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# THE EFFECT OF OMEGA - 3 FATTY ACIDS ON THE EXPERIMENTALLY INDUCED DIABETIC CARDIOMYOPATHY IN ADULT MALE ALBINO RATS

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## ABSTRACT

**Introduction:** Cardiovascular disease is responsible for about 80% of deaths among diabetic patients. Scientific evidence revealed that a diet rich in long chain omega -3 fatty acids help in the development of healthy brain, heart and immune system. **Aim of work:** The current study was designed to investigate the biochemical, histological and ultrastructural changes occurring in the diabetic heart of albino rats induced by streptozotocin (STZ) and the possible protective role of the omega-3 fatty acids. **Materials and Methods:** Forty five adult male albino rats were used in this study. The animals were divided into four groups, each group including 10 male rats except the group I was 15 rats**. Group I (control group)**, **Group II (Omega-3 group):** was given daily oral dose of 54mg/ rat omega -3 fatty acids dissolved in corn oil**. Group III (Diabetic group**): this group was given single intraperitoneal injection of streptozotocin (STZ), the dose was 60mg/kg**. Group IV (Diabetic and Omega-3 group):** in this group the rats were given both omega-3 fatty acids and STZ. The experiment continued for 6 weeks, then blood samples were collected for blood glucose level, creatine kinase-MB (CK-MB) and troponin-I estimation and statistical study was also done. Heart specimens were processed for light and electron microscopic study. **Result:** Light microscopic results of Group I and II showed the usual histological architecture of the myocardium. Group III showed discontinuous, widely spaced cardiomyocytes, vascular congestion and mononuclear cellular infiltration. Ultrastructural results showed discontinuous broken myofibril, mitochondria swollen of variable shapes and sizes. The blood glucose, CK-MB and troponin-I were significantly increased. Administration of omega-3 fatty acids to the diabetic rats (group IV) ameliorates the previous changes. **Conclusion:** Omega-3 fatty acids administration can protect the heart in cases of diabetic cardiomyopathy.

**KEYWORDS:** cardiomyopathy, STZ, omega -3 fatty acids, diabetes.

## INTRODUCTION

Diabetes and cardiac complication have become a public health problem of considerable magnitude.**[1]** Cardiovascular disease is responsible for about 80% of deaths among diabetic patients.**[2]** There is an increasing recognition that diabetic patients suffer from a cardiac insult termed 'diabetic cardiomyopathy'. Diabetic cardiomyopathy can be defined as myocardial disease in patients with diabetes that cannot be attributed to any other known cardiovascular diseases such as hypertension or coronary artery diseases.[3] The pathophysiology of diabetic cardiomyopathy is multi-factorial. The most important mechanisms of diabetic cardiomyopathy are metabolic disturbances as depletion of glucose transporter and increased free fatty acids.[2] Diabetes and its cardiac complications are associated with increased oxidative stress by increasing production of mitochondrial reactive oxygen species (ROS) by glucose auto-oxidation and by non- enzymatic glycation of proteins. Cell death is a major result of myocardial abnormalities and an important cause of various cardiomyopathy.[4]

Fatty acids species are classified by their varying degree of saturation into three main classes saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA). Omega -3 fatty acids are a group of poly-unsaturated fatty acids defined by a double bond at the 3rd carbon from the methyl end of the carbon chain.[5] Omega-3 polyunsaturated fatty acids (omega-3) are considered useful agents in the prevention of diabetes or at least in the reduction of insulin resistance.[6,7] Three major nutritionally important omega-3 fatty acids that are ingested in foods and used by the body are; Alpha-linolenic acid (ALA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Alpha-linolenic acid (ALA) being the primary omega-3 fatty acid.[8] The shorter chain omega-3 fatty

acid Alpha linolenic acid (ALA) can be converted to EPA, and the EPA is converted to DHA[9] Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been receiving a lot of attention lately because of their cardio-protective properties.[8]
Omega-3 fatty acids in Fish oil are one of the most important polyunsaturated fatty acids (PUFA) that have an anti-inflammatory and an antioxidant activity.[10,11] The dietary intake of Omega-3 (PUFA) could be useful in prevention of diabetes; as it reduced the activity of the pro-inflammatory processes which stimulated the body to attack its own insulin-producing cells.[12,13] Dietary intake of long chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) protects against heart disease.[9]

Omega- 3 polyunsaturated fatty acids (PUFAs) are called essential fatty acids as they fulfill essential functions, but the mammalian cell cannot synthesize them de novo. Their dietary sources include fish oil rich in (EPA) and (DHA).[14] Omega -3 fatty acids contain about 60% of long chain fatty acids DHA and EPA as combined. The most widely available source of EPA and DHA is cold water oily fish such as salmon, herring, mackerel, anchovies and sardines.[15]

According to the above data, this study was done to investigate the biochemical, histological and ultrastructural changes occurring in the diabetic heart of adult male albino rats induced by streptozotocin and the possible protective role of the omega-3 fatty acids.

## MATERIALS AND METHODS

45 adult male albino rats (200-210gm) were used in this experiment. The animals were purchased from the National Research Center (Cairo, Egypt), they were kept in clean properly ventilated cages, in the animal house of anatomy department, Faculty of medicine for girls Al-Azhar university. Free access to food & water was allowed throughout the experiment. The animals were left for one week for acclimatization.

The experimental animals were divided into four groups, each one including 10 male rats except the group I was 15 rats. The experimental period was six weeks. The design of the experiment was as follows: 1-Group I (Control group): The 15 animals of this group were divided equally into three subgroups. Group (I) were used as negative control where the animals didn't receive any oral or injected substances. Group (I a) were received by orogastric tube 1ml of corn oil /rat/day, while group (Ib) the animals were given a single intraperitoneal (i.p.) injection of 1ml of citrate buffer (pH 4.5) /rat. 2-Group II (Omega-3 group): the rats were treated with omega-3 fatty acids, in the form of Omega-3 fish oil capsule purchased from South Egypt Drug Industries Company (SEDICO). Each soft gelatin capsule contained 1000 mg fish oil. The capsule was evacuated, then, the content was dissolved in corn oil. The human therapeutic dose was 3000 mg/day.[16] The dose for rats was equivalent to the human dose according to,[17] so each rat was given 54mg/ rat/day. 3-Group III (Diabetic group): This group included 10 rats. Type I DM was induced in these animals by a single intraperitoneal injection of STZ at a dose of 60 mg/kg body weight. STZ was freshly dissolved in 0.1ml cold citrate buffer (pH 4.5).[18] STZ was purchased from Sigma Company, St. Louis, Mo, USA. To insure diabetes, blood glucose level was measured on the 3rd day of STZ injection following overnight fasting, using one Touch Ultra 2 glucose meter and strips. The rats with a blood glucose level above 250 mg/dl were considered diabetic.[19] 4-Group IV (Diabetic & Omega-3 group): in this group the rats were given STZ and omega-3 fatty acids as the previously mentioned regimens.

The experiment continued for 6 weeks, after which, the following parameters were estimated:-Body weight estimation was done to all animals, just before animal sacrifice.-Blood sampling and biochemical assay: the animals were exposed to ether inhalation, venous blood samples were withdrawn from the retro-orbital sinus and collected in glass tubes, for estimation of blood glucose level, serum cardiac troponin-I and serum creatine kinase- MB (CK-MB).

The histological study for cardiac tissue preparation was done by collecting the specimens from the lower one third of the left ventricle from all experimental groups, then fixed in 10% formalin,[20] and processed for paraffin blocks formation, serial sections of 5 Micron thickness were cut and stained with hematoxylin and eosin (H &E) for routine histological examination.[21] The slides were examined under the light microscope, the images were taken by a microscope (Leica) DM750 connected to a digital camera.

For electron microscopic examination small pieces 1x1 $mm^3$ of the left ventricle were collected from all experimental groups, fixed in 3% phosphate buffered gluteraldehyde for 24 hours and post fixed in 1% osmium tetraoxide in the same buffer for 1.5 hour. The cardiac pieces were further processed to form ultrathin sections. The ultrathin sections were cut, and double stained with urinyl acetate and lead citrate.[22] Where, it was ready for examination by Joel jem transmission electron microscope at 60 KV accelerating voltage. The electron microscopic study was done at the Regional center for mycology & Biotechnology AL-Azhar University.

Statistical study: was done for the following: i) body weight estimation, ii) blood glucose level, iii) serum cardiac troponin-I, and iiii) serum creatine kinase- MB. All data analyses were performed using SPSS Statistics for Windows, Version 17.0. (Chicago: SPSS Inc.2008).Descriptive statistics: data were presented as Mean ± standard deviation (SD). Analytical statistics: comparison between groups was done by using students t-test. Significance level: was set at $p<0.05$ for all

comparisons. Results were presented in tables and histograms.

## RESULTS

### I- Histological Results

### Light microscopic results

**Group I –Control Group:** Examination of H & E stained sections of the left ventricular myocardium of control group (group I) and subgroups (Ia and Ib) showed that they were histologically identical. In the longitudinal sections the cardiac fibromyocytes appeared as branching and anastomosing muscle fibers running in different directions with central elongated vesicular nuclei and acidophilic sarcoplasm. The intercalated discs, which represented end to end communication of muscle fibers could be detected. The cardiac muscle fibers were surrounded by a delicate sheath of connective tissue (C.T.) endomysium with a rich capillary network fig (1). The transverse section the myocardium of left ventricle showed that cardiac muscle fibers were acidophilic with central vesicular rounded nuclei. The cardiac muscle fibers appeared to be packed with numerous granules or dots, which represented the cut end of the myofibrils. There was a delicate supporting C.T filling the intercellular spaces and containing an extensive network of blood capillaries, also larger blood vessels contained RBCs could be recognized in between the muscle fibers fig (2).

**Group II (Omega -3):** Examination of H&E stained sections of the left ventricular myocardium of this group, showed that the longitudinal and transverse sections were more or less similar to the control group fig (3, 4).

**Group III (Diabetic group):** Examination of the H&E stained sections of the left ventricular myocardium in the longitudinal sections showed that the cardiac muscle fibers were widely separated from each other, the cardiac muscle fibers appeared broken and discontinuous. Some congested blood vessels, extravasated blood, and mononuclear cellular infiltration could be detected between the cardiac fibromyocytes. Intercalated discs within these destructed cardiac muscle fibers could not be seen fig 5: (A,B,C). In the transverse sections some of the cardiac muscle fibers were broken, discontinuous, fragmented and widely separated from each other. Some blood vessels in between these muscle fibers were congested and encouraged with blood also, hemorrhage could be recognized fig (6).

**Group IV (diabetic and omega-3 group):** Examination of H&E stained sections of the left ventricular myocardium showed that the longitudinal sections formed of branching and anastomosing cardiac muscle fibers with elongated vesicular nuclei, also small and wider thin walled blood capillaries could be recognized between them. Intercalated discs between the cardiac muscle fibers could be noticed. In spite of that, some cardiac fibromyocytes were disorganized and wide spaces could be noticed between them fig (7). The transverse section of the ventricular myocardium showed the cardiac muscle fibers were more or less similar to those of the control with slight vascular congestion was detected within some large blood vessels fig (8).

### Electron microscopic results

**Group I (Control group):** Examination of ultrathin sections of the left ventricular myocardium of this group showed that the cardiac fibromyocyte contained single elongated euchromatic nucleus and many parallel myofibrils, which were regularly arranged interspaced by columns of mitochondria. Each myofibril was formed of fine myofilaments, thick myosin filaments which presented within the dark A band and thin actin filaments within both light I and dark A bands. The dark A band was clearly seen within the myofibril, while the light I band could be seen in some sections and could not be seen in others. The middle zone of the light I band contained the dark prominent Z line, which repeated at a regular interval within the myofibril. The mitochondria of regular shapes and sizes were arranged in rows between the myofibrils, the mitochondrial cristae were easily recognized fig (9).

**Group II (Omega-3):** Examination of ultrathin sections of the left ventricular myocardium of this group showed that the cardiac fibromyocytes were similar to that of control group fig (10).

**Group III (diabetic group):** Ultrathin sections of left ventricular myocardium showed that the cardiac fibromyocyte contained elongated euchromatic nucleus, and many myofibrils and mitochondria. Some of myofibrils were discontinuous and interrupted or replaced by vacuoles. In spite of that, some other myofibrils were similar to the control, contained regular pattern of Z line. Mitochondria, were of variable sizes and shapes, even some of them were hugely enlarged formed what is called megamitochondria, some mitochondria were swollen, mitochondrial cristae could not be seen, these mitochondria were arranged in an irregular manner fig (11, 12).

**Group IV (diabetic and omega-3 group):** Examination of ultrathin sections of the left ventricular myocardium of this group showed that, the cardiac fibromyocyte contained single euchromatic nucleus, many myofibrils and mitochondria. The myofibrils were arranged in a regular parallel manner, showed dark A bands and prominent Z-line at a regular interval. The mitochondria were nearly arranged in a regular pattern between the myofibrils, mitochondrial cristae were prominent as those of the control, in spite of that, some of them were swollen and of irregular shape and size fig (13).

### II-Statistical Results

**For the body weight:** The body weight of the STZ-treated (group III) was significantly decreased as compared to that of the control (group I).The diabetic rats treated with Omega-3 (group IV) showed a

significant increase in body weight during the experimental period as compared with the diabetic group, table (1) and histogram (1).

**For blood glucose level:** Blood glucose of the diabetic (group III) was significantly increased as compared with the control (group I). The diabetic rats treated with Omega-3 (group IV) showed significant decrease in the blood glucose level as shown in table (2) and histogram (2).

**For serum cardiac enzyme markers:** In the present study, STZ induced changes in cardiac tissue enzyme markers, CK-MB and troponin-I as shown in table (3) and histogram (3, 4). In diabetic (group III) showed a significant increase in the activities of enzyme markers ($P<0.001$) as compared to the control group. Treatments with Omega-3 in (group IV) was significantly decreased the activities of these enzymes ($P<0.001$), but not reaching the control level.

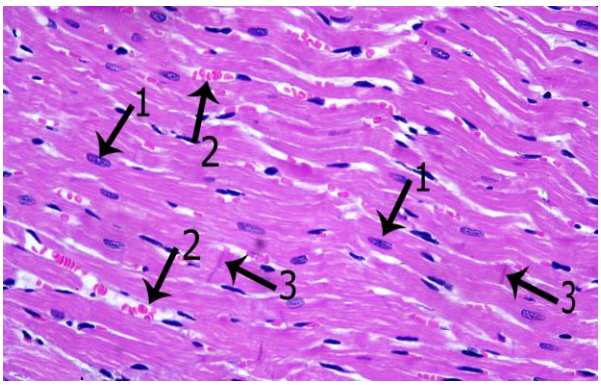



**Fig 1: Showing L.S. of cardiac muscle fibers contain vesicular elongated nuclei (1), blood capillaries in between (2), and intercalated discs (3).**

**Control H&E x 400**

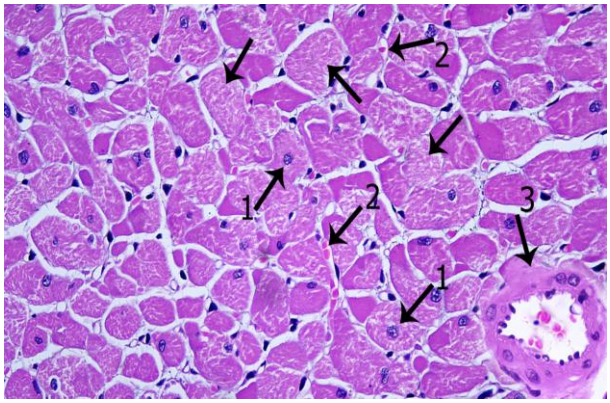



**Fig 2: Showing T.S. of cardiac muscle fibers with granular cytoplasm (arrows), contain central vesicular nuclei (1), blood capillaries in between (2), and larger blood vessel (3).**

**Control H&E x 400**

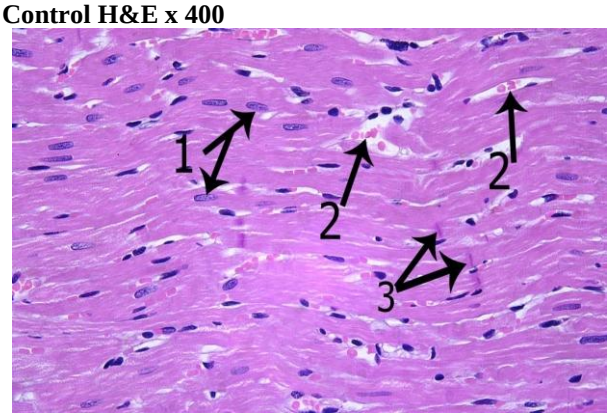



**Fig 3: Showing L.S. of cardiac muscle fibers contain central vesicular elongated nuclei (1), blood capillaries in between (2), and intercalated discs (3).**

**Group II H&E x400**

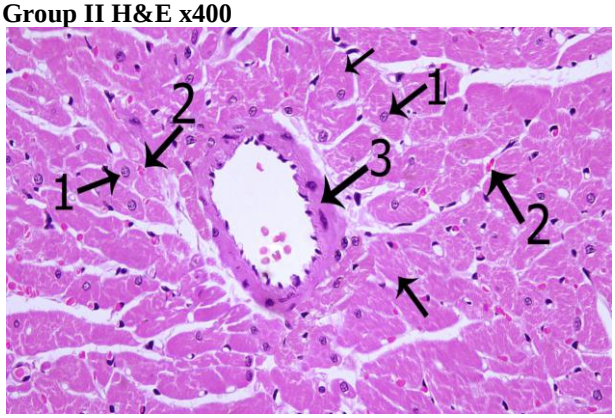



**Fig 4: Showing T.S. cardiac muscle fibers with granular cytoplasm (arrows), central vesicular nuclei (1), blood capillaries in between (2), and larger blood vessel (3).**

**Group II H&E x 400**

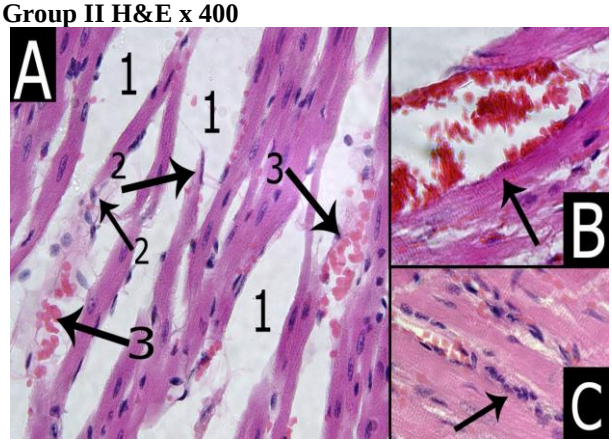



**Fig 5: A- showing wide spaces between the cardiac muscle fibers (1), discontinuous broken muscle fibers (2), extravasation of blood (3). B- showing congestion in blood vessel (arrow). C- showing mononuclear cellular infiltration (arrow).**

**Group III H&E x 400**

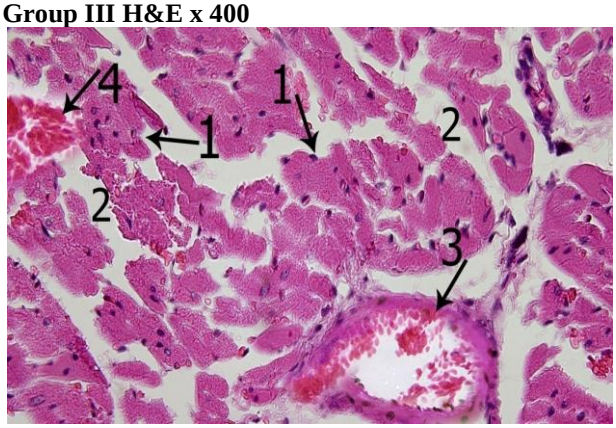



**Fig 6: Showing broken discontinuous cardiac muscle fibers (1), wide spaces between them (2), congestion in blood vessel (3) and hemorrhage (4).**

**Group III H&E x 400**

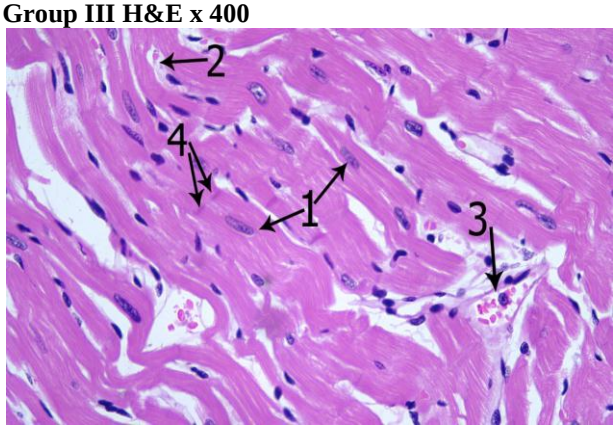



**Fig 7: Showing L.S. of cardiac muscle fibers with vesicular elongated nuclei (1), blood capillaries (2), wider thin wall blood capillary (3), intercalated discs (4).**

**Group IV H&E x 400**

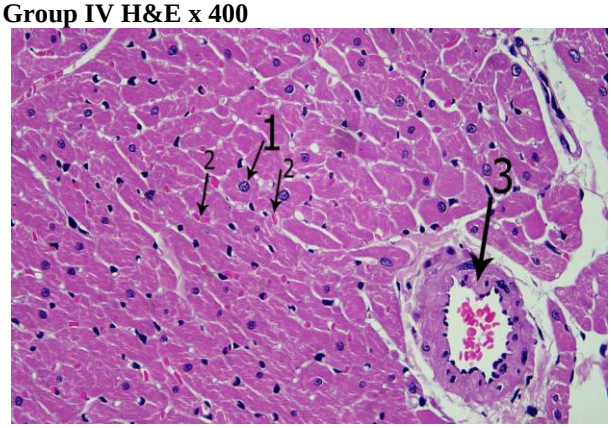



**Fig 8: Showing T.S. of cardiac muscle fibers which contain central rounded vesicular nuclei (1), blood capillaries in between (2), slightly congestion in larger blood vessel (3).**

**Group IV H&E x 400**

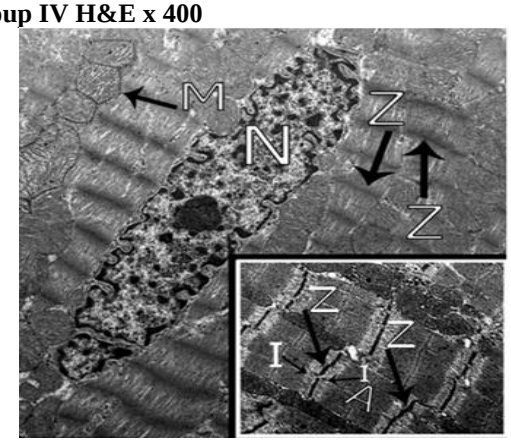



**Fig 9: showing cardiac muscle fiber contains euchromatic nucleus (N), mitochondria with prominent cristae (M), myofibril with a Z- line at regular interval (Z). In set: light I band with Z line at its center and dark A band.**

**Control TEM x 10000**

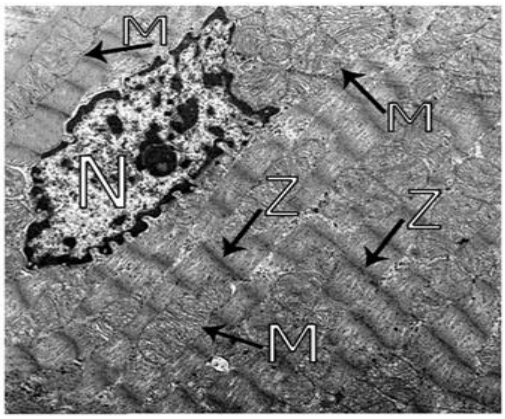



**Fig 10: Showing cardiac muscle fiber with euchromatic nucleus (N), mitochondria with prominent cristae (M), myofibril with a Z- line at regular interval (Z).**

**Group II TEM x 10000**

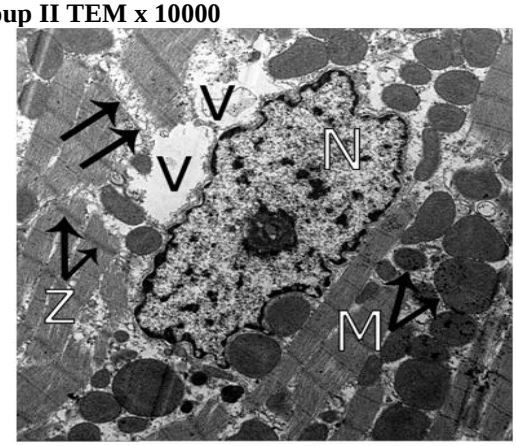



**Fig 11: Showing cardiac muscle fiber with euchromatic nucleus (N), many cytoplasmic vacuoles (V), myofibrils are broken and discontinuous (arrows), swollen variable shape and size mitochondria (M), also myofibril with a Z- line can be seen (Z).**

**Group III TEM x 10000**

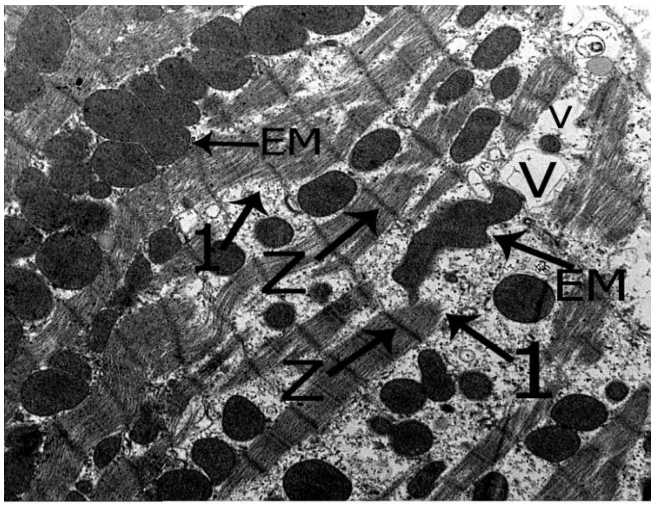



**Fig 12: Showing cardiac muscle fiber contains many vacuoles (V) discontinuous broken myofibrils (1), hugely enlarged mitochondria (EM) in spite of that, myofibril with a Z- line can be seen.**

**Group III TEM x 10000**

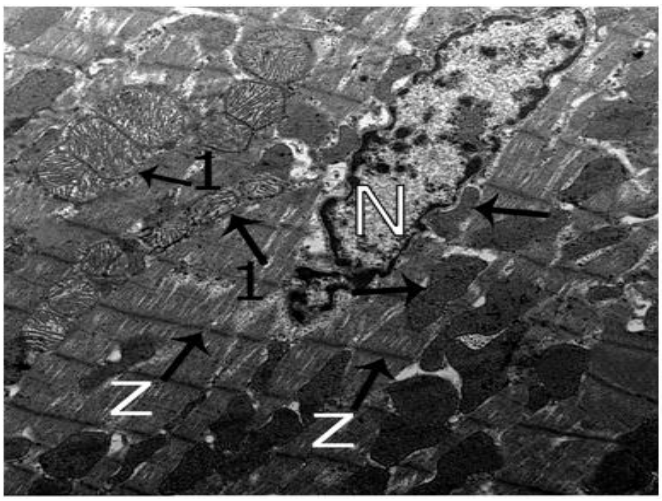


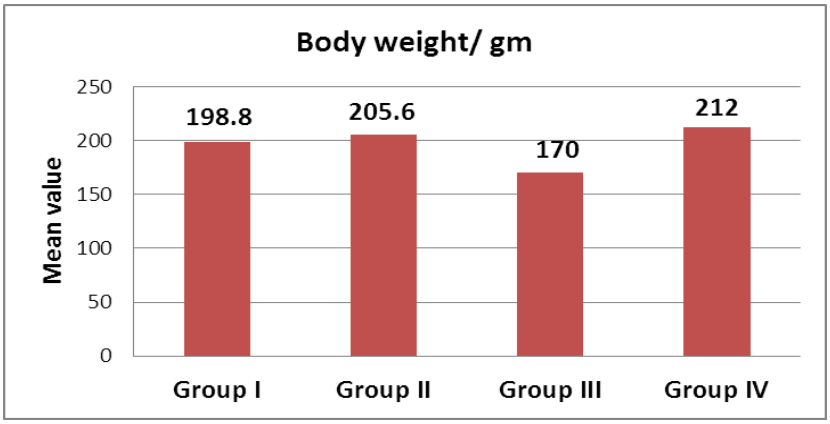

**Fig 13: Showing cardiac muscle fiber contains elongated euchromatic nucleus (N), mitochondria are arranged in rows with prominent mitochondrial cristae (1), swollen and irregular in shape mitochondria (arrows), Z- line can be seen at a regular interval (Z) within the myofibril.**

**Group IV TEM x 1000**

**Table 1: Mean values of rat body weight of the studied groups.**

| Groups | Group I (Control) | Group II (Omega-3) | Group III (Diabetic) | Group IV (Diabetic+ Omega-3) |
|---|---|---|---|---|
| **Rats' weight\ gm** Mean ± SD | 198.8 ± 7.7 | 205.6± 7.0[c] | 170.0±10.0[b] | 212.0±25.6[a,d] |

**a** significant increase in group IV compared with group III ($p<0.05$)
**b** significant decrease in group III compared to group I
**c** non- significant difference between Group I & II ($p > 0.05$)
**d** non- significant increase in group IV compared with group I& II ($p > 0.05$)

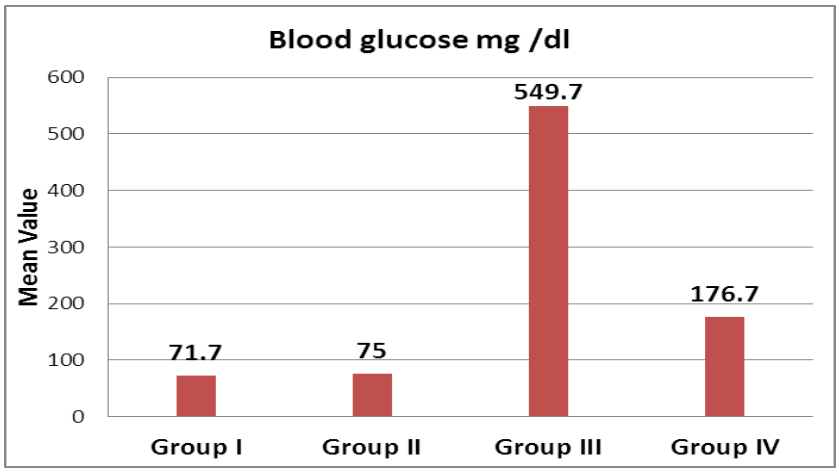



**Histogram 1: Mean values of rat body weight of the studied groups**

**Table 2: Mean values of blood glucose of the studied groups.**

| Groups | Group I (Control) | Group II (Omega-3) | Group III (Diabetic) | Group IV (Diabetic+ Omega-3) |
|---|---|---|---|---|
| **Serum glucosemg/dL Mean ± SD** | 71.7 ±12.6 | 75.0 ±13.2[c] | 549.7 ± 46.3 | 176.7 ± 25.1[a,b] |

**a** significant decrease in group IV compared with group III ($p < 0.05$)
**b** significant increase in group IV compared with group I& II ($p<0.05$)
**c** non- significant difference between Group I & II($p>0.05$)

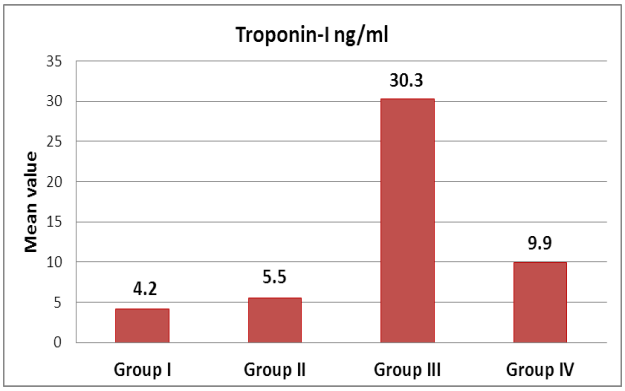



**Histogram 2: Mean values of blood glucose of the studied groups.**

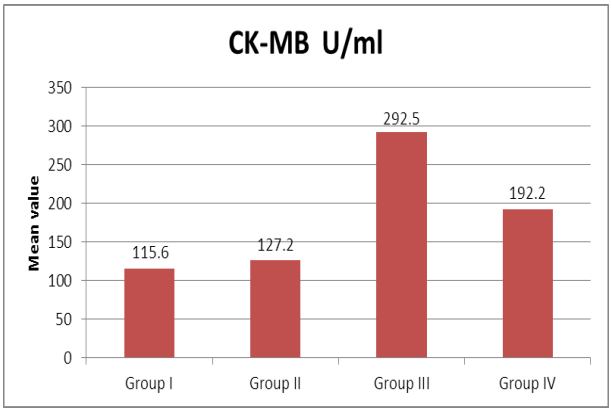
**Table 3: Mean values of serum cardiac enzyme markers of the studied groups.**

| Groups | Group I (Control) | Group II (Omega-3) | Group III (Diabetic) | Group IV (Diabetic+Omega-3) |
|---|---|---|---|---|
| **Troponin-I ng/ml** Mean ± SD | 4.2 ± 0.77 | 5.5 ± 0.8[c] | 20.3 ± 3.4 | 9.9 ± 2.2[a,b] |
| **CK-MBU/ml** Mean ± SD | 115.6 ± 20.9 | 127.2 ± 24.9[c] | 292.5 ± 54.3 | 192.2 ± 9.3[a,b] |

**a** significant decrease in group IV compared with group III ($p<0.05$)
**b** significant increase in group IV compared with group I& II ($p<0.05$)
**c** non- significant difference between Group I & II




**Histogram 2: Mean values of troponin-I of the studied groups.**




**Histogram 4: Mean values of CK-MBU/ml of the studied groups.**

## DISCUSSION

Cardiovascular disease was one of the main complications of diabetes, it was the major causes of death in diabetes mellitus.[23,2] Fish oil attracted the attention, and was used not only as nutraceutical but also as a source of potential pharmaceuticals.[24] Dietary fish oil, which contained omega-3 fatty acids, had beneficial effects on diabetes, autoimmune diseases, and some cardiovascular diseases.[25,26]

In the present study in diabetic (group III), the body weight was significantly decreased. The previous finding was in agreement with[27] who stated that diabetes caused by STZ was associated with intensive decline in body weight of diabetic rats,[28] mentioned that muscle wasting and loss of adipose tissues were responsible for the weight loss in diabetes and this was due to the increased rate of proteolysis and lipolysis for glucose generation in diabetic state. In omega-3 treated diabetic (group IV), the body weight was increased indicating the role of omega-3 in protecting against loss of weight. It was explained by[29] who stated that omega-3 supplementation induced the replacement of skeletal muscle mass loss. It was well established by stimulation of amino acid to enter in muscle protein synthesis.

In this study diabetic group (III), showed a significant increase in the levels of serum blood glucose. Similar biochemical changes were previously reported in other studies.[30] Some researchers[31,32] referred the significant elevation in blood glucose level of STZ induced diabetic rats to the reduction in the plasma insulin levels caused by selective necrosis of pancreatic beta cells. Further explanation was mentioned by[33] who said that, STZ after entering to beta cells caused damage to the DNA, this induced activation of poly ADP-ribosylation, a process that was more important for the diabetogenicity of streptozotocin. Omega-3 fatty acids treatment was significantly lowered fasting serum glucose, level in (group IV) The mechanism underlying the glucose-lowering effect was reported by[6,7] who stated that omega-3 polyunsaturated fatty acids are considered useful agents in the prevention of diabetes or at least in the reduction of insulin resistance.

In this study, the left ventricular myocardium of group II (omega-3) revealed a histological architecture similar to that of the control. These findings might be indicated that omega-3-PUFAs are beneficial and safe in cardiac tissue, which was attributed by some researchers to the great antioxidant effect of omega-3 PUFAs, which decreased the peroxide concentration and increased antioxidant defense enzymes in the heart.[34]

In the current work in diabetic group (group III) the cardiac muscle fibers were separated by wide spaces. The cardiomyocytes were broken and discontinuous. Extravasated blood and vascular congestion were also observed. The previous findings were explained by[35] who stated that the diabetes exhibited high oxidative stress due to persistent and chronic hyperglycemia which might deplete the activity of the antioxidative defense system and promoted the generation of free radicals, such models included of diabetes in rats and mice with alloxan or STZ. Oxidative stress produced by free radicals or reactive oxygen species (ROS), as evidenced by marked increase in production of lipid peroxidation products such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) and transient inhibition of endogenous antioxidant defense had been documented in myocardial infarction (MI).[36]

The electron microscopic examination of cardiac muscle fibers that showed most of the myofibrils contained dark A- bands, while the light I- bands were seen in some sections and not seen in others. This was explained by[37] who said that, if the cardiac muscle specimen in a highly contracted states the light I- band was practically obscured.

The electron microscopic examination diabetic (group III) in the current experiment showed that the cardiac fibromyocyte contained intracytoplasmic vacuoles which was in agreement with[38] who said that formation of intracytoplasmic vacuoles in the cardiac myocytes was thought to be due to dilatation of cytoplasmic and nuclear membranous components caused by intracellular fluid and electrolyte redistribution with loss of selective permeability of the cell membrane. Cytoplasmic

vacuolation also could be due to the inhibition of Na+/K+ ATP-ase and Na+/Ca++ exchanges across the cardiac cell membrane and to degeneration of a part of the smooth endoplasmic reticulum tubules.[39,40]

The presence of broken discontinuous myofibril within the cardiomyocytes of group (III) was explained by[40,41] who said that the lack of anti-oxidant defense enzymatic system together with increased reactive free radicals might facilitate the release of lysosomal enzymes into the cytosol with subsequent oxidation of protein back bone of these myofibrils causing their fragmentation. In the state of insulin deficiency (type 1 DM), there was a decrease in the protein synthesis, increase in the protein degradation, defective mitochondrial function with loss of myofibrils in the myocardium.[43]

The electron microscopic examination revealed markedly affected mitochondria in the form of swelling and variation in shapes and sizes in group (III) of the current experiment. These findings were in accordance with the results of[44] who referred these alterations to disruption of calcium homeostasis secondary to changes in the calcium-ATPase activity, and calcium content of mitochondria of cardiac myocytes. Others added that the possible inhibition of Na+/K+ -ATPase (the plasma membrane energy dependent sodium pump) could induced mitochondrial swelling secondary to intracellular sodium accumulation and osmotic gain of water.[45]

The megamitochondria were attributed to either fusion or fission of mitochondria. This was thought to be an adaptive mechanism to compensate and fulfill the need of high metabolic activity characteristic of the degenerated cells.[46] When the level of free radicals exceeds a certain level, mitochondria try to decrease intracellular reactive oxygen species (ROS) levels by decreasing the consumption of oxygen via megamitochondria formation.[47]

The histological changes in cardiac muscle fibers in diabetic group (group III) were supported biochemically by the serum cardiac enzyme markers; there were a significant elevation of CK-MB and troponin-I in group (III) of the current experiment. The previous findings were in agreement with[48] who said that the measurement of the CK-MB level was until recently the standard marker for myocyte death used in acute coronary syndrome, while cardiac troponin complex was made up of three distinct proteins (I,T and C) that were situated with tropomyosin on the thin actin filament that forms the skeleton of the cardiac myofilament. Others added that the confirmation of suspected myocardial infarction (MI) in the individual cauld be made through the detection of CK-MB and troponin-I in the blood. They were usually released into the blood stream within 3 to 12 hours after MI. Troponin-I levels remained elevated for up to 2 weeks from the time of the initial injury, thus it was regarded as an excellent marker for diagnosing MI that has recently occurred.[37] The improvement was evident by the significant decline in serum troponin-I and CK-MB. This was confirmed by other investigators[49] who reported that serum concentration of troponin –I correlated with the severity of cardiac injury and the prognosis. In group IV CK-MB and troponin-I were significantly decreased. This was explained by[14] who said that the omega -3 (PUFA) were beneficial and safe for cardiac tissue, they have great anti-oxidant effect which decreases the peroxide concentration and increases antioxidant enzymes in the heart.

In the current study in (group IV), there were improvement in the light and electron microscopic results. This improvement was explained by[14,50] who detected similar findings in their results and explained that by the supplementation with fish oil protected the myocardium against oxidative stress through its anti-oxidant, anti-thrombotic activity and thereby restores the structural and functional integrity of the myocardium. Also, it was improved the biological functions of the cells and its cell membrane such as signal transduction, ion channeling and ligand binding to nuclear receptors.[51] also added that the great cardioprotective effect of omega-3 PUFAs was due to the incorporation of EPA and DHA into the cell membranes. Omega-3 fatty acids had anti-inflammatory properties by reducing the expression of interleukin-1 beta and human leukocyte antigen class II alleles in activated human monocytes. In type 1 diabetes mellitus there was evidence of abnormal prostaglandin metabolism and omega-3 fatty acids (i.e. DHA and EPA) by its anti-inflammatory effect might be reduced the risk of disease development [52]. The most important function of omega-3 PUFAs was scavenging of free radicals, which diminished inflammatory response and oxidation of lipoprotein particles, notably low density lipoproteins. These molecular processes had distinct cardioprotective effects.[53]

## CONCLUSION

Diabetes has a deleterious effect on the histological structure of cardiac muscle. The omega-3 fatty acids have the ability to protect and ameliorate many of these changes.

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