, A. K., & Meyerholz, D. K. (2013). Principles for valid histopathologic scoring in research. *Veterinary Pathology, 50*(6), 1007-1015. https://doi.org/10.1177/0300985813485099

Hamady, J. J., & Al-Okaily, B. N. (2022). Alveolar gene expression of tight junction protein in nicotine rats treated with zinc and vitamin D. *International Journal of Health Sciences, 6*(S9), 232-246. https://doi.org/10.53730/ijhs.v6nS9.12218

Ho, H. J., & Shirakawa, H. (2022). Oxidative stress and mitochondrial dysfunction in chronic kidney disease. *Cells, 12*(1), 88. https://doi.org/10.3390/cells12010088

Hortobagyi, D., Grossmann, T., Kirsch, A., Winter, C., Roblegg, E., & Gugatschka, M. (2024). Exploring the anti-inflammatory potential of topical hyaluronic acid for vocal fold injury in a rat model. *European Archives of Oto-Rhino-Laryngology, 281*(1), 301-310. https://doi.org/10.1007/s00405-023-08278-1

Khudair, N. T., & Al-Okaily, B. N. (2022). Renal ameliorating effect of resveratrol in hydrogen peroxide induced male rats. *Iraqi Journal of Veterinary Science, 36*(3), 571–577. https://doi.org/10.33899/ijvs.2022.130939.1898

Kovesdy, C. P. (2022). Epidemiology of chronic kidney disease: an update. *Kidney International Supplements, 12*(1), 7-11. https://doi.org/10.1016/j.kisu.2021.11.003

Lee, J. Y., Son, Y. K., Lee, M. H., Lee, S. M., Kim, S. E., & An, W. S. (2023). Omega-3 fatty acids upregulate Nrf2 expression and attenuate apoptosis, inflammation, and fibrosis in a rat model of cyclosporine-induced nephropathy. *Kosin Medical Journal, 38*(3), 184-192. https://doi.org/10.7180/kmj.23.112

Li, J., Zuo, H. L., Huang, H. Y., Zhang, T., Bai, J. W., & Huang, H. D. (2025). Dietary Omega-3 PUFAs in Metabolic Disease Research: A Decade of Omics-Enabled Insights (2014–2024). *Nutrients, 17*(11), 1836. https://doi.org/10.3390/nu17111836

Li, P., Ma, Y., Wang, X., Li, X., Wang, X., Yang, J., & Liu, G. (2024). The protective effect of PL 1-3 on D-galactose-induced aging mice. *Frontiers in Pharmacology, 14*, 1304801. https://doi.org/10.3389/fphar.2023.1304801

Liu, B., Hu, Y., Tian, D., Dong, J., & Li, B. F. (2024). Assessing the effects of tempol on renal fibrosis, inflammation, and oxidative stress in a high-salt diet combined with 5/6 nephrectomy rat model: utilizing oxidized albumin as a biomarker. *BMC Nephrology, 25*(1), 64. https://doi.org/10.1186/s12882-024-03495-0

Lobato, T. B., Santos, E. S. D. S., Iser-Bem, P. N., Falcão, H. D. S., Gimenes, G. M., Pauferro, J. R. B., & Gorjão, R. (2024). Omega-3 Fatty Acids Weaken Lymphocyte Inflammatory Features and Improve Glycemic Control in Nonobese Diabetic Goto-Kakizaki Rats. *Nutrients, 16*(23), 4106. https://doi.org/10.3390/nu16234106

Martinovic, J., Stojanovic, I. G., Nesic, S., Todorovic, A., Bobic, K., Stankovic, S., & Drakulic, D. (2025). Chronic oral D-galactose induces oxidative stress but not overt organ dysfunction in male Wistar rats. *Current Issues in Molecular Biology, 47*(3), 161. https://doi.org/10.3390/cimb47030161

Pan, H., Feng, W., Chen, M., Luan, H., Hu, Y., Zheng, X., & Mao, Y. (2021). Alginate oligosaccharide ameliorates d-galactose-induced kidney aging in mice through activation of the Nrf2 signaling pathway. *BioMed Research International, 2021*(1), 6623328. https://doi.org/10.1155/2021/6623328

Partridge, L., Deelen, J., & Slagboom, P. E. (2018). Facing up to the global challenges of ageing. *Nature, 561*(7721), 45–56. https://doi.org/10.1038/s41586-018-0457-8

Pawluk, H., Kołodziejska, R., Grześk, G., Woźniak, A., Kozakiewicz, M., Kosinska, A. & Kozera, G. (2022). Increased oxidative stress markers in acute ischemic stroke patients treated with thrombolytics. *International Journal of Molecular Sciences, 23*(24), 15625. https://doi.org/10.3390/ijms232415625

Peng, R., Liu, K., Li, W., Yuan, Y., Niu, R., Zhou, L., & Wu, T. (2021). Blood urea nitrogen, blood urea nitrogen to creatinine ratio and incident stroke: the Dongfeng-Tongji cohort. *Atherosclerosis, 333*, 1-8. https://doi.org/10.1016/j.atherosclerosis.2021.08.011

Qiu, L., Ma, Z., Li, J., Wu, Z., Dai, L., Long, R., Hu, L., Sun, J., Hu, M., & Li, Y. (2024). Development of a spontaneous model of renal interstitial fibrosis in NOD/SCID mice: Aging-induced pathogenesis. *PloS One, 19*(12), e0315437. https://doi.org/10.1371/journal.pone.0315437

Saeed, A. I., Rieder, S. A., Price, R. L., Barker, J., Nagarkatti, P., & Nagarkatti, M. (2012). Acute lung injury induced by Staphylococcal enterotoxin B: disruption of terminal vessels as a mechanism of induction of vascular leak. *Microscopy and Microanalysis: the Official Journal of Microscopy Society of America, Microbeam Analysis Society, Microscopical Society of Canada, 18*(3), 445–452. https://doi.org/10.1017/S1431927612000190

Sala-Vila, A., Fleming, J., Kris-Etherton, P., & Ros, E. (2022). Impact of α-linolenic acid, the vegetable ω-3 fatty acid, on cardiovascular disease and cognition. *Advances in Nutrition, 13*(5), 1584-1602. https://doi.org/10.1093/advances/nmac016

Sarkar, D., & Fisher, P. B. (2006). Molecular mechanisms of aging-associated inflammation. *Cancer Letters, 236*(1), 13–23. https://doi.org/10.1016/j.canlet.2005.04.009

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols, 3*(6), 1101–1108. https://doi.org/10.1038/nprot.2008.73

Sinuani, I., Beberashvili, I., Averbukh, Z., & Sandbank, J. (2013). Role of IL-10 in the progression of kidney disease. *World Journal of Transplantation, 3*(4), 91–98. https://doi.org/10.5500/wjt.v3.i4.91

Sundaram, T. S., Giromini, C., Rebucci, R., Pistl, J., Bhide, M., & Baldi, A. (2022). Role of omega-3 polyunsaturated fatty acids, citrus pectin, and milk-derived exosomes on intestinal barrier integrity and immunity in animals. *Journal of Animal Science & Biotechnology, 13*(1), 40. https://doi.org/10.1186/s40104-022-00690-7

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's theory and practice of histological techniques. In Elsevier eBooks. https://doi.org/10.1016/c2015-0-00143-5

Wang, C. C., Chang, T. Y., Peng, P. J., Chan, D. C., Chiang, C. K., & Liu, S. H. (2024). Role of advanced glycation end-products in age-associated kidney dysfunction in naturally aging mice. *Life Sciences, 354*, 122984. https://doi.org/10.1016/j.lfs.2024.122984

Wati, F. A., Budiningsih, F., Wijaya, D. T., Arifin, A., & Susilo, R. S. B. (2025). The effect of D-galactose on inflammaging markers, appendicular muscle mass, fat-to-muscle ratio, and physical endurance to accelerate aging in mice. *Journal of Research in Pharmacy, 29*(2), 713-721. https://doi.org/10.12991/jrespharm.1664941

Xie, S. H., Li, H., Jiang, J. J., Quan, Y., & Zhang, H. Y. (2021). Multi-Omics Interpretation of Anti-Aging Mechanisms for ω-3 Fatty Acids. *Genes, 12*(11), 1691. https://doi.org/10.3390/genes12111691

Zhou, M., Zhang, Y., Shi, L., Li, L., Zhang, D., Gong, Z., & Wu, Q. (2024). Activation and modulation of the AGEs-RAGE Axis: Implications for inflammatory pathologies and therapeutic interventions-A review. *Pharmacological Research, 206*, 107282. https://doi.org/10.1016/j.phrs.2024.107282

فعالية اوميغا ٣ على بعض الجوانب الفسيولوجية لكلى الجرذان المسنة

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المستخلص

الخلفية: الكلى حساسة بشكل خاص لتأثيرات الشيخوخة، إذ قد يحدث خلل كبير في توازن الجسم الداخلي نتيجة لتغيرات فسيولوجية مرتبطة بالتقدم في العمر، وقد يؤدي ذلك إلى أن الأوميغا ـ٣ يمتلك دوراً في تقليل الشيخوخة المرتبطة بالكلى. هدفت هذه الدراسة الى تقييم مدى فائدة أوميغا ٣ في الحد من اختلال وظائف الكلى وتوازن الأكسدة والاختزال ومؤشرات الالتهاب لدى الجرذان المعالجة بدال كاللاكتوز. **المنهجية:** قسم ستون جرذا ذكرا بالغا عشوائيا الى اربع مجموعات تجريبية على النحو التالي: الجرذان في مجموعة السيطرة (T1) حُقنت تحت الصفاق بمحلول مائي طبيعي وأعطيت عن طريق الفم فول الصويا لمدة ٩٠ يوما، اما المجموعة الثانية (T2) حُقنت داخل الصفاق بدال كاللاكتوز بجرعة ١٥٠ ملغم/كغم من وزن الجسم وأُعطيت اوميغا ٣ بجرعة ٧٥ ملغم/كغم من وزن الجسم لمدة ٩٠ يوما، بينما المجموعة الثالثة (T3A) حُقنت بنفس الجرعة من دال كاللاكتوز داخل الصفاق بجرعة ١٥٠ ملغم/كغم من وزن الجسم لمدة ٦٠ يوما وأُعطيت اوميغا ٣ بجرعة٧٥ ملغم/كغم من وزن الجسم لمدة ٣٠ يوما، والمجموعة الرابعة (T3B) حُقنت بدال كاللاكتوز داخل الصفاق بجرعة ١٥٠ ملغم/كغم من وزن الجسم لمدة ٩٠ يوما. جُمعت عينات دم من الجرذان المخدرة لتقدير مستويات نيتروجين اليوريا في الدم، والسيستاتين سي وبروتين الكاربونيل والكتليز و عامل النخر الورم الفا (TNF-α)، والانترلوكين IL-1B في المصل، كما جُمعت عينات من انسجة الكلى لأجراء الفحص المرضي النسيجي ولقياس التعبير الجيني IL-10 في الكلى. **النتائج:** أظهرت النتائج الى انخفاض ملحوظ في انزيم الكتليز، الى جانب ارتفاع ملحوظ في مستويات نيتروجين اليوريا في الدم، والسيستاتين سي، وبروتين الكاربونيل، TNF-α، IL-1B في مجموعة T3B بالإضافة إلى ذلك، أظهرت مجموعة T2 انخفاضًا في الآفات الالتهابية الكلوية، وتحسنًا في التعبير الجيني لجين IL-10. كما بيّنت النتائج أن إعطاء اوميغا ٣ لمجموعتي T2 وT3A حسّن بشكل ملحوظ وظائف الكلى، ومعايير الالتهاب، وسلامة حاجز الكلى، والمعايير المتعلقة بالإجهاد التأكسدي، والتعبير الجيني لجين IL-10، والتشوهات النسيجية المرضية. **الاستنتاجات:** يظهر الأوميجا ٣ تأثيرا وقائيا ضد اختلال وظائف الكلى الناتج عن الدال كاللاكتوز في الجرذان المعالجة، ويتضح من تعديل اختبارات وظائف الكلى والتغيّر النسيجي المرضي عبر قدراته المضادة للأكسدة والالتهابات.

الكلمات المفتاحية: الشيخوخة، دال كاللاكتوز، وظائف الكلى، المؤشر الحيوي المؤكسد، الاوميغا٣