Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 62(1): 130-136 July 2019
DOI: 10.5455/ajvs.55680

The Possible Effect of Omega 3 Fatty Acids Against Cardiovascular Diseases in Rats: Biochemical and Histopathological Studies

Nada Sallai, Mohamed El-Adl*, Gehad El-Sayed
Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Mansoura University.

Key words:
Omega 3 fatty acids, Doxorubicin, Cardiovascular diseases, cardiotoxicity, Antioxidant.

***Correspondence to:**
drmohamedalymaher@hotmail.com

Received: 01 June 2019
Revised: 30 June 2019
Accepted: 01 July 2019

ABSTRACT

This research is designed for detect the possible protective effect of omega 3 poly unsaturated fatty acids (PUFA) against cardiovascular diseases (CVD) in rats. Fifty rats were divided into five groups each group contain ten rats. The first group, control group, received control diet without any additives. The second group was injected intraproteneally with doxorubicin 2.5mg/kg b.wt in 6 injected equally and alternatively in 6 injections for 2 weeks. The third group was given omega 3 fish oil 400mg/kg b.wt daily for one month via intragastric tube. The fourth group was injected with doxorubicin as second group, and then given omega 3 fatty acids for 2 weeks. The fifth group, was given fish oil omega 3 fatty acids for 2 weeks, and then was injected with doxorubicin as second group. Serum samples were collected for assessment of heart muscle function through (CK, LDH enzymes). Heart was divided longitudinally to three parts taken for antioxidant enzymes activities determination, gene expression and *histopathological examination.* The result of CK and LDH concentrations in serum showed significant elevation in doxorubicin injected groups, decrease in groups received omega 3. Superoxide dismutase (SOD), glutathione S transferase (GST) and Catalase (CAT) activities were elevated in groups received omega 3. GSH concentration was also elevated in groups received omega 3. The gene Rp6kb1 expression showed improvement in groups received omega 3. Finally, we can conclude that omega 3 have a significant protective and therapeutic effect on cardiovascular diseases.

1. INTRODUCTION

The omega 3 PUFA was steadily interested since the observation that Greenland Eskimos have a low incidence of cardiovascular diseases due to diet rich in fatty fish (Saravanan *et al.*, 2010).

Main dietary source of omega 3 fatty acids are fish containing ecosapentanoic acid (EPA) and docosahexanoic acid (DHA), as well as nuts, seeds and vegetables. Marine sources may be a helpful method for both primary and secondary prevention of CVD. Omega 3 exerts the cardio-protective effect through several mechanisms, including reducing arrhythmia and modifying production of prostaglandins, which reduce inflammation and improve platelet and endothelial function (Deflippis and sprling, 2006). Up till, no grave adverse effect of omega 3 have been identified.

Doxorubicin is a potent, fundamental and highly effective drug in several types of cancer as breast cancer, soft tissue sarcoma of the esophagus and leukemia. But it is utility is still limited due to it is specific toxicity to heart tissue (Zhons et al., 2001).

After several time of DOX administration Congestive heart failure, cardiomyopathy and cardio electrography changes demonstrated (Lenaz and Page, 1976). The mechanisms of the cardiotoxicity effect of DOX, include, decreased activity of Na+, K+ pumping mechanism, free radical induced myocardial injury, lipid peroxidation, mitochondrial damage. Increased oxidative stress and release of free radicals,

including superoxide anion (O^{-2}) and other reactive oxygen intermediates as well as endogenous antioxidant deficit have been suggested to play major role in DOX -induced cardiomyopathy and heart failure (Ahmed et al., 2005).

This study aimed to demonstrate cardio-protective effect of omega3 polyunsaturated fatty acid against doxorubicin induced myocardial toxicity in spreque dawley rats through the biochemical analysis of myocardial function (CK and LDH), analysis of antioxidant activity in heart tissue homogenate (SOD, GST, CAT and GSH), histopathological expression of myocardial tissue and gene expression of Rps6kb.

2. Materials and methods

2.1. Chemicals

Omega3 a capsule 1000mg was supplied from South Egypt Industries Company, Egypt, Doxorubicin Hcl (50mg) was supplied from EIMC united pharmaceuticals.

2.2. Animals: the study was carried out on fifty rats weighted from 150 to 200 gm kept for one week to accommodate to laboratory condition in faculty of veterinary medicine in Mansoura University and the experiment continued to one month later.

2.3. Experimental design

Fifty rats were divided equally into five groups ten rats in each group as followed: first group (control group) received diet without any additives, second group injected IP with doxorubicin 2.5mg/kg b.wt injected equally and alternatively 6 injections for 2 weeks (Swamy et al., 2012), third group received omega 3 fish oil 400mg/kg b.wt daily through gastric intubation for one month (Uygur et al., 2014). Fourth group injected with doxorubicin as second group then treated with omega3 fish oil, fifth group receive omega 3 as in third group for 2 weeks then injected with doxorubicin as in second group. All experimental diet was designed according to NRC (1995).

2.4. Blood and tissue Sampling

At the end of experiment rats were anasethized with thiopental sodium and blood samples were centrifuged for serum collection used to determine:

2.4.1. Biochemical analysis

Creatine kinase (CK) and lactate dehydrogenase (LDH) activities were determined according *to* Szasz et al., (1976) *and* Decker and Lohmann-Matthes, *(1988) respectively*. Rats were dissected and specimens from heart tissues were collected and divided longitudinally to three parts, first part was preserved in ice cooled phosphate buffer saline for evaluation of antioxidant activities, second part preserved in RNA later for determination of Rps6kb1 gene expression and third part was fixed in 10% neutral buffered formalin for histopathological examination

2.4.2. Antioxidant analysis

Heart tissues were homogenized with PBS, pH 7.4, where centrifugation performed and supernatant collected for enzymatic activity determination. SOD determined by (Nishikimi et al, 1972), GST measured by colorimetric method according to method of Habig and Jakoby, (1981). Catalase measured according to method of Aebi, (1984), GSH concentration measured by the colorimetric method according to Beutler *et al.,* (1963).

2.4.3. RNA extraction for Rps6kb1 gene

Quantitative reverse transcriptase (qRT-PCR) PCR was done to determine the Rps6kb1 gene expression. The extraction and purification of total RNA was done according to the instruction of RNeasy mini kit (Qiagen, Cat. No.74104) RNA concentration and quality was checked with Quawell, nanodrop spectrophotometer (USA). An equivalent of 1µg of RNA was transferred into cDNA (Thermo Fisher, Cat. No. EP0441), where 10 µm of forward and reverse primers were mixed with 10 µl of sybr green master mix (Intron, South Korea, Cat. No. 204141). The designed primers were listed in the Table 1. The Reverse transcription was done at 50 ℃/30 min. After that, the amplification was done and included 40 cycles with primary denaturation at 94 ℃/15 min, secondary denaturation at 94 ℃/ 15 sec, annealing at 60 ℃/30 sec for β-actin and at 55 ℃/30 sec for Rps6kb1, extension at annealing at 72 ℃/30 sec. this method according to Yuan *et al.,* (2006).

2.5. Histopathological studying

The heart tissue was fixed in 10% neutral buffer formalin. The specimens were embedded in paraffin wax. Blocks was stained with hematoxylin and eosin and examined by light microscopy according to **Wood and Ellis, (1994)**

2.6. Statistical analysis

Data were expressed as mean and it is standard error and was analyzed by using one way analysis of Variance ANOVA for the determination of statistical differences between means at significant level of 0.05, where least significance difference test was used as a post-hoc test (McCormick *et al.,* 2017).

Table 1 Primers pair with their accession number

Gene	Primer sequence	Reference/Accession number
βactin	TCCTCCTGAGCGCAAGTACTCT GCTCTAGTAACAGTCCGCCTAGAA	**Bannei et al., (2010)**
Rps6kb1	GCTCATACAAAAGCAGAGCGG GATTTCAGCCAAGTAAAAGCAAGC	**Current study (NM_031985.1)**

3. RESULTS

3.1. Effect of omega3 on CK and LDH enzymes concentrations in blood

Results showed increase in both CK and LDH concentration in group 2 and group 4 which injected with DOX when group 3 and group 5 received omega3 showed decrease in their concentration (Table 1).

3.2. Effect of omega3 on antioxidants activities on rats' heart

The result showed improvement in SOD, GST and Catalase activities in group 3 and group 5 which received omega3 also GSH concentration improved in same groups (Table 2)

3.3. Effect of omega 3 on Rps6kb1gene expression

Group3 which received omega 3 showed an increase in the gene expression, group2, group4 and group 5 showed a decrease in gene activity (Table 2)

3.4 Histopathological examination

In group 1, a longitudinal section of myocardium was presented and showed normal myocardial fibers (Fig.1). In group 2, a marked degree of myocardial necrosis was found (arrow) with the presence of interstitial tissue fibrosis (arrow head) (Fig. 2). In the third group, normal myocardium (arrow) was found that was separated with blood capillaries. In Fig. 4, focal degeneration of myocardium with loss of striation and marked eosinophilia in sarcoplasm (arrow). Moreover, a moderate degree of myolysis was also observed in the fourth group. A mild degree of myolysis was further extended to the fifth group in cardiac fibers (arrow) (Fig.5).

Table (2): Effect of omega 3 fatty acids on CK and LDH enzyme activities in serum of rats (Mean±SEM).

Animal groups	Mean activity of CK in serum (U/ML)±SEM	Mean activity of LDH in blood (U/ML)±SEM
Group1	119.000±11.239^{c}	380.666±12.197^{bc}
Group2	290.000±10.263^{a}	689.000±16.093^{a}
Group3	96.666 ±7.753^{c}	318.000±14.573^{c}
Group4	210.000±14.177^{b}	465.333±51.017^{b}
Group5	138.000±17.039^{c}	378.000±15.307^{b}

Means with different letters in the same column are significantly differed at (p< 0.05).

Table (3): Effect of omega 3 fatty acids on myocardium antioxidant status and Rps6kb1 relative expression in rats (Mean±SEM).

Animal groups	*Mean SOD activity (U/gm)±SEM*	*Mean GST activity(U/gm)±SEM*	*Mean catalase activity (U/gm)±SEM*	*Mean GSH concentration (mg/gm)±SEM*	*Rps6kb1 relative expression*
Group1	650.3±3.179^{a}	0.843±0.127^{c}	2.050±0.425^{b}	6.490±0.338^{b}	1±0.02^{c}
Group2	323.0±3.78^{d}	2.636±0.409^{a}	2.216±0.158^{b}	4.506±0.266^{c}	8.87±0.005^{a}
Group3	512.6±4.05^{b}	2.230±0.176^{ab}	3.146±0.136^{a}	7.096±0.306^{ab}	0.88±0.01^{c}
Group4	434.3±27.41^{a}	0.793±0.096^{bc}	1.803±0.095^{b}	4.196±0.358^{a}	5.21±0.06^{b}
Group5	645.6±554^{c}	1.513±0.169^{c}	2.566±0.881^{ab}	8.25±0.397^{c}	6.92±0.05^{ab}

Means with different letters in the same column are significantly differed at (p< 0.05).

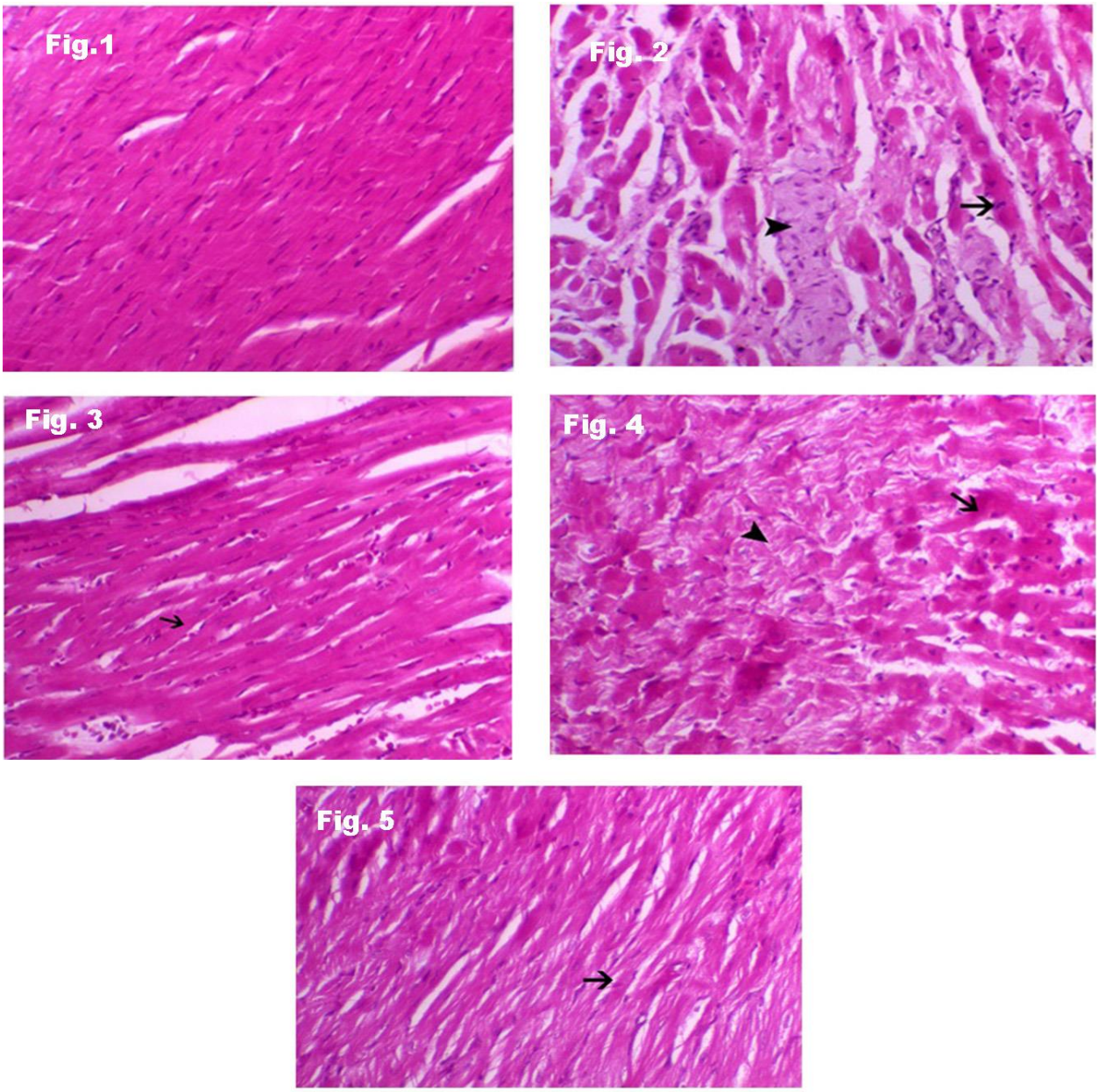

Fig.1. A longitudinal section of myocardium was presented and showed normal myocardial fibers. **Fig. 2**: marked degree of myocardial necrosis was found (arrow) with the presence of interstitial tissue fibrosis (arrow head). **Fig.3**: Normal myocardium (arrow) was found that was separated with blood capillaries. **Fig. 4**: focal degeneration of myocardium with loss of striation and marked eosinophilia in sarcoplasm (arrow)., a moderate degree of myolysis was also observed in the fourth group. **Fig.v5**: A mild degree of myolysis was further extended to the fifth group in cardiac fibers (arrow)

4. DISCUSSION

According to table (1) mean serum concentration of CK in Group (2) which intoxicated with Dox (were significantly higher more than 2 folds compared to control group. This result was supported with (Venkatesan et al, 2010). In contrast, group 3(received omega 3 PUFA 400mg/kg b.wt. dialy for month) showing significantly decreases in mean serum concentration of CK even lower than control group. When Group (4) showing improved result of serum CK concentration compared to Group (2). In Group (4) dox intoxicated myocardium then receive omega 3 PUFA 400mg/kg b.wt by gastric intubation for 2 weeks lead to decrease serum CK concentration. When omega 3 PUFA was received before Dox. Injections by 2 weeks as in group 5 minimized the effect of Dox on myocardial cells. Group (2) showed significantly higher serum LDH concentration in near to 2 folds compared to control group. In contrast, Group (3) lower concentration in LDH even control group. When Group (5) which pretreated with omega 3 PUFA showing an improvement in LDH concentration. In Group (4), (receive omega 3 PUFA for 2 weeks after DOX injections) there is an improvement in LDH result compared to Group (2) which injected with DOX only. These results were supported by (Venkatesan *et al.,* 2010). SOD activity of heart tissue were illustrated in table (2). SOD activities were lowered in group 2 which injected with DOX than control group. In contrast, group 5 which prophylaxes with omega 3 PUFA significantly increased. Group 4, which treated with omega 3 PUFA after DOX intoxication, showed slightly improvement of SOD activity compared to group 2. Group 3 which received omega3 improved SOD activities even more than control group as known that DOX increase oxygen free radicals and cause lipid peroxidation in the myocardium (Fadilliglu et al., 2003). Various studies showed that DOX significantly decrease SOD activities in myocardial tissues (Mohan *et al.,* 2006). GST activity of heart tissue were illustrated in table (2). The GST activities in group 2 significantly increased over control group to overcome the stress of DOX toxicity in myocardial tissues. Group 4 showed decrease in GST activities comparing to control group. Group 3 show increase in GST activity. Group 5 showed improvement in GST activities compared to group 4 which mean that pretreated with omega 3 PUFA before intoxication with DOX by 2 weeks improve GST activities in myocardial tissues which clarify anti-inflammatory and antioxidant properties of omega 3 FA. These results supported with (Mohan *et al.,* 2006). In table (2), the activity of CAT in group 3 and group 5 increased over the control group which demonstrated the antioxidant properties of omega 3 FA. In contrast, in group 4 is decreased. It looks like that myocardial tissue attempt to detoxify the oxygen free radicals but cannot able to. Group 2 these results supported with (Fadillioglu *et al.,* 2004). Table (2) showed a decrease in group 2 and group 4 compared to control group. In contrast group 3 and group5 showed significantly increase in GSH activity even more than control group which mean that omega 3 PUFA administration improved GSH concentration in heart tissues. Our results are in agreement with literature data on acute cardiotoxicity after DOX administration (Alpsoy et al., 2013). In table (2) we showed significant increase in Rps6kb1 gene in group 2 and 4 which intoxicated with DOX comparing to control group. Group 3 which received omega 3 PUFA only showed noteworthy decrease in Rps6kb1. In group 2 (dox injected): the animal fur become scruffy and developed a pink tinges in the later days of observation period was followed by red exudates around the eyes and nose and decrease in body weight. Necrosis was also observed in site of the dox injection. These changes were less pronounced in case of n-3 FA treated animals (group 4), which accounts for the effective cell protecting of n-3 FA with anti-inflammatory and antioxidant effect This result supported by (*Swamy* et al., 2012). In histopathological examination of heart tissue, the control group exhibited myocardial fibers with normal structure and regular morphology of myocardial cells according to (Fig. 2). On the other hand, group 2 (dox induced), In Fig. 3, severe morphological changes in heart tissues showing marked degree of myocardial necrosis, myophagia and marked interstitial tissue fibrosis, defuse myocardial degeneration sever myolysis and fibrils fragmentation (Swamy et al., 2012). In group 3, which received omega 3, showed normal myocardial fibers separated with blood capillary. In group 4, animal intoxicated with Dox then treated with omega 3 showing focal myocardial degeneration associated with loss of fiber striation

with marked eosinophils sarcoma associated with moderate degree of myolysis. In group 5 (Fig. 5) which pretreated with omega 3 then intoxicated with Dox showed an improved in histological appearance, mild degree of myolysis of the cardiac fibers have shown a benefit effect of fish omega 3 on anthracycline induced cardiotoxicity. In present study, rats which injected by Dox suffered from marked myocardial necrosis and fibrosis comparing to which treated with omega 3 which have mild myolysis group 4,5 this results supported by numerous studies (Uygur *et al.,* 2014).

It can be concluded from the result of the study that the protective effect of supplementation of omega 3 fatty acid in cardiotoxicity induced rats is more efficient in treated group with omega 3 fatty acids through enhancing antioxidant status in heart tissues in rats.

REFERENCES

Aebi, H., 1984. Catalase in vitro. In Metho ds in enzymology 1.5
;121-126.

Ahmed, H.H., Mannaa, F., Elmegeed, G.A., Doss, S.H., 2005. Cardioprotective activity of melatonin and its novel synthesized derivatives on doxorubicin-induced cardiotoxicity. Bioorganic & medicinal chemistry, 13(5), pp.1847-1857.

Alpsoy, S., Aktas, C., Uygur, R., Topcu, B., Kanter, M., Erboga, M., Karakaya, O., Gedikbasi, A., 2013. Antioxidant and anti-apoptotic effects of onion (Allium cepa) extract on doxorubicin-induced cardiotoxicity in rats. Journal of Applied Toxicology, 33(3), pp.202-208.

Banni, M., Messaoudi, I., Said, L., El Heni, J., Kerkeni, A., Said, K., 2010. Metallothionein gene expression in liver of rats exposed to cadmium and supplemented with zinc and selenium. Archives of environmental contamination and toxicology, 59(3), pp.513-519.

Beutler E., Duron O, Kally MB. 1963. Improved method for the determination of blood glutathione. J. lab. clin. Med., 61, pp.882-888

Decker, T., Lohmann-Matthes, M.L., 1988. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. Journal of immunological methods, 115(1), pp.61-69.

DeFilippis, A.P., Sperling, L.S., 2006. Understanding omega-3's. American heart journal, 151(3), pp.564-570.

Fadillioglu, E., Oztas, E., Erdogan, H., Yagmurca, M., Sogut, S., Ucar, M., Irmak, M.K., 2004. Protective effects of caffeic acid phenethyl ester on doxorubicin-induced cardiotoxicity in rats. Journal of Applied Toxicology: An International Journal, 24(1), pp.47-52.

Habig, W.H., Jakoby, W.B., 1981. Assays for differentiation of glutathione S-Transferases. In Methods in enzymology (Vol. 77, pp. 398-405). Academic press

Lenaz, L., Page, J.A., 1976. Cardiotoxicity of adriamycin and related anthracyclines. Cancer treatment reviews, 3(3), pp.111-120 .

Lin, C.Y., Wheelock, Å.M., Morin, D., Baldwin, R.M., Lee, M.G., Taff, A., Plopper, C., Buckpitt, A., Rohde, A., 2009. Toxicity and metabolism of methylnaphthalenes: Comparison with naphthalene and 1-nitronaphthalene. Toxicology, 260(1-3), pp.16-27.

Majumder, D., Das, A., Saha, C., 2017. Catalase inhibition an anti cancer property of flavonoids: A kinetic and structural evaluation. International journal of biological macromolecules, 104, pp.929-935.

McCormick, K., Salcedo, J., Peck, J., Wheeler, A., 2017. SPSS statistics for data analysis and visualization. John Wiley & Sons.

Mohan, I.K., Kumar, K.V., Naidu, M.U.R., Khan, M., Sundaram, C., 2006. Protective effect of CardiPro against doxorubicin-induced cardiotoxicity in mice. Phytomedicine, 13(4), pp.222-229.

Narayanan, D., Xi, Q., Pfeffer, L.M., Jaggar, J.H., 2010. Mitochondria control functional CaV1. 2 expression in smooth muscle cells of cerebral arteries. Circulation research, 107(5), pp.631-641

Nishikimi, M., Rao, N.A., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications, 46(2), pp.849-854.

Saravanan, P., Davidson, N.C., Schmidt, E.B., Calder, P.C., 2010. Cardiovascular effects of marine omega-3 fatty acids. The Lancet, 376(9740), pp.540-550

Swamy, A.V., Gulliaya, S., Thippeswamy, A., Koti, B.C.., Manjula, D.V., 2012. Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. Indian journal of pharmacology, 44(1), p.73.

Szasz, G., Gruber, W., Bernt, E., 1976. Creatine kinase in serum: 1. Determination of optimum reaction conditions. Clinical Chemistry, 22(5), pp.650-656

Uygur, R., Aktas, C., Tulubas, F., Alpsoy, S., Topcu, B., Ozen, O.A., 2014. Cardioprotective effects of fish omega-3 fatty acids on doxorubicin-induced cardiotoxicity in rats. Human & experimental toxicology, 33(4), pp.435-445.

Venkatesan, B., Prabhu, S.D., Venkatachalam, K., Mummidi, S., Valente, A.J., Clark, R.A., Delafontaine, P., Chandrasekar, B., 2010. WNT1-inducible signaling pathway protein-1 activates

diverse cell survival pathways and blocks doxorubicin-induced cardiomyocyte death. Cellular signalling, 22(5), pp.809-820

Wood, E and Ellis, R. 1994. Laboratory histopathology a complete Reference. 1st ed., Churchill. Living stone, Eden berg, Hong Kong, London, Madrid, New York and Tokyo.

Yuan, J.S., Reed, A., Chen, F., Stewart, C.N., 2006. Statistical analysis of real-time PCR data. BMC bioinformatics, 7(1), p.85..

Zhou, S., Palmeira, C.M., Wallace, K.B., 2001. Doxorubicin-induced persistent oxidative stress to cardiac myocytes. Toxicology letters, 121(3).