Original Article

Possible Impact of Cod Liver Oil on Ovariectomy-Induced Skeletal Muscle Damage in Adult Female Albino Rats: A Histological, Histochemical, and Immunohistochemical Study

Sara Abdel Hafez

Department of Histology and Cell Biology, Faculty of Medicine, Minia University, Egypt

ABSTRACT

Introduction: After menopause, women live more than one-third of their lifetimes, which is characterized by a low estrogen level associated with multiple diseases. Medical specialists are interested in the health advantages of cod liver oil (CLO), that contains omega-3 fatty acids, according to encouraging studies.

Aim of the Work: The study's goal was to investigate the impact of ovariectomy (an experimental model of menopause) on skeletal muscle structure and to investigate the efficacy of CLO in restoring the muscular deficit caused by hormone deprivation) ovariectomy).

Material and Methods: Twenty-four adult female albino rats were included in the study. They were separated into equal four groups: sham, CLO, ovariectomized (OVX), and CLO-OVX groups. The gastrocnemius muscle underwent histological, histochemical, and immunohistochemical examinations.

Results: The findings revealed a variety of morphological changes in the OVX group in both skeletal muscle and nerve fibers. The Glee's stain revealed myoneural junctions' degeneration. Succinate dehydrogenase stain was reduced in type II skeletal muscle fibers. Acetylcholine esterase activity was significantly decreased. It was detected a significant decrease in anti-desmin activity in addition to a significant increase in anti-CD 86 activity in this group if compared to the sham and CLO groups. Interestingly, all the previously listed changes were improved in the CLO-OVX group.

Conclusion: It could be concluded that a significant adverse impact of ovarian hormones deficiency on skeletal muscle structure. Additionally, CLO supplementation has a favorable effect on skeletal muscle in rat ovariectomized model, and this could provide an alternative way to avoid the negative effects of high-dose replacement hormone therapy.

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Key Words: Cod liver oil, ovariectomy, rat, skeletal muscle.

Corresponding Author: Sara Abdel Hafez, MD, Department of xxxxxxxxxxxxxxxxx, Faculty of Medicine, Minia University, Egypt, **Tel.**: +20 10 6147 9147, **E-mail:** sara.abdelhafez@mu.edu.eg

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INTRODUCTION

Menopausal women experience a variety of symptoms and diseases as a result of estrogen shortage. Menopause is a natural component of the ageing process, as a result of surgical removal of the ovaries, or as a result of treatment such as chemotherapy[1]. Estrogen is known to control metabolic, cardiovascular, brain, and bone formation[2]. Reductions in activity and locomotion with ageing as well as disuse damage, pose significant dangers to aged women due their lower capacity for functioning and longevity as compared to men[3,4].

Previous studies examined the biochemical and physiological alterations in muscle tissue produced by estrogen deprivation, as well as the impact of estrogen substitution treatment on skeletal muscle mass and performance[5]. In this study, an ovariectomized (OVX) rat strain was used as a postmenopausal model.

Skeletal muscle is believed to be estrogen sensitive since its estrogen receptors are identical to those found in normal target organs. These receptors appear to be functioning, as evidenced by scattered research demonstrating estrogen's various effects on skeletal muscle. For example, estrogen appears to reduce skeletal muscle injury, as evaluated by the release of cytosolic enzymes or other indicators[6].

Cod liver oil (CLO) is enriched in omega-3 fatty acids and eicosatetraenoic acid. Additionally, it contains vitamin D, A, and docosahexaenoic acid. Consumption of CLO has been linked to a variety of health advantages[7]. Consumption of seafood and various marine oils may be good for our health. Observations over the years have indicated that certain human populations, such as Eskimos, have a lower incidence of coronary heart disease, which can be ascribed to their seafood-rich diet. It was reported that CLO is one such food. Several health advantages have been connected with CLO ingestion; it impact on body's hemostatic function and lipid content[8].

The goal of this work was to estimate the possible protective impact of nutritional supplements (CLO) in improving the muscle of ovariectomized rats. The muscle was evaluated via utilizing histological, histochemical, immunohistochemical, and morphometric method.

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MATERIAL AND METHODS

Ethical approval and animal handling

The current study was approved (Approval NO. 548/2022) and carried out in conformity with criteria of the research ethical of Faculty of Medicine- Research Ethics Committee, "FMREC", Minia University, Egypt. The rat study followed the ARRIVE (Animal Research: Reporting of In Vivo Studies) principles and was conducted with the UK Animals (Scientific Procedures) Act of 1986 and its related rules, as well as EU Directive 2010/63/EU on animal studies. Twenty-four seemingly healthy female albino rats weighing 170-185 g were acclimated for two weeks. They were about 8–10 weeks old. The rats were housed in plastic cages at 21°C with a Twelve-hour Cycle of light and dark. Throughout the trial, the rats were supplied with food freely and had access to clean, fresh water.

Experimental design

Chemicals and drugs

Cod liver oil: Each 2.5 ml of 100% virgin cod liver oil solution contains 2.5g of total fat, Vitamins A (2500 IU) and D-3 (250 IU), Vitamin E3 (3 IU), Omega-3 Fatty acids (600 mg), DHA (Docosahexaenoic acid) 350 mg, and EPA (E cosapentaenoic acid, 250 mg). NutraPro International, based in Hong Kong.

Animals

Animals were equally divided into four groups; each one was formed of 6 rats. Sham operated group: Animals were subjected to sham surgery then the animals were sacrificed after eight weeks. Cod liver oil (CLO): rats were given CLO for 8 weeks in a dose (5 ml/kg body weight) orally[7]. The animals undergo scarification after eight weeks. Rats in the Ovariectomized (OVX) group had bilateral ovariectomy, and the rats undergo sacrificed eight weeks following ovariectomy. Rats in the CLO-OVX group underwent ovariectomy followed by everyday CLO as in previous dose. Eight weeks after the ovariectomy, the animals were sacrificed.

Establishment of estrous cycle

For the following 15 days, smears of the vagina were taken between 8 a.m. and 9.00 a.m. Two drops of normal saline were flushed into the vaginal canal using a pipette tip that was carefully positioned 2 mm deep into the vagina. The pipette tip was then licked with a vaginal fluid and normal saline combination. The smear was ejected onto a glass slide that had been tagged with the female identity numbers and date, dyed with violet crystals, then the slides was then examined using a light microscope. The four-phase cycle of rats lasts only 4-5 days. The metestrus phase lasts 21 h and has equal amounts of both leucocyte cells and nucleated cornified epithelial cells (Figure 1A). Diestrus cycle phase takes about 57 h and is characterized by dominated leucocytes (Figure 1B). The 3rd phase, known as the proestrus, is characterized by aggregated large nucleated epithelial cells persisting 3-12 h (Figure 1C). Anucleated cornified cells appear in closely aggregated clusters characterizing the estrous phase, which lasts 12 h (Figure 1D). The study included animals with three normal estrus cycles (15 days) and considered the commencement of the experimental design. To avoid fluctuations in hormone levels during the estrous cycle, the animals began the experiment during the diestrus phase[9].

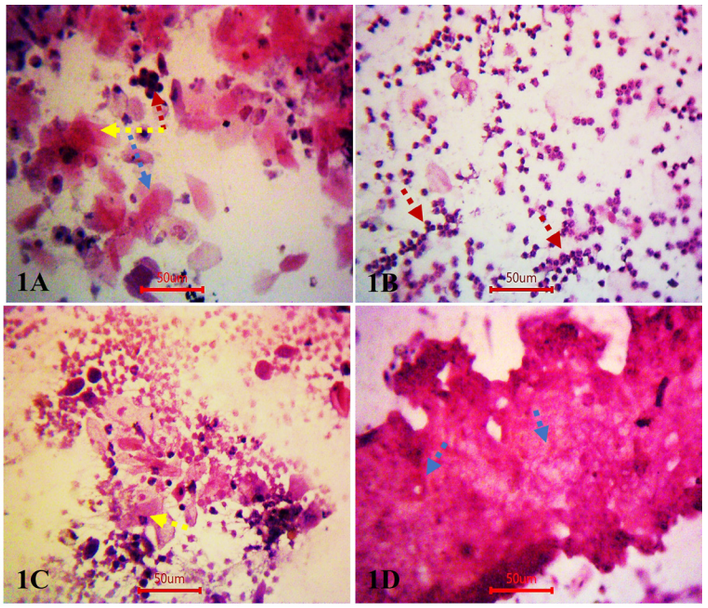


Fig. 1: Photomicrographs of smears of rat's vaginal stained with crystal violet: a) Metestrus phase showing leucocytes (red arrow), cornified cells (blue arrow) and nucleated epithelial cells (yellow arrow). b) Diestrus phase showing abundant leucocytes (red arrows). C: Proestrus phase showing aggregation of large nucleated epithelial cells (yellow arrows). D: Estrous phase showing abundant anucleated cornified cells (blue arrow) noticed in closely packed cluster.
(H&E x 400, scale bar=50 µm)

Ovariectomy (OVX)

Light ether was used for rat anesthesia. A 10 g/100 mL solution of povidone iodine was used to disinfect rat skin. A three-centimeter-long incision was formed in the middle of the lower part of the body and tail end, as well as a double dorsolateral incision in the muscle. Ovaries enclosed by body fat were discovered Once the abdominal cavity opened. The fallopian tube junctions and uterine horns were incised, and the ovaries were taken out. Catgut sutures were used for muscle approximation and skin closure. Gentamycin (6 mg/kg) was injected intramuscularly after surgery. Following the operation, the animals were allowed to move about inside their steel cage. In the sham procedure, Rats' abdominal cavities were opened, but ovaries remained intact, exposing them to the identical stress as the procedure. The same antiseptic technique and wound closure steps were carried out as in the ovariectomy group[10].

Sample dissection and preparation.

At the end of the current study, the animals were sacrificed using 1.9% inhaled diethyl ether (0.08 ml/ liter container capacity). For tissue preparation, skeletal muscle tissue samples were obtained. The right limb's gastrocnemius muscle was dissected and separated into 2 parts. For paraffin sections, the 1st half was promptly fixed in 10% formal saline and stained with hematoxylin and eosin, Masson`s Trichrome, Glee's technique, and immunohistochemical analysis. 2nd part was processed for succi¬nate dehydrogenase and acetylcho¬line esterase stain.

Histological studies[11]

Hematoxylin and Eosin (H&E) to detect morphological alternation.

Masson`s Trichrome to detect collagen fibers.

Glee's method to detect nerve fiber changes.

Immunohistochemically

Diluted rabbit monoclonal anti-desmin was used (ab32362), 1:2000; Abcam for desmin protein detection. Positive control was Human skeletal muscle (not included). Desmin staining pattern: positive reactions occur as brown cytoplasmic coloring. Diluted rat monoclonal anti Cluster of Differentiation CD 68 (anti-CD68) was used (ab8158), 0.5 - 5 µg/ml.: Abcam, for macrophage detection. Positive control was mouse lung (not included). For D68 Positive expression showed as brown cytoplasmic staining. Following deparaffinization, the slides were followed by hydration then washed with 0.1 M phosphate buffer saline. After being treated with H2O2 in methanol and subsequently washed in tris-buffer saline (TBS), endogenous peroxidases were inhibited. normal goat serum diluted 1:50 with TBS and 0.1% bovine serum albumin prevented nonspecific binding of IgG for 30 minutes. Followed by a 4 C ° overnight dilution of the 1ry antibodies, the slides were treated for thirty minutes with biotinylated a goat anti-rabbit 2ry antibody (1:1,000), washed with buffer, and dried. Followed by another 30 minutes, Vectastain ABC kits (Avidin Biotinylated Horse Radish Peroxidase Complex) were incubated. Ten minutes after washing, the substrate, diaminobenzidine tetrahydrochloride in Water that was distilled, was applied for about five-ten minutes. Following that, Hematoxylin (H) was utilized as a counterstain[12]. For both immune antibodies, negative control slides were done, following the same immune steps but with omitting primary antibody (slides not included).

Histochemical study

The second portion of muscle was quickly transferred to the cryostat for histochemical labelling of succinate dehydrogenase, which indicates mitochondrial oxidative capacity[13] and Acetylcholine esterase (AChE); the peripheral and central nervous systems' cholinergic synapses are significantly impacted by it[14].

Image capture

Images (H&E, histochemistry, and immunehistochemistry) are recorded digitally via a digital camera (LC20, Germany) attached to an Olympus BX40 microscopy and mounted on the computer using LCmicro program.

Morphometric studies

Morphometric analysis of equally magnified pictures was conducted through the use of Image J software program (National Institutes of Health, MD, USA). Ten separate non-overlapping randomly chosen fields were used from each slide from each group. The sections were counted in X 400 to investigate these variables:

1. The mean number of damaged fibers was detected and scored for quantification of muscle fibers (using H&E stain). Damaged muscle fibers were classified as mild (up to about 20% of damaged fibers), moderate (up to 50% of affected fibers), or severe more than 50% of affected muscle fibers[15].
2. The mean surface area fraction of desmin immunostained positive cells
3. The mean surface area fraction of CD 68 immuno positive stained cells.

Statistical study

Prism (Graph Pad Software, San Diego, California, USA; www.graphpad.com) version 7.01 for Windows was used to analyze the quantitative data. Mean and standard deviation were used to express the results. Using a Tukey-Kramar post hoc test after a one-way ANOVA, several groups were compared. A statistically significant *P-value* is one that is less than 0.05.

RESULTS

Survival rat

No deaths were reported in any group.

Hematoxylin and eosin results

Cross sections (CS) of rat gastrocnemius muscle from both sham and cod liver oil (CLO) groups revealed the same histological structure. The muscle was made of bundles and divided by perimysium connective tissue. The bundles were formed of polyhedral muscle fibers which had numerous oval peripheral vesicular nuclei underneath the sarcolemma (Figures 2A1,2B1). Additionally, longitudinal skeletal sections (LS) displayed parallel, elongated, cylindrical non-branched muscle fibers. The skeletal muscle fibers showed regular striations (Figures 2A 2, 2B 2). While in ovariectomized (OVX) group, the alignment of the muscular fibers was disorganized. The muscle fibers were of varied sizes and shapes. Some muscle fibers were apparently shrunk (Figure 3A). Furthermore, CS showed nuclear internalization with inflammatory cell infiltration in-between the myofibers (Figure 3B). It was noticed in LS sections, focal degenerated muscular areas and muscle fibers fragmentation and widening of endometrium (Figure 3C). Disrupted striations, splitting, and branching of the muscle fibers were also detected. Darkly stained muscle nuclei were a clear finding among the section (Figure 3D). It was noticed muscle fibers with undulated disrupted sarcolemma (Figure 3E). Multiple large vacuoles replace and interrupt the muscle fibers continuity were noticed in this group. Some sections showed lightly stained foci. Others showed dilated blood vessels associated with inflammatory cell infiltration (Figure 3F). Regarding CS from CLO-OVX group, most of the fibers appeared more or less normal. A few lightly stained foci and few apparently shrunken muscle fibers among the apparently normal muscle fibers were observed (Figure 4A). In LS, the peripheral vesicular nuclei that form the nuclear chain looked to be normal. There was still plenty of place between muscle fibers. Another finding showed a decrease in inflammatory cell infiltration. Additional magnification revealed organized muscular fibers with distinct striations (Figure 4B).

Masson`s Trichrome results

Sham and CLO groups displayed collagenous connective tissue within the perimycin (Figures 5A,B). While OVX group displayed condensation of collagen fibers seen in perimycin and were observed surrounding the dilated congested blood vessels (Figures 5C1,2). Meanwhile CLO- OVX group showed less collagen bundles seen in perimycin than OVX group (Figure 5D).

Immunohistochemical results

Immunohistochemical staining for anti- desmin

Both sham and CLO groups exhibited a strong cytoplasmic positive reactivity of desmin in muscle fibers (Figures 6A,B). The OVX group slides revealed faint muscle cytoplasmic expression. (Figure 6C). CLO-OVX, on the other hand, showed more desmin cytoplasmic expression in muscle fibers, whereas other muscle fibers showed less expression than OVX group (Figure 6D).

Immunohistochemical staining for anti- CD34

Both the sham and CLO groups showed macrophages with weak CD34 cytoplasmic expression (Figures 7A,B). While OVX group showed strong in macrophage cytoplasmic expression that seemed to be increased in size (Figure 7C). Meanwhile, CLO-OVX revealed less cytoplasmic expression in the previously mentioned cells that appeared to be of small size (Figure 7D).

Histochemical results

Glee's staining results

Both the sham and CLO groups sections revealed intact nerve fiber with uniform diameters. On the surface of the muscle fibers, the myoneural junctions (MNJ) appeared as hen legs (Figures 8A,B). The OVX group showed considerable localized nerve fiber injury. This was expressed as a breakdown of the silver impregnation as compared to the sham group's homogeneous staining. Some nerve fibers were found to be weakening along their length (Figure 8C). Meanwhile, CLO-OVX showed nerve fibers with more or less normal. Restoration of MNJ's appearance was frequently seen among the sections (Figure 8D).

Succinic dehydrogenase histochemistry

Succinic dehydrogenase staining of type II fibers with low intensity and a small number of type I fibers with high intensity were present in the TS of skeletal muscle tissue in both the sham and CLO groups (Figures 9A,B). In contrast, the OVX group's type II fibers lacked succinic dehydrogenase staining in both the central and eccentric regions. The staining intensity in the center of the type I fibers appeared to be decreasing (Figure 9C). While CLO-OVX group showed muscle fibers – mostly type II and few type I. Additionally, it was observed that the staining intensity in the center of a few type II fibers appeared to diminish (Figure 9D).

Acetyl choline esterase (AChE)

In every group, acetyl choline esterase was observed in the motor end plate areas. In both the CLO and sham groups. AChE staining revealed that the neuromuscular junctions (NMJ) were compact, brown, oval, circular, or elliptical. They were scattered along the periphery of the muscle fibers (Figures 10A,B). While the OVX group revealed that some parts of the NMJ were faintly stained, (Figure 10C). Furthermore, in CLO-OVX group, the appearance of NMJs was similar to that of the sham and CLO groups (Figure 10D).

The morphometric results

The OVX group showed moderate muscle fiber degradation, whereas the CLO-OVX group, the muscle fiber degeneration was mild (Histogram 1A). The mean area fraction of desmin immuno stained cells was decreases in OVX than sham and CLO groups (Histogram 1B). While CD 68 immune stained cells' mean area fraction was increased in OVX than the sham and CLO groups (Histogram 1C). While CLO-OVX group showed reverse of the previous morphometrical study.

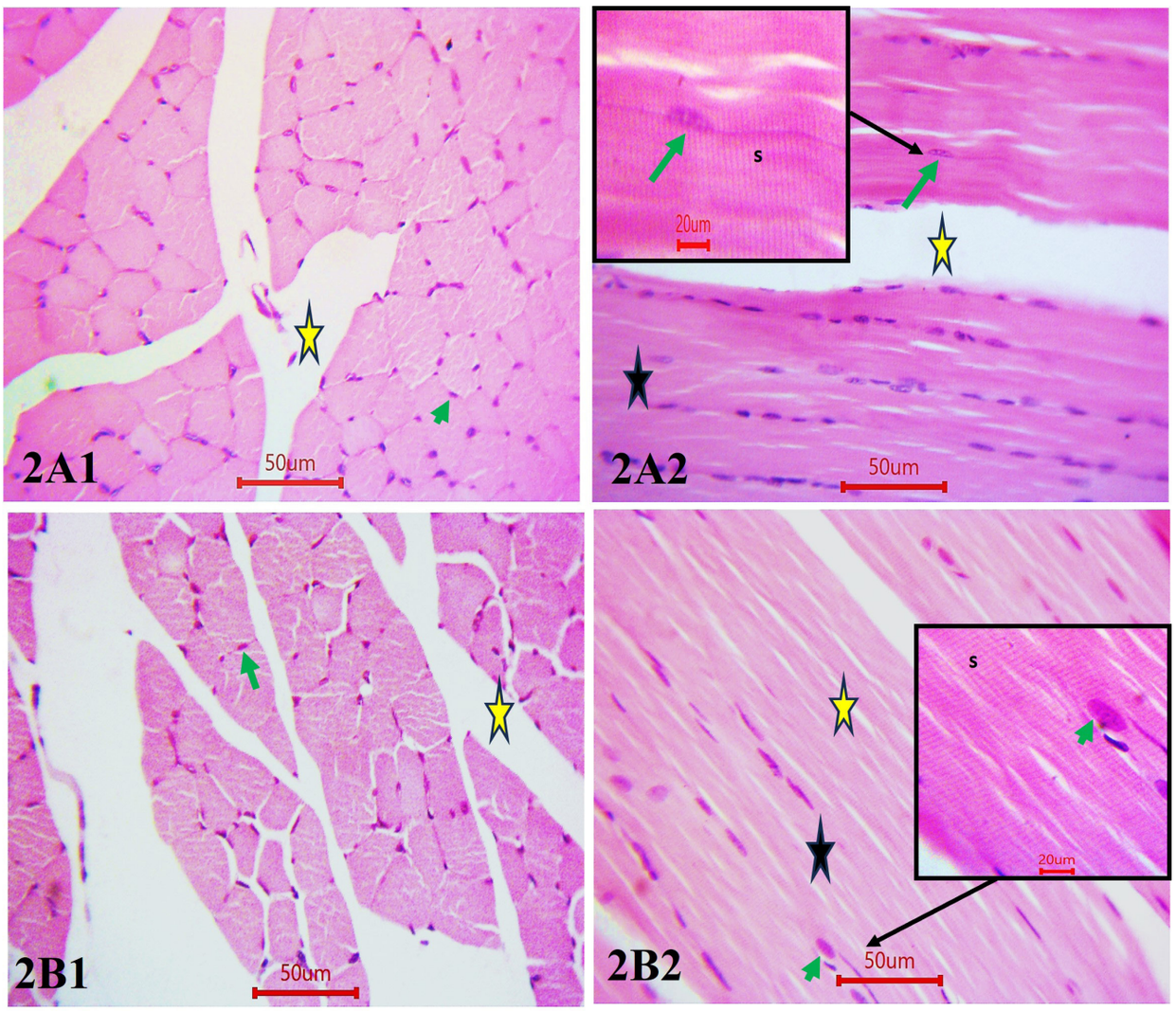


Fig. 2: Photomicrographs of rat's gastrocnemius muscle from: A & B) Sham and CLO group. A1 & B1) Cross (CS) sections showing perimysium connective tissue divides the muscle bundles (yellow stars). Notice the polyhedral muscle fibers appear with acidophilic cytoplasm and oval peripheral vesicular nuclei (green arrows). A2 & B2) Longitudinal (LS) skeletal sections showing cylindrical, elongated, parallel, unbranched muscle fibers (black stars) with regular striations (s) and numerous spindles shaped flattened peripheral nuclei underneath the sarcolemma (green arrows). Insets showing higher magnification of evident vesicular nucleus (green arrow) and transversely striated pattern of muscle fibers (s).

(H&E x 400, scale bar=50 µm, inset x 1000)

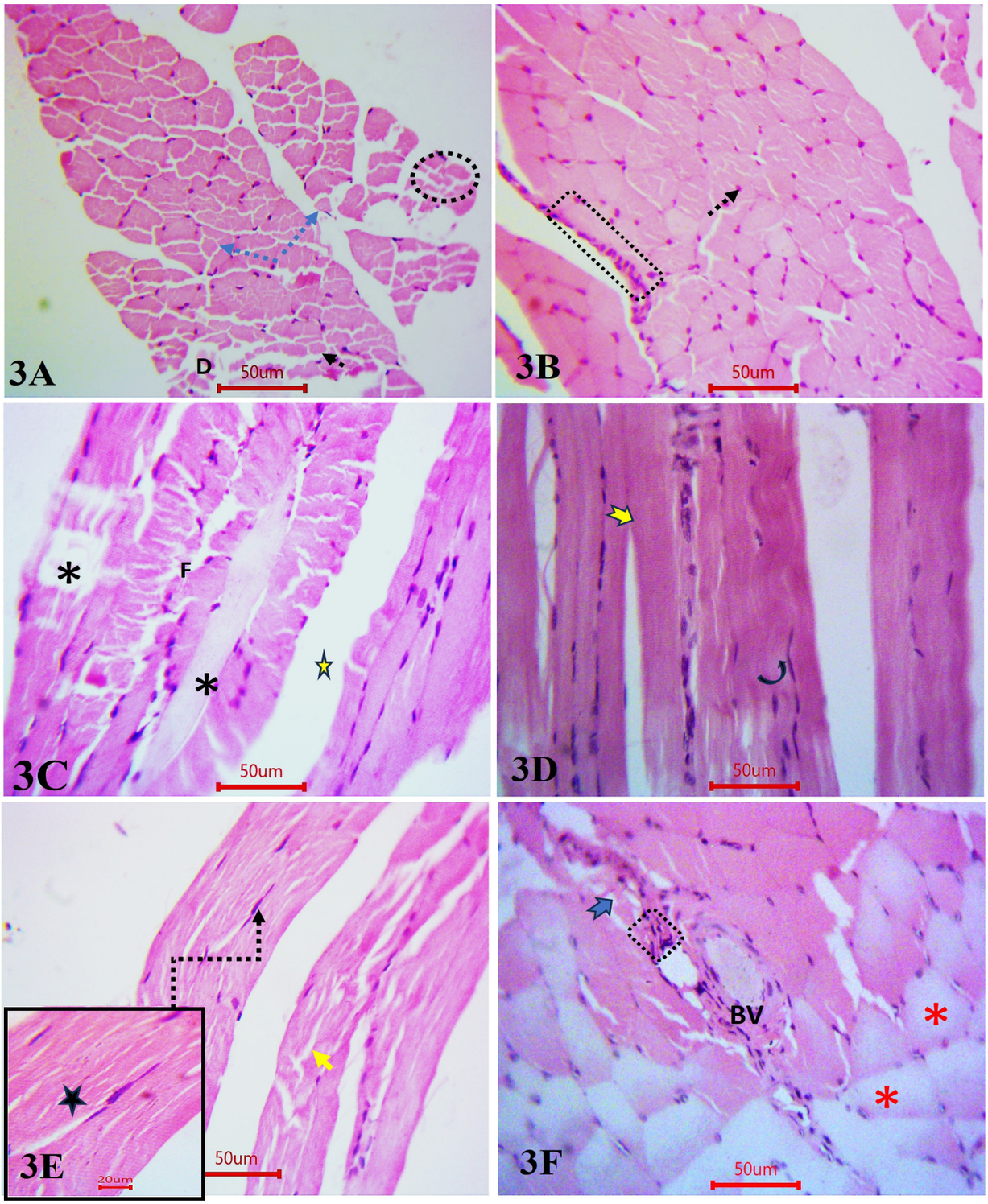


Fig. 3: Photomicrographs of rat's gastrocnemius muscle from OVX group showing: A) CS showing variable size and shape muscle fibers (circle). Some muscle fibers are apparently shrunk (black arrows). B) CS showing internalization of nuclei (arrow) and inflammatory cell infiltration (rectangle). C) LS showing focal areas of degeneration (black asterisks) and fragmented muscle fibers (F). Notice the wide spacing between muscle fibers (yellow star). D) LS showing splitting branching of the muscle fibers (notched arrow). Notice densely stained nuclei (curved arrow). E) LS showing undulated disrupted sarcolemma (yellow arrow). Insets: Higher magnification showing focal loss of striated pattern of muscle fibers (black star). F) LS showing numerous large vacuoles (notched arrow) interrupt and replace the continuity of the skeletal muscle fibers. Blood vessels (BV) appear dilated with associating inflammatory cell infiltration (rectangle). Lightly stained foci (red asterisks) are also noticed.

(H&E x 400, scale bar=50 µm, inset x 1000)

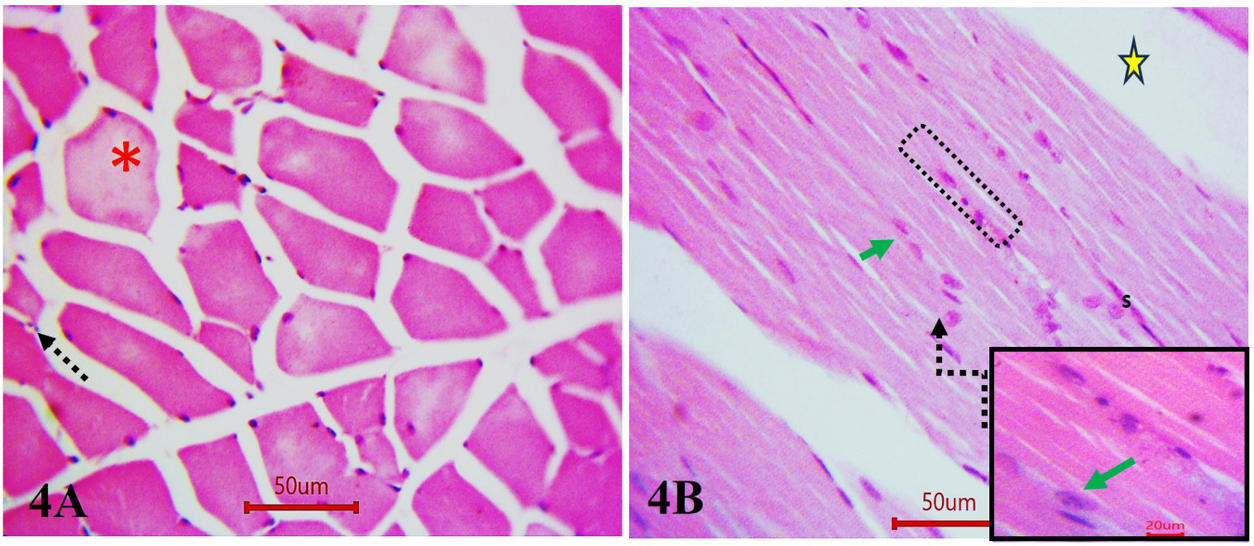


Fig. 4: Photomicrographs of rat's gastrocnemius muscle from CLO-OVX group showing: A) Cross sections showing muscle fibers more or less similar to that of the sham and CLO groups. Notice a few lightly stained foci (red asterisk) and few apparently shrunken muscle fibers (black arrow) among the apparently normal muscle fibers. B) Longitudinal section showing apparent normal appearance of eccentric vesicular nuclei that forming nuclear chain (green arrows). Notice the wide spacing between muscle fibers (yellow star). Notice the less inflammatory cell infiltration (square). Higher magnification showing vesicular nucleus (green arrow) and prominent striations and well-organized muscular fibers (s).

(H&E x 400, scale bar=50 µm, inset x 1000)

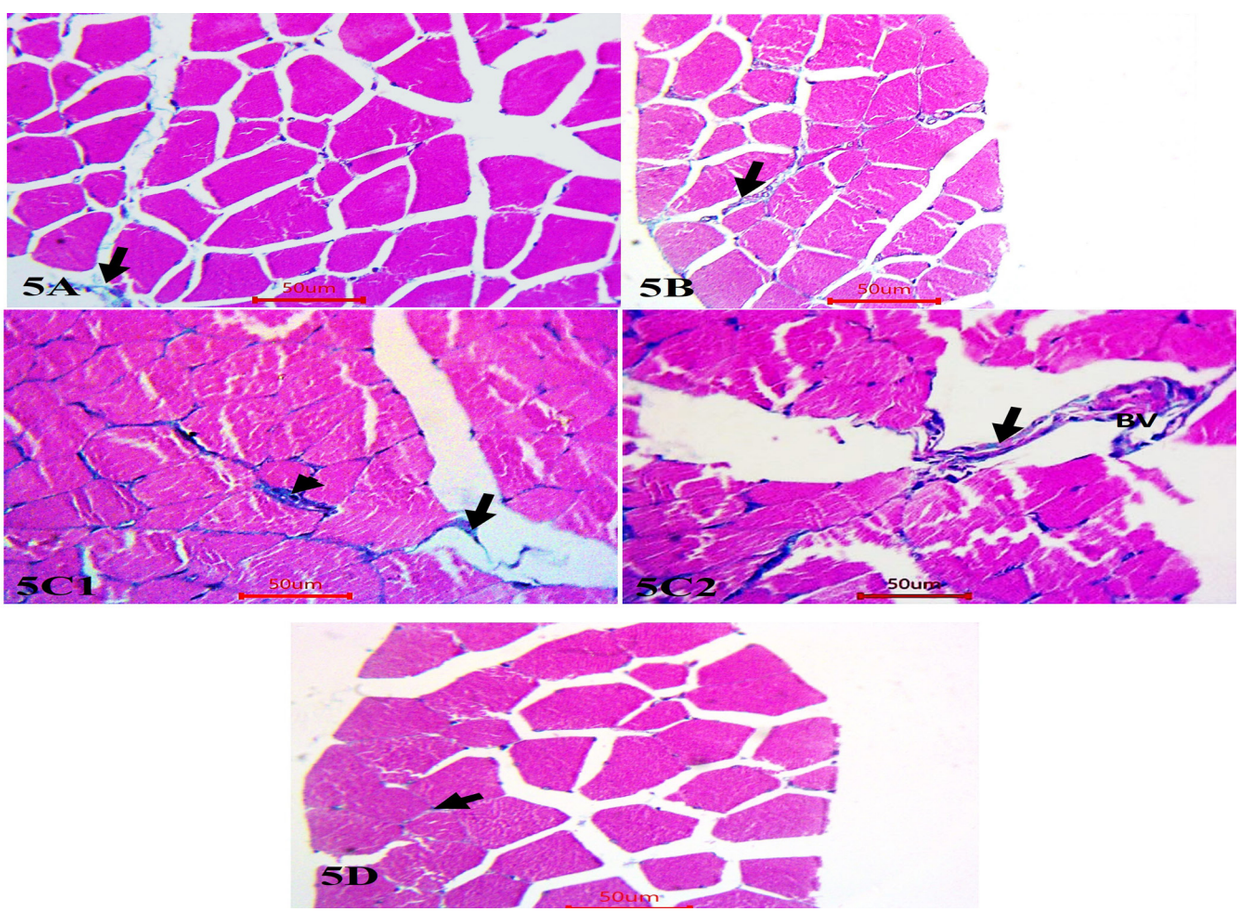


Fig. 5: Photomicrographs of cross sections of rat`s gastrocnemius muscle of: A & B) Sham and CLO groups showing collagenous connective tissue (black arrows) in perimycin. C) OVX group showing C1) Condensation of collagen bundles (black arrow) within perimycin and endomysium (arrowhead). C2) Condensation of collagen bundles surrounding dilated blood vessels (BV). D) CLO-OVX group showing less collagen bundles (black arrow) within perimycin.

(Masson`s Trichrome x400, scale bar=50 µm)

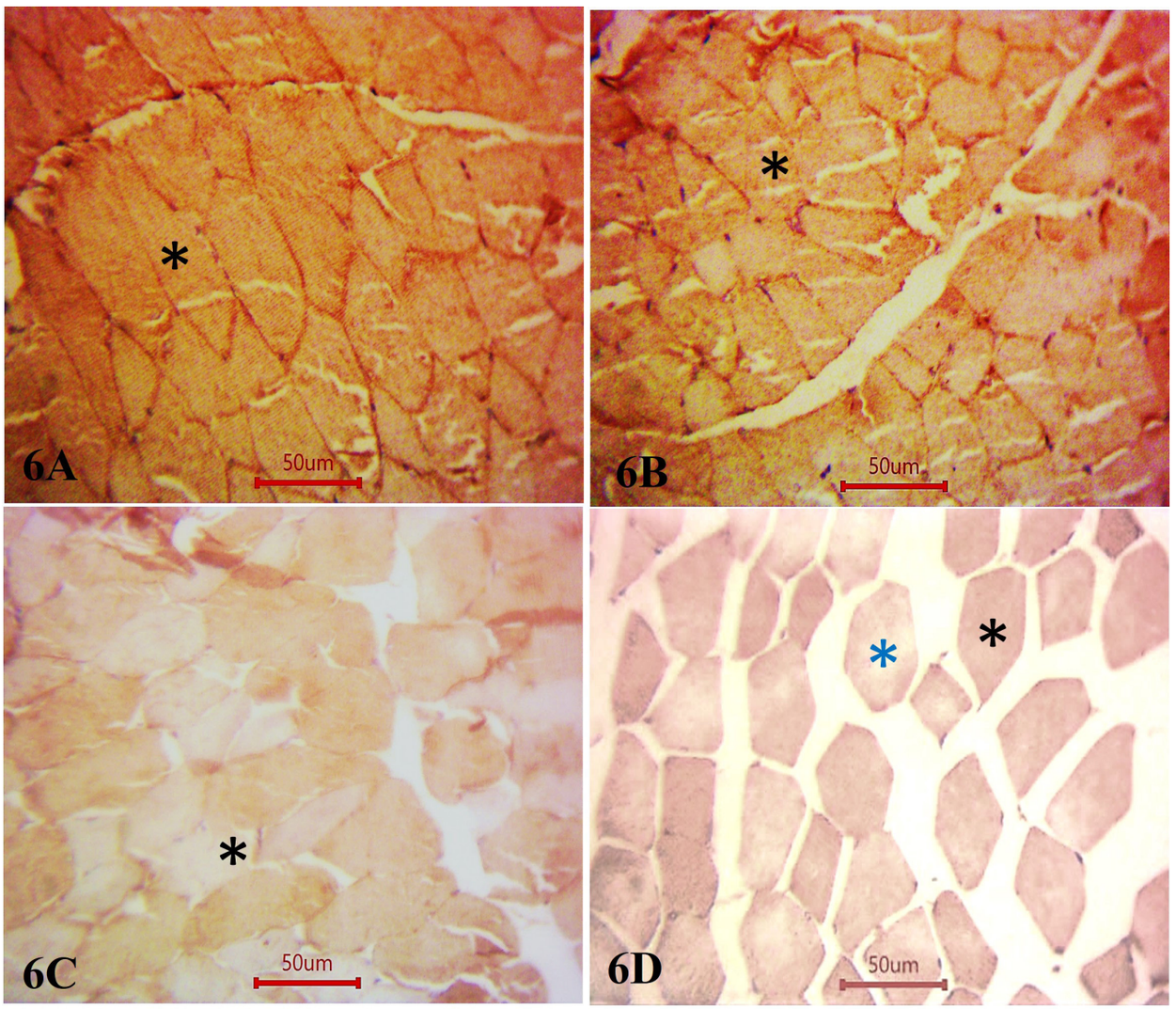


Fig. 6: Photomicrographs of rat`s gastrocnemius muscle of: A & B) Sham and CLO groups showing strong intense desmin cytoplasmic expression in muscle fibers (asterisks). C) OVX group showing faint desmin cytoplasmic expression in muscle fibers (black asterisks). D) CLO-OVX group showing apparent more desmin cytoplasmic expression in muscle fibers (black asterisks) while some muscle fibers showing faint cytoplasmic expression (blue asterisks).
(Anti desmin x 400, scale bar=50 µm)

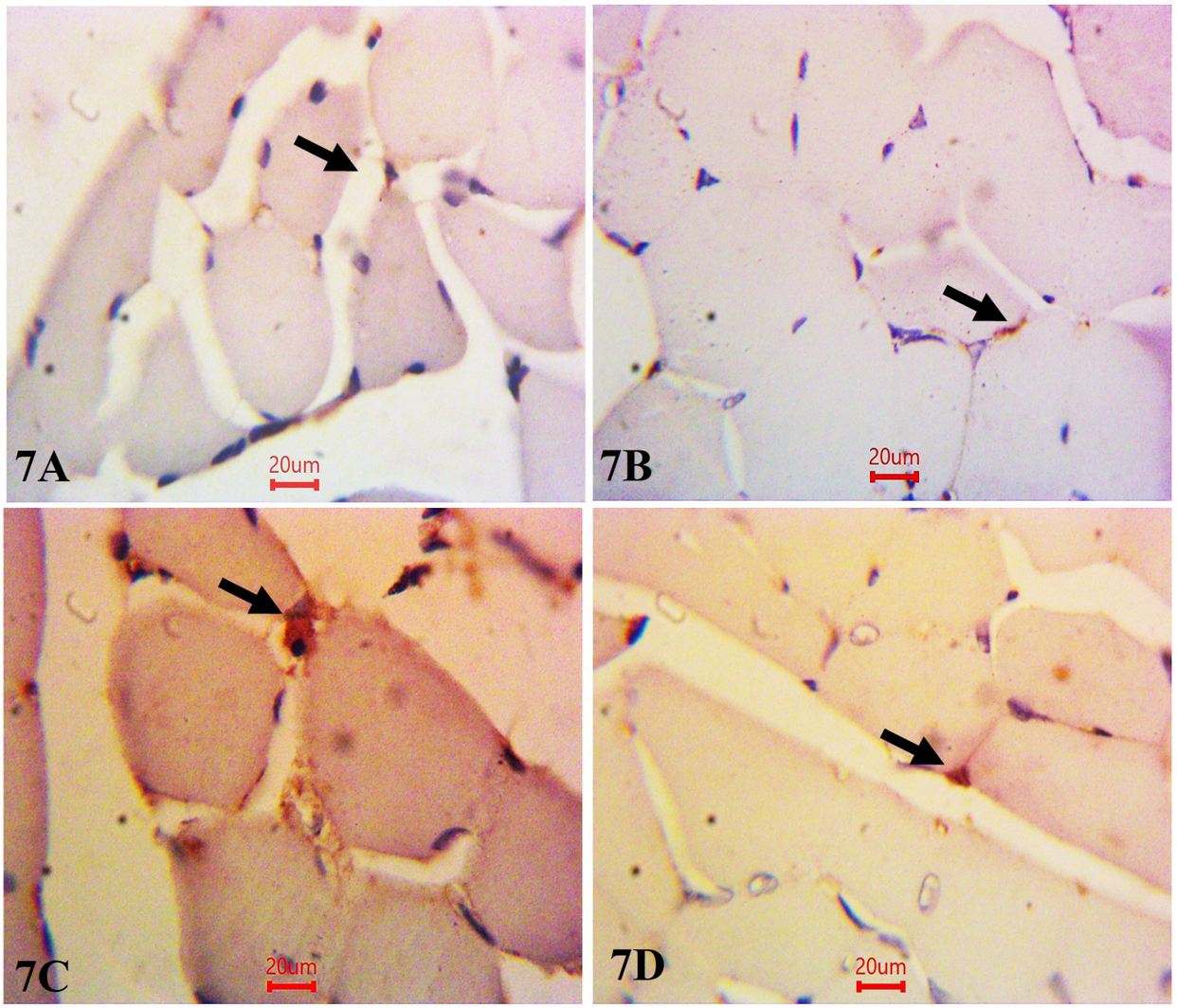


Fig.7: Photomicrographs of rat`s gastrocnemius muscle of: A & B) The sham and CLO showing macrophage with faint CD 68 cytoplasmic expression (arrows). C) OVX group showing intense cytoplasmic expression in apparently enlarged size macrophage (arrow). D) CLO-OVX group showing less cytoplasmic expression in apparently small size macrophage (arrow).

(Anti CD34 x 400, scale bar=50 μm)

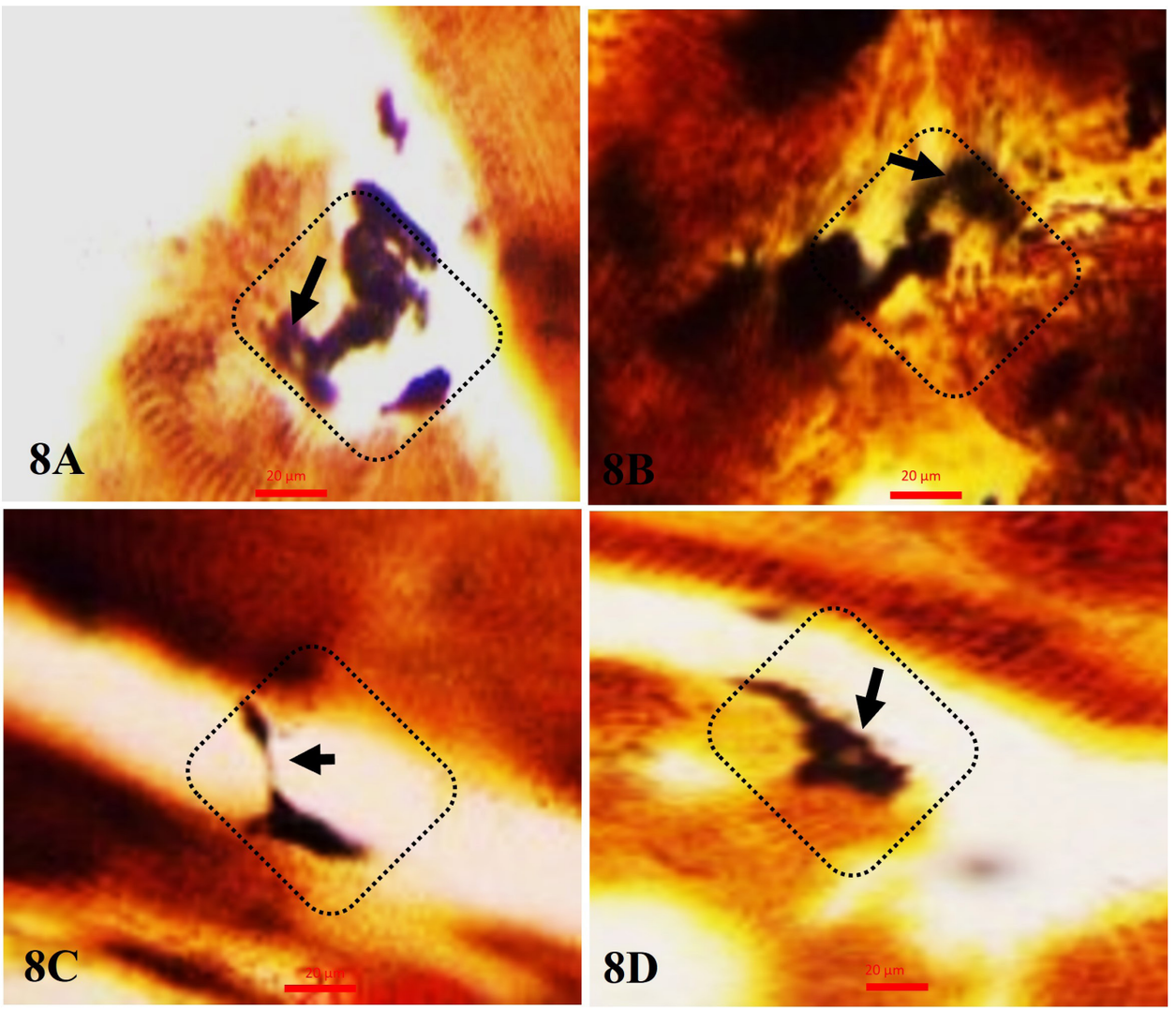


Fig. 8: Photomicrographs of rat`s gastrocnemius muscle of: A & B) Sham and CLO groups showing uniformly diameter, intact nerve fiber (rectangles). Notice the myoneural junctions (thick arrow) with hen leg appearance of nerve terminals on the surface of the muscle fibers. C). OVX group showing shrinkage of the nerve terminals and breakup of the nerve fiber staining (rectangles) with focal nerve fiber thinning (arrow). D) CLO-OVX showing nerve fiber with more or less normal (rectangles). Notice the restoration of myoneural junction's appearance (thick arrow).

(Glees x 1000, scale bar=50 µm)

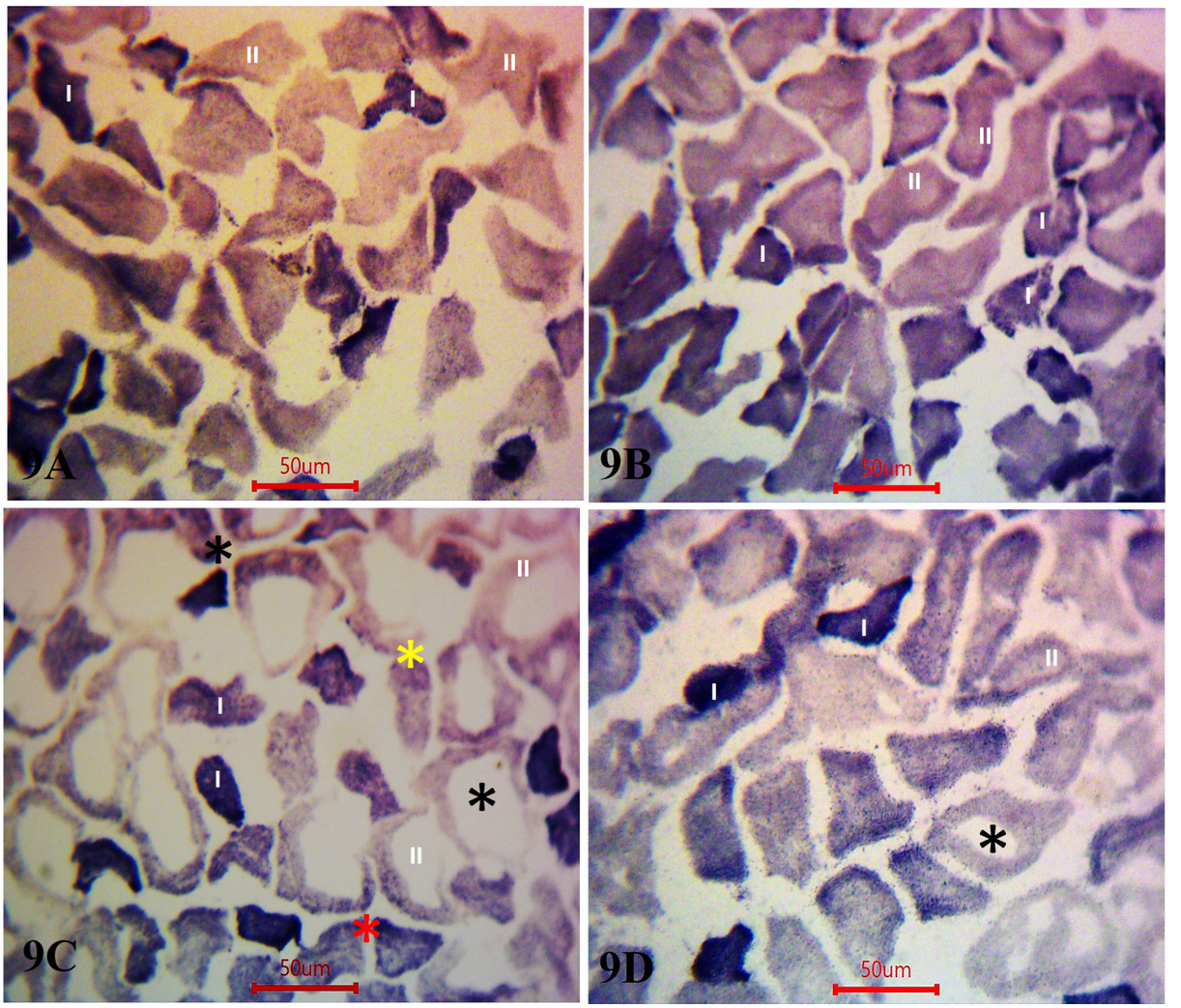


Fig. 9: Photomicrographs of rat`s gastrocnemius muscle of: A & B) Sham and CLO groups showing that the muscle consisting mostly of type II fibers (II) with less intensity and few type I fibers (I) with a high intensity of SDH staining. C) OVX group showing type II fibers with central (black asterisk) and eccentric (red asterisk) loss of staining. Notice the apparent decrease in the intensity of staining in the center of type I fibers (yellow asterisk). D) CLO-OVX group showing fibers more or less normal. Mostly type II (II) and few t type I (I) are observed. Notice the apparent decrease in the intensity of staining in the center of a few type II fibers (black asterisk).

(Succinic dehydrogenase histochemistry X400, scale bar=50 µm)

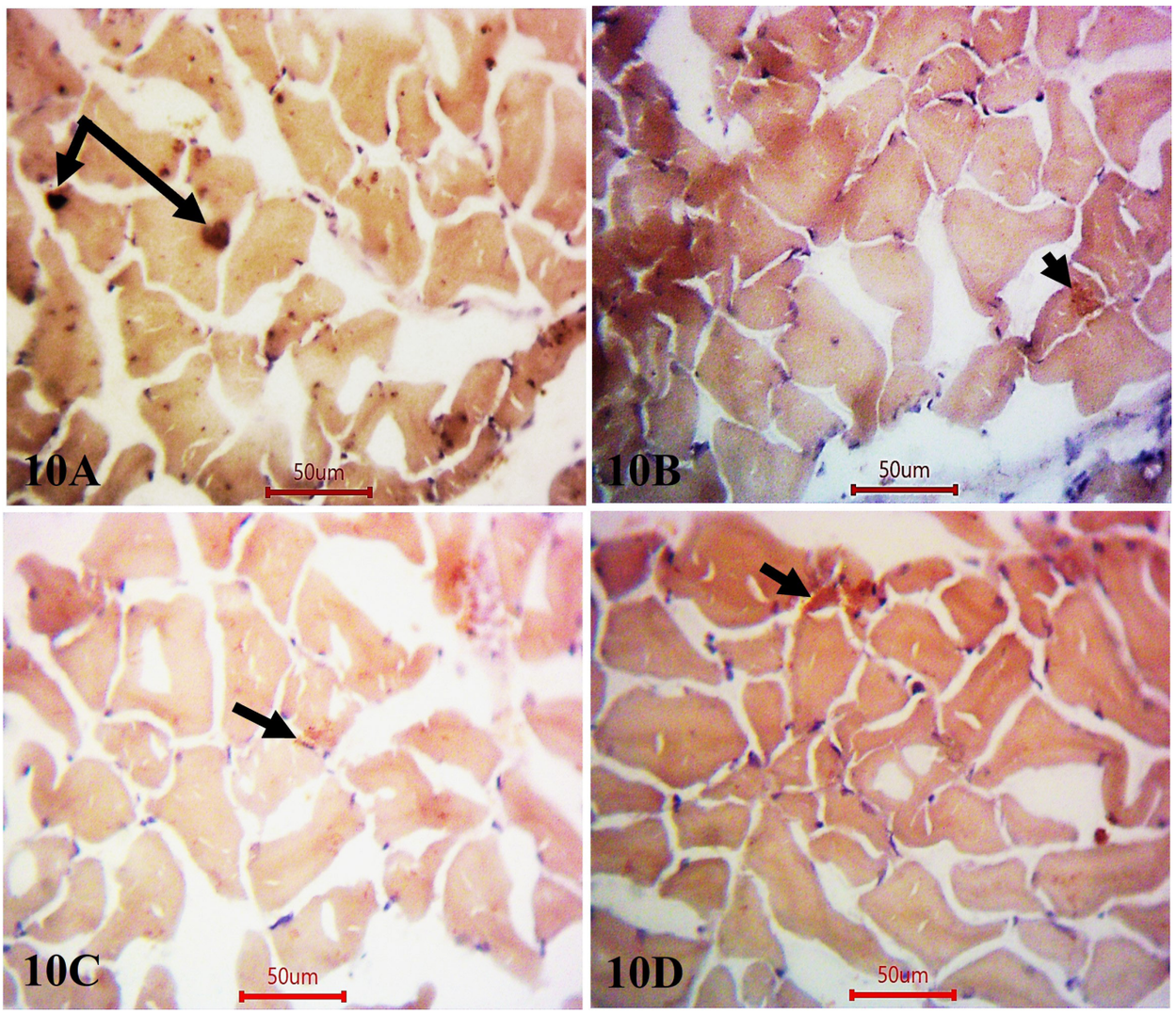
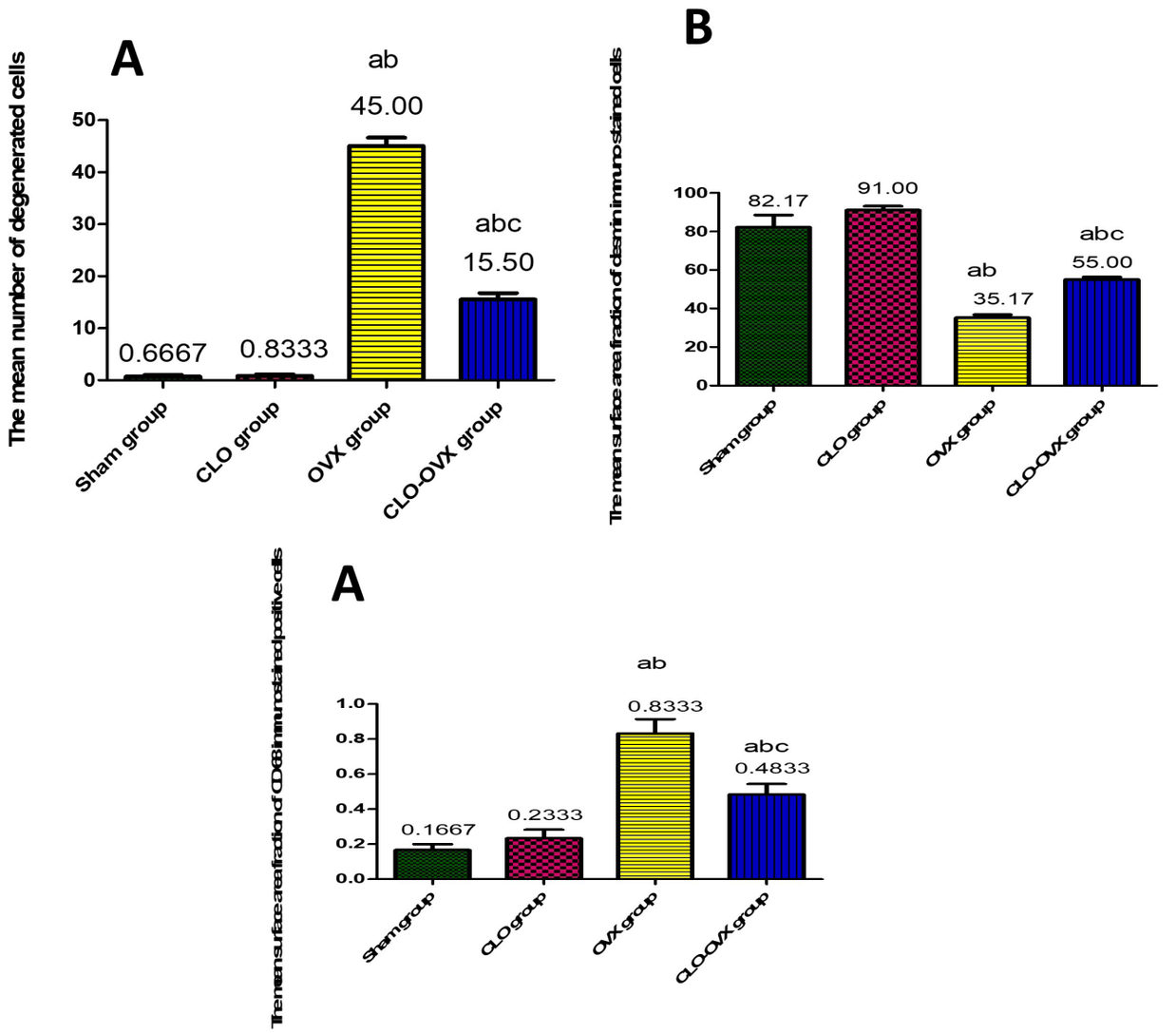


Fig. 10: Photomicrographs of rat`s gastrocnemius muscle of: A & B) sham and CLO groups showing brown, oval, or round and compact NMJ (neuromuscular junction) areas (arrows) distributed along the muscle fibers border. C) OVX group showing focal lightly stained NMJ (arrows). D) CLO-OVX group showing NMJs' appearance (arrow) more or less the same as that sham &CLO groups

(AChE histochemistry x 400, scale bar=50 µm)



Histogram 1,2,3: Showing quantitative analysis of A) Mean number of degenerated cells per section. B) Mean surface area fraction of desmin of immuno-stained cells. C) Mean surface area fraction of CD 68 of immuno-stained cells. Data are expressed as mean ± SD. of 6 rats per group. a significantly different from sham group, b significantly different from CLO group, c significantly different from OVX group, and d significantly different from CLO-OVX group. $P \leq 0.05$.

DISCUSSION

The goal of this current study was to asse the histological alternations caused by ovariectomy on skeletal muscle injuries in adult female albino rats. The ovariectomized rat was used as a postmenopausal model for postmenopausal muscular damage. And to investigate the efficacy of CLO to restore the muscular deficit caused by hormone deprivation.

The muscle fibers of skeletal muscle are classed as slow- and fast-twitch fibers (types I and II)[16]. The Gastrocnemius muscle garnered interest in experimental study as it comprises both type I (slow-twitching) and type II (fast-twitching) muscle fibers[17]. Therefore, gastrocnemius was used for this study.

This study showed muscle fiber degeneration and inflammation in ovariectomized (OVX group). Compiling evidence has shown these results before[18]. It is possible that the degenerative alterations that impacted the muscular fibers were the trigger for the inflammatory response. As previously indicated, degenerated muscle fibers release several inflammatory mediators that contribute to inflammatory cell infiltration[19]. Centrally placed nuclei were found in this work, it is line with a previous study which stated that many markers have been suggested as evidence of muscle fiber breakdown in degenerative myopathies[20]. The spitted muscle fibers noticed in this study might be contributed deficient oxygen delivery and metabolite exchange to the larger and hypertrophied fibers[21]. This is in accordance with Navarrrete, et al., (2020) who docomented the ovarian hormone insufficiency has a vital role in skeletal muscle growth and function defect[22]. According to Shu et al., 2023, estrogen deprivation causes a significant down-regulation of myofibril construction, raising the risk of several disorders associated with muscle failure[23]. After menopause, myofibrils revealed a decrease in protein synthesis and energy metabolism[24].

Hematoxylin and eosin finding were confirmed by scoring muscle fibers degeneration. The OVX group showed a considerable increase in collagen fiber deposition. It is in line with Mori et al., 2011 who demonstrated that oxidative stress induced by OVX promotes cardiac fibroblast proliferation, phenotypic change, and collagen synthesis. Furthermore, estrogen deficiency induced oxidative stress may worsen transforming growth factor-mediated myocardial fibrosis in pressure-loaded hearts[25].

Neuromuscular junctions (NMJs) are the interconnection between the neurological and skeletal muscle systems, can receive pathogenic information from both pre- and post-synaptic sources. As a result, NMJs are an excellent indicator for overall motor health[26]. Using the Glees stain, it was possible to see degenerative alterations in the nerve fibers. These changes may manifest as thinning or disorganization of their neurofibrils, resulting in the breakdown of nerve fibers. Neuronal degeneration can cause muscle atrophy. Despite functional denervation may activate numerous mechanisms that lead to morphological degeneration of NMJs and altered AChR turnover with ageing[27] and it is matched with OVX group's Gless staining findings, which exhibited disorganized thinning NMJ.

Succinic dehydrogenase (SDH), an essential component of complex II of the respiratory chain of the mitochondria, had been shown to serve a critical role in elevated respiratory rates, making its function a credible marker of mitochondrial oxidative capability. Since SDH molecules are found in the inner membranes of mitochondria, it has been shown that the physicochemical characteristics of the membranes such as fluidity and the phospholipid/cholesterol mole ratio have a significant influence on how well the membranes function[28]. Possibly, the mitochondrial morpho functional state, as the organelle's capacity of appropriate adenosine triphosphate (ATP) provision, can be measured by assessing SDH activity using SDH stain[29]. This study revealed an apparent decrease in SDH activity that appeared in type II (lightly stained) fibers. Both types I and II were also affected as the dose was increased. In the central core disease myopathy, all muscle fibers exhibited an absence of oxidative enzyme reaction (SDH) both centrally and eccentrically. When analyzed ultra structurally, these patches represent areas of mitochondrial absence and myofilament deformation[30].

All cholinergic synapses in the peripheral and central nervous systems require AChE, as was previously mentioned. It rapidly hydrolyzes acetylcholine generated by nerve endings. It was widely documented, the nature of electrical impulses, muscle electromechanical action, and neuronal substances delivered by axonal transportation all have an impact on AChE activity. After skeletal muscle denervation, AChE mRNA levels decrease[31]. Additionally, it was reported previously that ovariectomy modifies acetylcholinesterase activity in brain locations, although not uniformly, and affects only qualitative elements of cognitive function, which could be improved with estrogen replacement[32]. It has been reported that ovariectomy-lead to hormone deficiency after a period of four weeks, when associated with A1-42 accumulating in the hippocampal dorsal part, can hinder memory via reducing release ACh and 7nAChR activity with no triggering apoptosis in the dorsal hippocampus's CA1 area[33].

Desmin levels decreased in the OVX group. In the cervix, estrogen appears to influence desmin protein expression through translation. Desmin S-nitrosylation may possess a role in the peripartum uterus[34].

In this study, a significant rise in CD 68 expression was seen in the OVX group. It was previously observed that macrophage concentration was increased on the 7th day of recovery following ovariectomy. However, macrophage chemotaxis in ovariectomized female soleus muscles can be cyclic during the first week after reload[35]. The identification of ovarian hormone-sensitive muscle recovery pathways will help in the development of future pharmacological therapies for the fast effective repair of

muscle tissue following a period of inactivity via stromal macrophage control[36].

It was noticed an amelioration of skeletal muscle injury following CLO intake. These findings showed the importance of cod liver oil (CLO) in avoiding postmenopausal skeletal muscle damage. It was stated before that hippocampal SOD, GSH, and TAO levels were raised by CLO and imipramine. Additionally, it reduced the increased lipid peroxidation. Antioxidant capabilities of unsaturated fatty acids in CLO may play a protective effect in lowering lipid peroxidation with subsequent enhancing antioxidant activities, explaining the antidepressant role of CLO[7]. Cod liver oil is enriched in vitamin D and A, as well as vital omega-3 fatty acids. Vitamin A plays an important function in the upkeep of several vital biological processes. The hydrophobic chain of polyene units may be responsible for vitamin A's antioxidant effect. Previous research has also looked into vitamin A's antioxidant activity in terms of its ability to protect against neurodegenerative and cardiovascular disorders. Vitamins in CLO may have played a beneficial function in reducing the response to stress-mediated inflammatory and generation of free radical[7].

The reported anti-inflammatory differences in a prior study between consumers of CLO and other omega-3 fatty acid products could be due to the supplement's quality, namely its level of fatty acids oxidation[37].

Patients who were given CLO had a lower risk of myocardial infarction. This could potentially prevent all ailments associated with elevated cholesterol and lipid levels, such as cardiovascular disease. As a supplement, adding CLO to rosuvastatin may assist lower the dose of rosuvastatin, which may aid in alleviation their unpleasant responses. In patients who are contraindicated for statins (cholesterol Lowering drug), CLO can be suggested as an alternative; nevertheless, clinical evidence[8].

Additionally, it suppresses the building up and release of very low-density lipoproteins, which lowers the synthesis of triacylglycerol by activating sterol receptor element-binding protein-1c. However, it increases the voltage needed for membrane depolarization, lengthens the relative refractory time, and blocks voltage-gated sodium channels. Because of all these benefits, omega-3 fatty acids have received a lot of attention for their ability to lower cardiac events[38].

It was previously found that rats exposed to a 9-week vitamin A deficiency (VAD) thickened their cortical bones. Previous research suggested that supplementing with retinoic acid, the active form of vitamin A, was less effective than feeding whole food CLO to VAD rats in order to restore the thickness of their deficient rat's bone[39]. Additionally, it was noticed that CLO supplements containing n-3 fatty acids can be employed as NSAID-sparing medicines in rheumatoid arthritis patients[40].

These findings indicate that omega-3 poly unsaturated fatty acids promote anabolism of muscle by increasing dietary stimulus sensitivity that is mainly independent on Protein kinase B activity[41]. It could provide an explanation for the NMJ's return to normal in CLO-OVX groups, suggesting improved axon quality. Omega-3 fatty acid supplementation combined with resistance training could enhance muscle function in the elderly[42].

Consuming omega-3 fatty acids inside mitochondria probably changes the flexibility of the membrane and makes it easier for electrons to pass through and produce superoxide. After fish oil dietary supplements, the tendency for succinate-induced mitochondrial H2O2 release rises 1.3 times, according to an analysis of mitochondrial reactive oxygen species emission[43].

In this study, it was detected that a significant increase in acetylcholine esterase in CLO-OVX group if compared to OVX groups. It has previously been shown that dietary fish oil improved the ability to contract of isolated rat intestines caused by acetylcholine and eicosanoid[44].

In addition, it was noticed an increase in desmin immunoreactivity in CLO-OVX group when compared to OVX group. Cod liver oil has been observed to enhance demine in colon cancer in rat livers[45].

The CLO-OVX group exhibited a significant decrease in CD68 immunoactivity in contrast to the OVX group. Omega-3 fatty acid supplement had been shown to lower biomarker of inflammation in patients suffer from variety of chronic inflammatory or non-inflammatory disorders[37]. During a murine model of muscle damage and restoration, the creation of traditional mediators of pro-inflammation such as prostaglandins/leukotrienes and pro-resolving mediators such as resolving was seen. The infiltrated leukocytes (macrophages and neutrophils) generated these mediators, and their temporal fluctuation was linked to a phenotypic shift for these leukocytes. The formation of an anti-inflammatory phenotype in macrophages was linked to the resolution phase[46].

CONCLUSION

In this study, it was demonstrated a significant adverse impact of ovarian hormones deficiency on skeletal muscle structure. It could be concluded that CLO supplementation has a favorable effect on skeletal muscle in a rat ovariectomized model, and that this could provide an alternative way to avoid the negative effects of high-dose replacement hormone therapy.

ABBREVIATIONS

OVX: ovariectomized; **CLO:** cod liver oil; **H&E:** Hematoxylin and Eosin; **AChE:** acetylcho¬line esterase; **CD 68:** Cluster of Differentiation 68; **CS:** cross sections; **LS:** longitudinal sections; **NMJs:** neuromuscular junctions; **SDH:** Succinic dehydrogenase.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير المحتمل لزيت كبد سمك القد على إصابة العضلات الهيكلية الناجمة عن استئصال المبيض في إناث الجرذان البيضاء البالغة؛ الدراسة النسيجية والكيميائية النسيجية وهستوكميائيه المناعية

ساره محمد نجيب عبد الحافظ

قسم الهستولوجى و بيولوجيا الخليه كليه الطب جامعه المنيا مصر

المقدمة: تقضي المرأة أكثر من ثلث حياتها بعد انقطاع الطمث، والذي يتميز بانخفاض مستوى هرمون الاستروجين المرتبط بأمراض متعددة. أثارت الفوائد الصحية لزيت كبد سمك القد، الذي يحتوي على أحماض أوميجا ٣ الدهنية، اهتمام المتخصصين الطبيين بسبب النتائج الواعدة.

الهدف من البحث: كان الهدف من هذا العمل هو تحديد آثار استئصال المبيض (نموذج تجريبي بعد انقطاع الطمث) على بنية العضلات والهيكل العظمي والتحقيق من قدرة كبد سمك القد على تقليل الخلل العضلي الناجم عن نقص الهرمون.

مواد وطرق البحث: تم استخدام أربعة وعشرون من إناث الجرذان البيضاء البالغة في الدراسة. وتم تقسيمهم إلى أربع مجموعات متساوية: مجموعه تعرضت لعمليه فتح بطن و اعاده اغلاقها، و مجموعه زيت كبد سمك القد، ومجموعه تعرضت لعمليه استئصال المبايض، و مجموعه تناولت زيت كبد سمك القد بعد عمليه استئصال المبايض . تمت معالجة عضلة الساق لإجراء التحليلات النسيجية والكيميائية النسيجية الهستوكمائيه المناعية.

النتائج: أظهرت النتائج في مجموعة استئصال مبايض عن مجموعة متنوعة من التغيرات المورفولوجية في كل من العضلات الهيكلية والألياف العصبية. كشفت صبغه الجليس عن خلل في الوصلات العضلية العصبية. لوحظ انخفاض في صبغة هيدروجيناز السكسينات في ألياف العضلات الهيكلية من النوع الثاني. انخفض نشاط أستيل كولين بشكل ملحوظ. وقد لوحظ أيضا انخفاض كبير في نشاط مضاد الديسمين بالإضافة إلى زيادة كبيرة في التفاعل الإيجابي المناعي لنشاط السى دى ٨٦ في هذه المجموعة بالمقارنة مع كل من المجموعتين التي تعرضت لعمليه فتح بطن واعاده اغلاقها، ومجموعه زيت كبد سمك. ومن المثير للاهتمام أن جميع التغييرات المذكورة سابقًا تم تحسينها في المجموعة التي تناولت زيت كبد سمك القد بعد عمليه استئصال المبايض.

الاستنتاج: يمكن استنتاج وجود تأثير سلبي كبير لنقص هرمونات المبيض على البنية العضلية الهيكلية. يمكن أن نستنتج أن مكملات زيت كبد سمك لها تأثير إيجابي على العضلات الهيكلية في نموذج استئصال المبيض لدى الفئران، وأن هذا يمكن أن يوفر طريقة بديلة لتجنب الآثار السلبية للعلاج بالهرمونات البديلة بجرعة عالية.