Effect of Morin on Fluoxetine (Prozac)-Induced Structural Changes of Mice Skeletal Muscle: Light and Electron Microscopic Study

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Original Article

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ABSTRACT

Introduction: Fluoxetine (Prozac) is an antidepressant drug with an adverse effect on the tissues with its prolonged use. Morin is natural polyphenol with different health benefits due to its antioxidant, and anti-inflammatory actions.

Aim of the Work: This work designed to evaluate the effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle by means of light and electron microscopic study.

Materials and Methods: 30 mice (50-70 gm) were used for 3 weeks and divided into 6 groups (5 rats each); Group 1: Control, Group 2: Morin low dose (MLD): mice received low dose of morin 30 mg/kg/day orally dissolved in distilled water, Group 3: Morin high dose (MHD): mice received high dose of morin 100 mg/kg/day orally dissolved in distilled water, Group 4: Prozac group (PR): mice received an intraperitoneal injection of 0.06 mg PR/mice/day in normal sterile saline. Group 5: Prozac +Morin low dose: (PR+MLD): mice that received an intraperitoneal injection of 0.06 mg PR/mice/day in normal sterile saline preceded by one hour with a low dose of morin (30 mg/kg/day) dissolved in distilled water orally, and Group 6: Prozac +Morin high dose: (PR+MHD): mice that received an intraperitoneal injection of 0.06 mg PR/mice/day in normal sterile saline preceded by one hour with a high dose of morin (100 mg/kg/day) dissolved in distilled water orally. Oral doses were by an intragastric tube.

Results: PR group showed vacuolations, cellular infiltrations, small, dark central nuclei at H&E sections, decreased PAS +ve reaction, significantly increased collagen area percentage, significantly increased Cytochrome-C area percentage, myofibrils with wide separation, accumulations of mitochondria of different sizes and shapes, and degenerated nucleolar membrane with vacuolated nucleoplasm at electron microscope. On the other hand, these previous changes were ameliorated by morin dose dependently.

Conclusion: Morin dose dependently could protect against fluoxetine induced skeletal muscle degenerative changes in mice.

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Key Words: Cytochrome C; electron microscope; fluoxetine; morin: skeletal muscle.

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INTRODUCTION

Fluoxetine (FLX) is a central nervous system 5-hydroxytryptamine, serotonin (5-HT) reuptake inhibitor. It has been used in the treatment of multiple psychiatric disorders like bulimia nervosa, depressive, premenstrual dysphoric as well as obsessive-compulsive disorders[1,2].

It was believed that fluoxetine effect is specific without affecting the other tissue functions[3]. Nevertheless, recent studies proposed that; the intake of FLX for a long time could lead to adverse effects on different tissues like liver, kidney besides ovary. Besides sexual dysfunction and teratogenic effects[4].

Additionally, FLX long term intake could lead to adverse effects on the locomotor system because of its special effects on the structure and function of the somatosensory system[5].

These might be due to the effect of FLX on the level of the antioxidants leading to excess production of free radicals with the occurrence of oxidative stress. In addition to lipid as well as protein peroxidation[6].

Also due to its effect on the serotonin receptors, especially following its repeated administrations. So, desensitization of the pre-synaptic 5-HT1A and 5-HT auto-receptors. Consequently, augmentation of extracellular 5-HT[6].

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Morin is a 3,5,7,2′,4′-pentahydroxyflavone natural polyphenol with different health benefits. It is isolated as a yellow pigment from Moraceae family and can be extracted from the leaves, fruits, stems as well as branches of many plants[7].

Morin has a potent antioxidant, anti-inflammatory, antidiabetic, antitumor, antihypertensive, antibacterial, and neuroprotective properties. It also protects the DNA damage by the released free radicals besides inhibition of the occurrence of lipoprotein (low-density) oxidation. Moreover, it could modulate the activity of several enzymes inducing systemic protection. In addition, its chronic administration is well tolerated suggesting its ability to be used either alone or in combination with other drugs, to prevent different systemic pathologies[8].

Furthermore, morin can exhibit a dose-dependent increase in insulin secretion as well as the intracellular calcium concentration. It also suppresses lipogenesis, inflammation as well as the oxidative stress activities, which is beneficial in condition of high fat diet induced obesity[9].

Form the previously mentioned data; the present work was designed to evaluate the possible protective role of morin in fluoxetine (Prozac)-induced structural changes of mice skeletal muscle. For this, a light besides an electron microscopic study were used.

MATERIALS AND METHODS

Fluoxetine (Prozac) 60mg Capsules: ADVANZ Pharma, Capital House, 1st Floor, 85 King William Street, London, EC4N 7BL, UK

30 mice were used weighing from 50 - 70 gram; obtained from (animal house, Tanta University, Egypt). Mice were placed at hygienic environment with normal room temperature and humidity 60%. They were allowed to have a free access to diet and water ad libitum. For achievement of the safety measures of the experiment; the guideline for the animal care were positioned according to that of Tanta University Committee, Institution of Research Ethics with the ethics code is 34869/8/21.

Experimental groups

Group 1: Control (5 mice): that further subdivided into subgroup1a: 2 mice that left without treatments & subgroup 1b: 3 mice that received an intraperitoneal injection of saline for 3 weeks.

Group 2: Morin low dose (MLD) group: 5 mice that received low dose of morin 30 mg/kg/day dissolved in distilled water orally by an intragastric tube for 3 weeks.

Group 3: Morin high dose (MHD) group: 5 mice that received high dose of morin 100 mg/kg/day dissolved in distilled water orally by an intragastric tube for 3 weeks

Group 4: Prozac group (PR group): 5 mice that received an intraperitoneal injection of 0.06 mg PR/mice/day in normal sterile saline for 3 weeks[5].

Group 5: Prozac +Morin low dose: (PR+MLD): 5 mice that received an intraperitoneal injection of 0.06 mg PR/mice/day in normal sterile saline preceded by one hour with a low dose of morin (30 mg/kg/day) dissolved in distilled water orally dissolved in distilled water for 3 weeks[10].

Group 6: Prozac +Morin high dose: (PR+MHD): 5 mice that received an intraperitoneal injection of 0.06 mg PR/mice/day in normal sterile saline preceded by one hour with a high dose of morin (100 mg/kg/day) dissolved in distilled water orally dissolved in distilled water for 3 weeks[11].

At day 21; pentobarbital sodium was injected intraperitoneal in a dose of 60 mg/kg to anesthetize mice[12]. After that, 0.5-1 cm of the gastrocnemius muscle was extracted immersed in 10% formalin buffered saline followed by embedding into paraffin wax. Later, 5 µm sections obtained for light microscopic assessment. Considering the electron microscopic examination; muscle samples were fixed in 4% phosphate-buffered glutaraldehyde.

Light microscopic examination[13]

Haematoxylin & Eosin (H&E)

Muscle specimens were deparaffinized, hydrated then stained with hematoxylin and eosin. Finally, sections were dehydrated, cleared and mounted in Canada balsam Nuclei were stained blue while, the cytoplasm stained pink.

Periodic Acid Schiff's (PAS)

Sections were deparaffinized, rehydrated, oxidized by 0.5% periodic acid the, stained with Schiff's reagent. Finally, sections were counterstained with hematoxylin, then dehydrated, cleared with Xylol and mounted. The Neutral mucopolysaccharides appeared with magenta coloration.

Mallory's trichrome

Sections were deparaffinized, then rehydrated and washed in distilled water. Then, re-fixation in Bouin's solution and staining with Wiegert's iron hematoxylin and Biebrich scarlet-acid fuchsine. Later, sections were differentiated in phosphotungstic acid solution then put in aniline blue stain and differentiate in 1% acetic acid. Finally, dehydration, and clearance. The collagen fibers were stained blue, and nuclei red.

Cytochrome-C immunohistochemistry[1]

Muscle sections were deparaffinized, rehydrated, placed in a buffer solution then, were boiled in a microwave oven 3 minutes for antigen retrieval (Kenmore, USA). After that sections were cooled down at a room temperature then were placed in 0.3% hydrogen peroxide/methanol for 15 minutes (dilute 30% H_2O_2 with methanol). Later, block the non-specific protein binding sites by adding a blocking solution. This was followed by addition of a primary antibody at 4 °C; rabbit polyclonal anti-Cytochrome-C (1:100) (Abcam, England). Then, the secondary antibody

(Termo, USA) was added 30 minutes at room temperature. At last, sections treated by DAB, then counterstaned by Mayer's hematoxylin.Finally, sections were dehydrated, cleared and mounted. Negative control was obtained by staining of the sections PBS instead of the primary antibody (Figure 8A). Positive control is human kidney (ab76107, Abcam, USA) (Figure 8B). Results: cytoplasmic brown immunohistochemical reaction.

Transmission electron microscopy[14]

First, fixation of specimens in 4% glutaraldehyde then, post-fixation in 1 % osmium tetroxide for 2h at room temperature. Later, 1 mm3 sections were attained, dehydrated and embedded in epoxy resin. After that, 70 - 90 nm sections were obtained using the ultramicrotome (Leica, Austria). Then, loaded over 200 mesh copper grids. Followed by uranyl acetate and lead citrate staining. Finally, examination of the sections using an electron microscope (JEOL-JEM-100 SX, Japan); Electron Microscopy Unit, Faculty of Medicine, Tanta University.

Morphometric analysis

Java Image J software program (1.6.0. 2017. USA) was used for the assessment of area percentage of collagen fibers as well as the area percentage of cytochrome-c immunostaining. For this, ten randomly selected images magnification x 400 of each experimental group were selected.

Statistical analysis

Values were presented as means ± SD. By which the statistical significant difference between the different experimental groups was evaluated using the two-sample Student's t-test. Furthermore, to define significant differences in-between PR and morin treated groups (MLD& MHD), one-way ANOVA was used. A *P value* less than 0.05 considered significant.

RESULTS

H&E

Examination of the longitudinal section (LS) of the gastrocnemius muscle of the control group revealed the normal histological structure of the skeletal muscle. It consisted of parallel, non-branching and cylindrical fibers with acidophilic sarcoplasm and transverse striations. These fibers were connected together by connective tissue endomysium with flat nuclei of fibroblasts. Regarding the nuclei of the muscle fibers, they appeared vesicular, elongated, and peripheral in position (Figure 1A). For MLD & MHD groups; they appeared like control with parallel, non-branching and cylindrical fibers with acidophilic sarcoplasm and transverse striations. Connected together by endomysium with flat nuclei of fibroblasts besides vesicular, elongated, and peripheral nuclei (Figures 1B,C).

As regards Prozac (PR) group; it showed muscle fiber vacuolations, and mononuclear cell infiltrations. Besides, wavy fibers with splitting. The nuclei of the muscle fibers seen small in shape, dark and central in position (Figure 2 D,E).

Examination of PR+MHD group revealed nearly normal picture with cylindrical fibers have acidophilic sarcoplasm and transverse striations with peripheral vesicular nuclei. While the PR+MLD group showed splitting of some fibers with mild mononuclear cellular infiltrations (Figure 2 F,G)

PAS results

Control group besides MLD& MHD groups of PAS stained skeletal muscle sections showed normal distribution with strong PAS +ve reaction (Figure 3A-C).

Prozac (PR) group showed decrease in PAS +ve reaction with faint distribution in some skeletal muscle fibers. Considering the PR+MHD group; it showed nearly normal PAS +ve reaction. While in the PR+MLD group; some muscle fibers appeared with moderately increased PAS +ve reaction (Figure 4D-F)

Mallory's trichrome results

The control group as well as the MLD & MHD groups showed some blue-stained collagen fibers situated in-between the muscle fibers (Figure 5A-C).

Prozac (PR) group showed markedly increased deposition of collagen fibers in-between the muscle fibers. The PR+MHD group exposed nearly normal picture with some collagen fibers situated in-between the muscle fibers in contrast to the moderate amounts of collagen fibers in the PR+MLD group (Figure 6D-F)

Cytochrome-C immunohistochemical results

As regards the negative control; it showed no cytoplasmic reaction while the kidney positive control showed positive brownish cytoplasmic reactions of the renal cortical structures (Figure 7A,B).

The transverse sections of the control as well as MLD&MHD groups showed skeletal muscle fibers with mild cytoplasmic relations for cytochrome C (Figure7C-E).

Prozac (PR) group showed increased cytoplasmic immunohistochemical reaction. For the PR+MHD group; it exhibited a picture similar to the control group with skeletal muscle fibers have mild cytochrome C cytoplasmic. Oppositely, the PR+MLD group revealed moderate cytoplasmic immunostaining for cytochrome C (Figure 8F-H).

Electron microscopic (EM) results

The electron microscopic examination of the control (Figure 9 A1&A2), as well as MLD (Figure 10 B1&B2) & MHD (Figure 10 C1&C2) groups revealed myofibrils situated in the sarcoplasm parallel to the long axis of the myofibers with alternating light (I) and dark (A) bands. Additionally, at the center of the A band, there was an electron-lucent narrow region (H zone) bisected by a dark electron-dense line (M line). Moreover, Z-line was seen at the middle of the light band. These all besides mitochondria and flat euchromatic nucleus.

In PR group, there was disarranged myofibrils with wide separation and accumulations of mitochondria of

different sizes and shapes. For the nucleus; it was seen with degenerated nucleolar membrane and vacuolated nucleoplasm (Figure 11D1,D2). For PR+MLD group; mild improvement seen with disarranged fibrils. While in PR+MHD Group; nearly normal EM picture with nearly normal myofibrils, mitochondria, and nucleus (Figure 11E,F).

Morphometric results

Considering the area percentage of collagen fibers; it showed significant increase in the PR group when compared to the control group conversely, significantly decreased at groups PR+MLD and PR+MHD when compared to the PR group. On the other hand, a non-significant difference was seen at groups MLD and MHD when compared to the control group (Figure 12).

As regards the morphometric results of the area percentage of cytochrome-c immunostaining; it showed significant increase in the PR group as compared to control. Conversely, significant decreased at groups PR+MLD and PR+MHD when compared to the PR group. Instead, a non-significant difference was seen at groups MLD & MHD when compared to the control group (Figure 13).

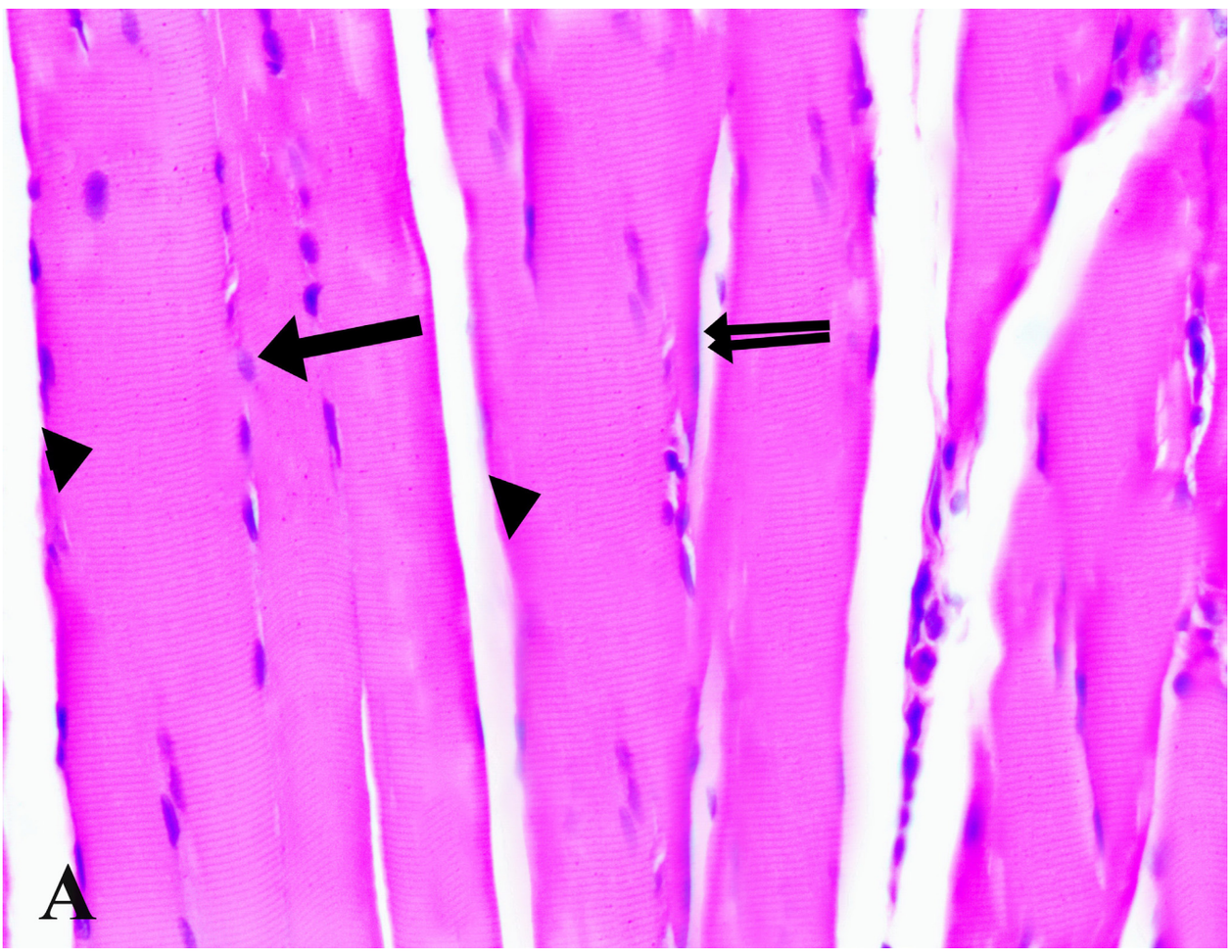

Fig. 1A: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle of the control group showed, parallel, non-branching and cylindrical fibers with acidophilic sarcoplasm and transverse striations (►), vesicular elongated peripheral nuclei (→) and endomysium with flat nuclei of fibroblasts (double arrow) (H&E x400).

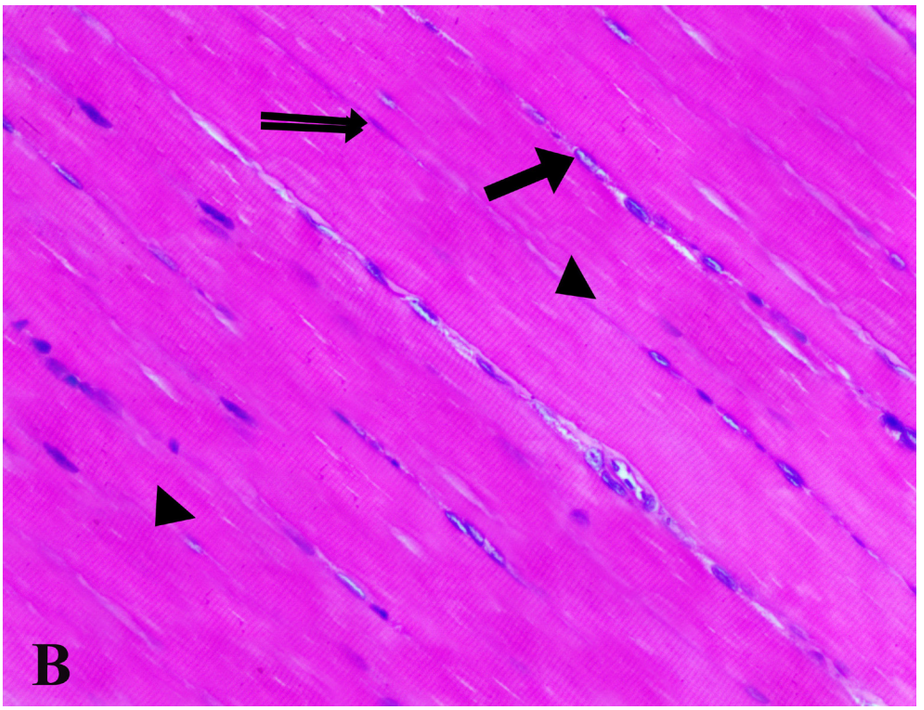

Fig. 1B: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle of MLD group showed, parallel, non-branching and cylindrical fibers with acidophilic sarcoplasm and transverse striations (►), vesicular elongated peripheral nuclei (→) and endomysium contained flat nuclei of fibroblasts (double arrow) (H&E x400).

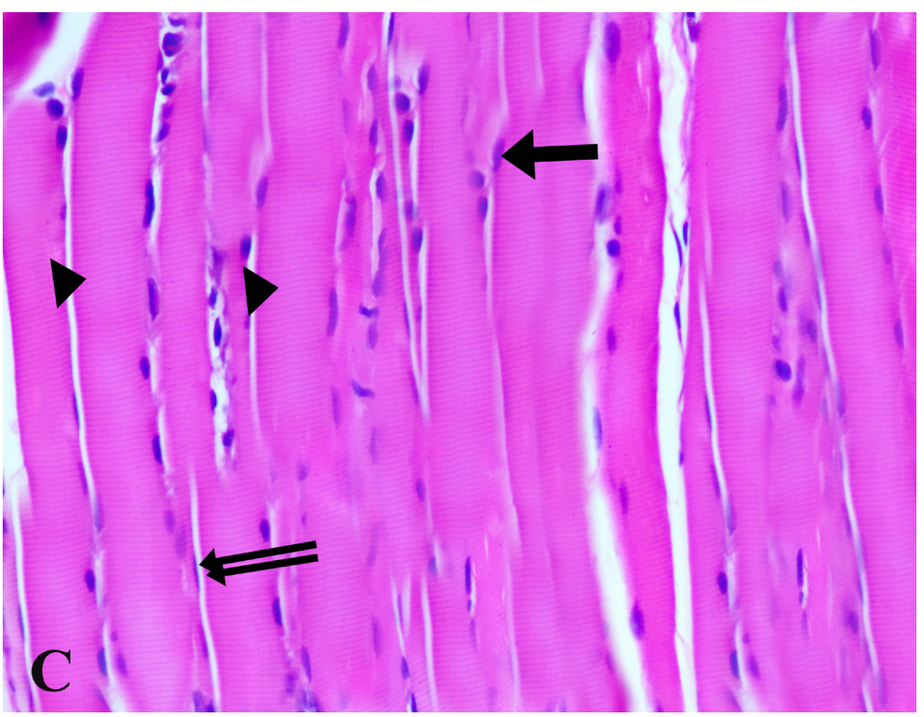

Fig. 1C: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle of MHD group showed, parallel, non-branching and cylindrical fibers with acidophilic sarcoplasm and transverse striations (►), vesicular elongated peripheral nuclei (→) and endomysium with flat nuclei of fibroblasts (double arrow) (H&E x400).

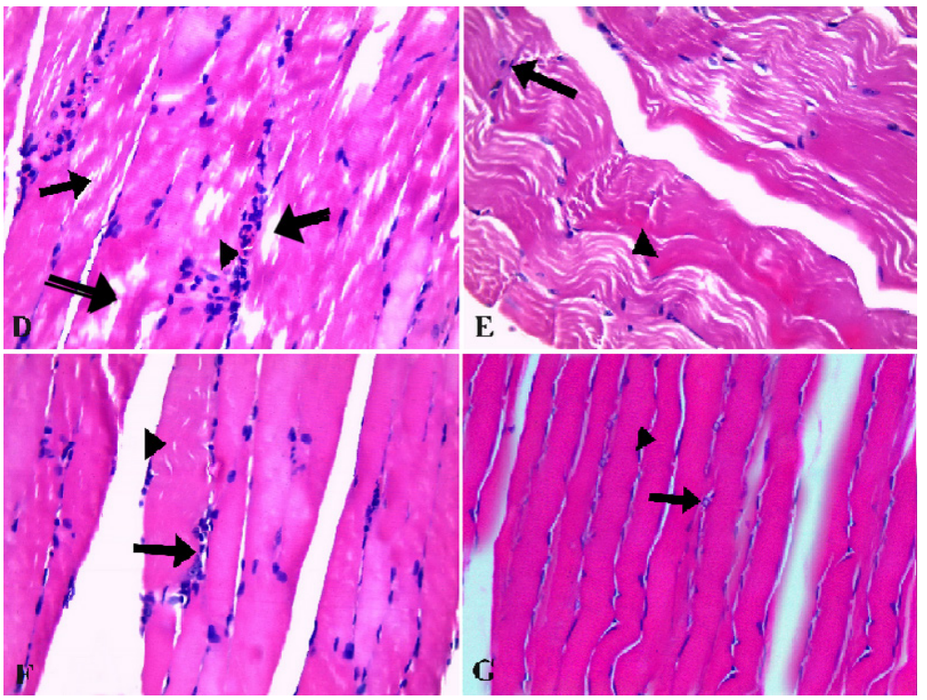

Fig. 2: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle stained by H&E (x400)
D) Prozac (PR) group: muscle fiber vacuolations (→), and mononuclear cell infiltrations (►). E) PR group: wavy fibers with splitting (►) and small, dark and central nuclei (→). F) PR+MLD group: splitting of some fibers (►) and mild mononuclear cellular infiltrations (→). G) PR+MHD group: cylindrical fibers with acidophilic sarcoplasm and transverse striations (►), and peripheral vesicular nuclei. (→).

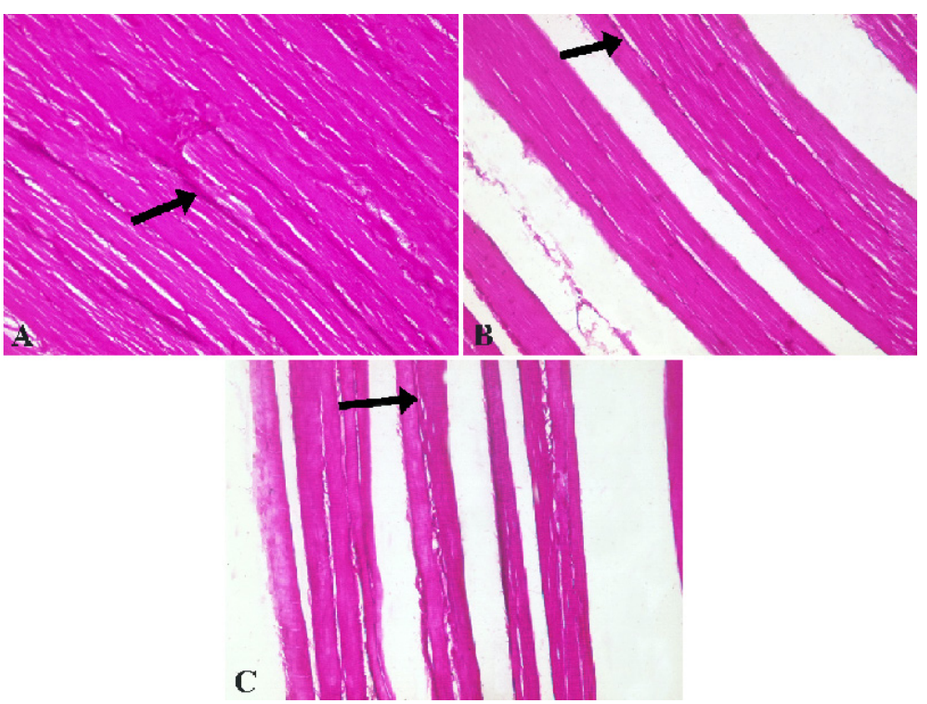

Fig. 3: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle stained by PAS (x400).
A,B &C) Control group, MLD& MHD groups respectively: normal distribution with strong PAS +ve reaction (→).

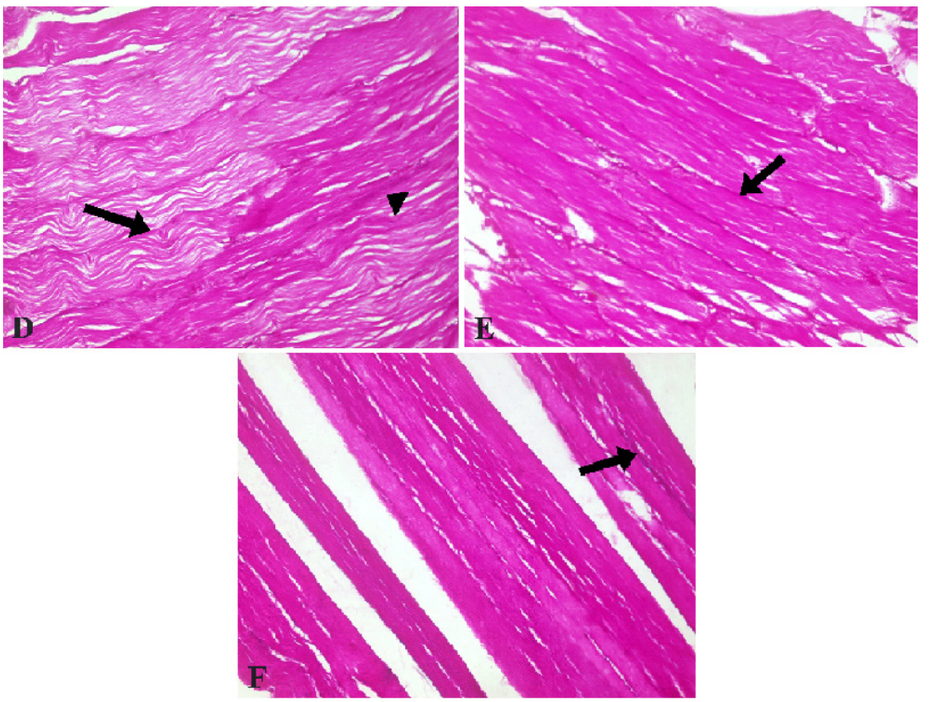

Fig. 4: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle stained by PAS (x 400).
D) Prozac (PR) group: decrease in PAS +ve reaction with faint distribution in some skeletal muscle fibers (→) While others were still normal (►). E) PR+MLD group: some muscle fibers appeared with moderately increased PAS +ve reaction (→). F) PR+MHD group: nearly normal of PAS +ve reaction (→).

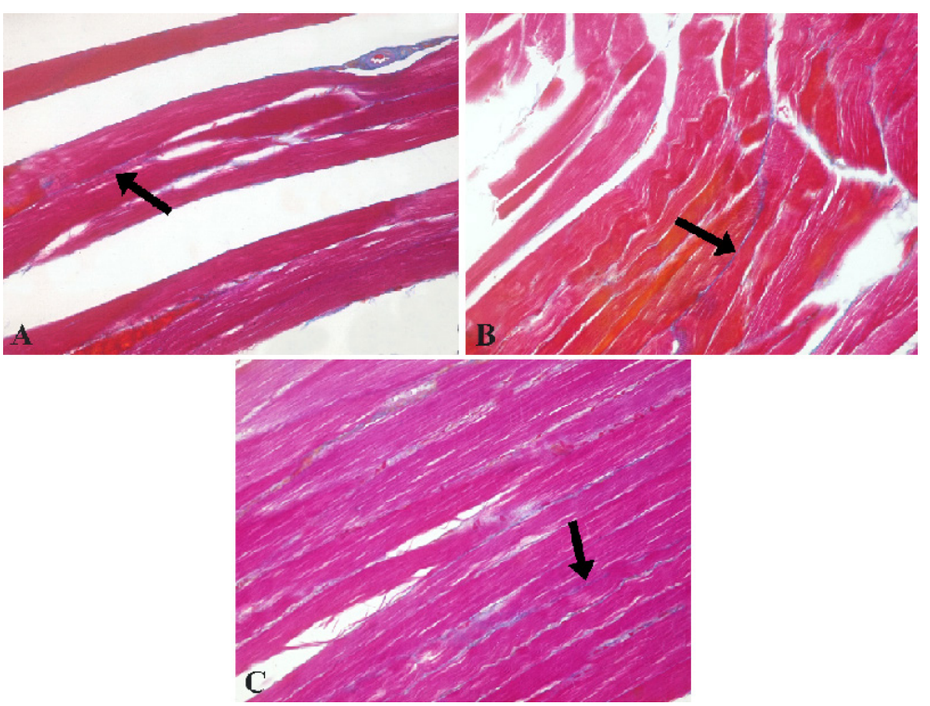

Fig. 5: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle stained by Mallory's trichrome (x400)
A, B&C) Control, MLD & MHD groups respectively: some blue-stained collagen fibers situated in-between the muscle fibers (→).

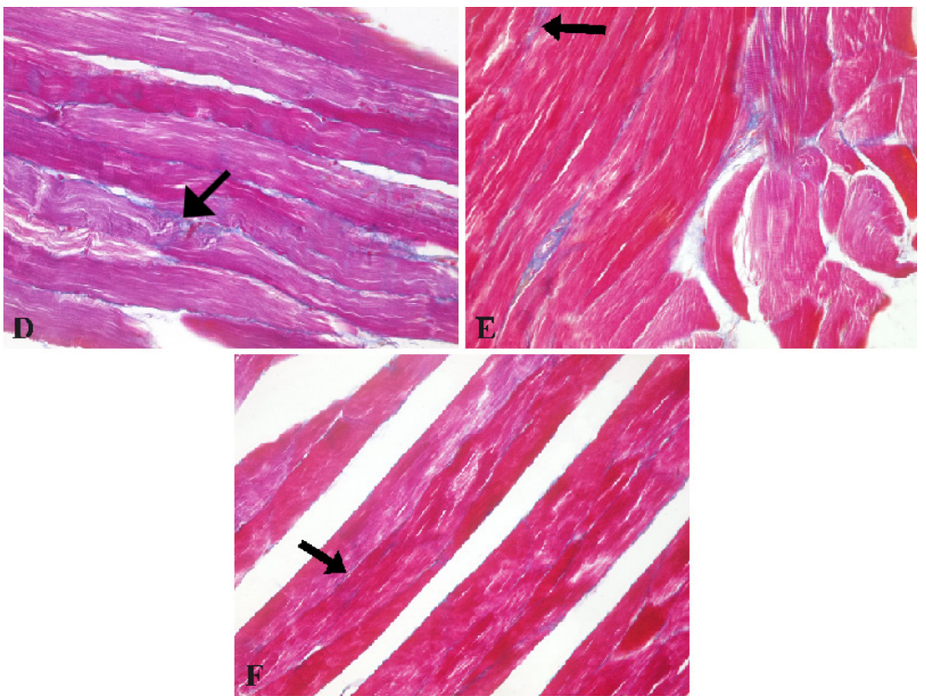

Fig. 6: Effect of morin on fluoxetine (Prozac)-induced structural changes of mice skeletal muscle stained by Mallory's trichrome (x400)
D) Prozac (PR) group: markedly increased deposition of collagen fibers in-between the muscle fibers (→). E) PR+MLD group: moderate amounts of collagen fibers (→). F) PR+MHD group: some collagen fibers situated in-between the muscle fibers (→).

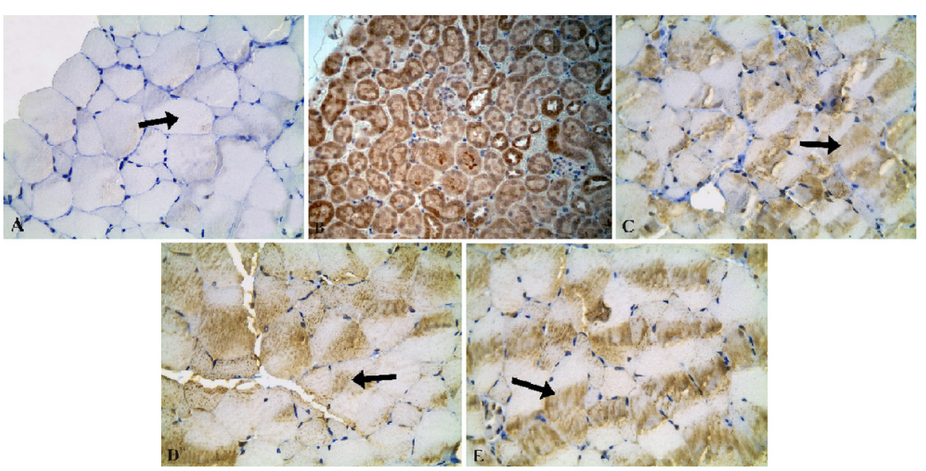

Fig. 7: Effect of morin on fluoxetine (Prozac)-induced cytochrome C immunohistochemical changes of mice skeletal muscle (x400).
A) Negative control: no cytoplasmic immunohistochemical reaction (→).
B) Kidney positive control showed positive brownish cytoplasmic reactions of the renal cortical structures. C, D&E) Control, MLD&MHD groups respectively: showed skeletal muscle fibers with mild cytoplasmic relations for cytochrome C (→).

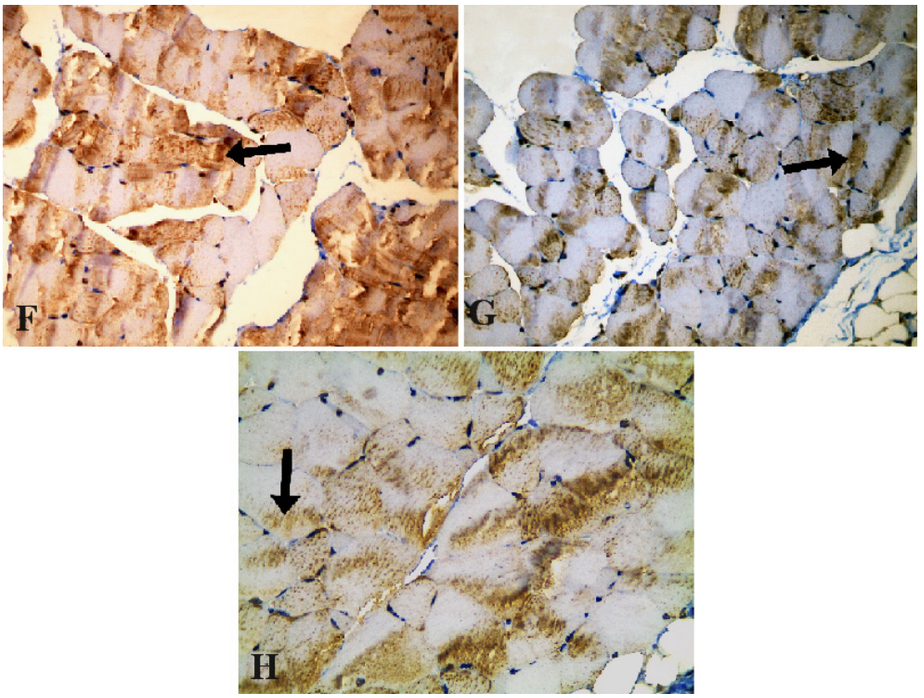

Fig. 8: Effect of morin on fluoxetine (Prozac)-induced cytochrome C immunohistochemical changes of mice skeletal muscle (x400).
F) Prozac (PR) group showed increased cytoplasmic immunohistochemical reaction (→). G) PR+MLD group showed moderate cytoplasmic immunostaining for cytochrome C (→). H) PR+MHD group showed skeletal muscle fibers with mild cytochrome C cytoplasmic (→).

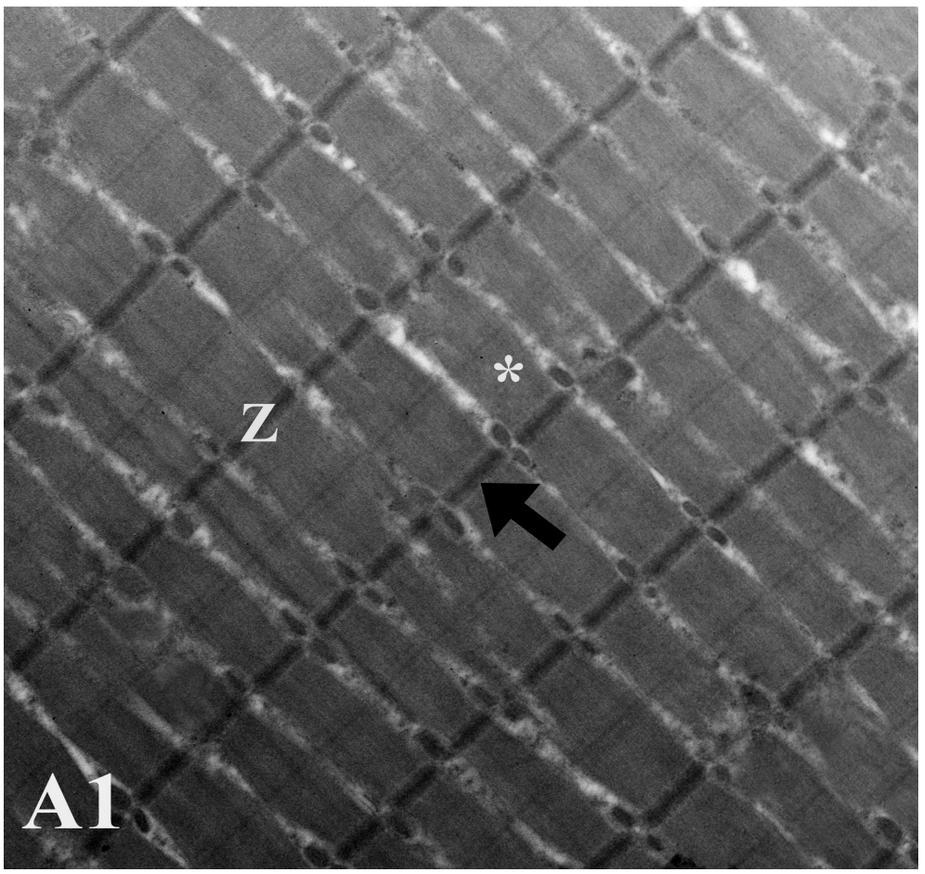

Fig. 9A1: Effect of morin on fluoxetine (Prozac)-induced EM changes of the mice skeletal muscle of the control group showed, myofibrils situated in the sarcoplasm parallel to the long axis of the myofibers with Z-line at the middle of the light band (Z), alternating light (→) and dark (*) bands. (x 2000).

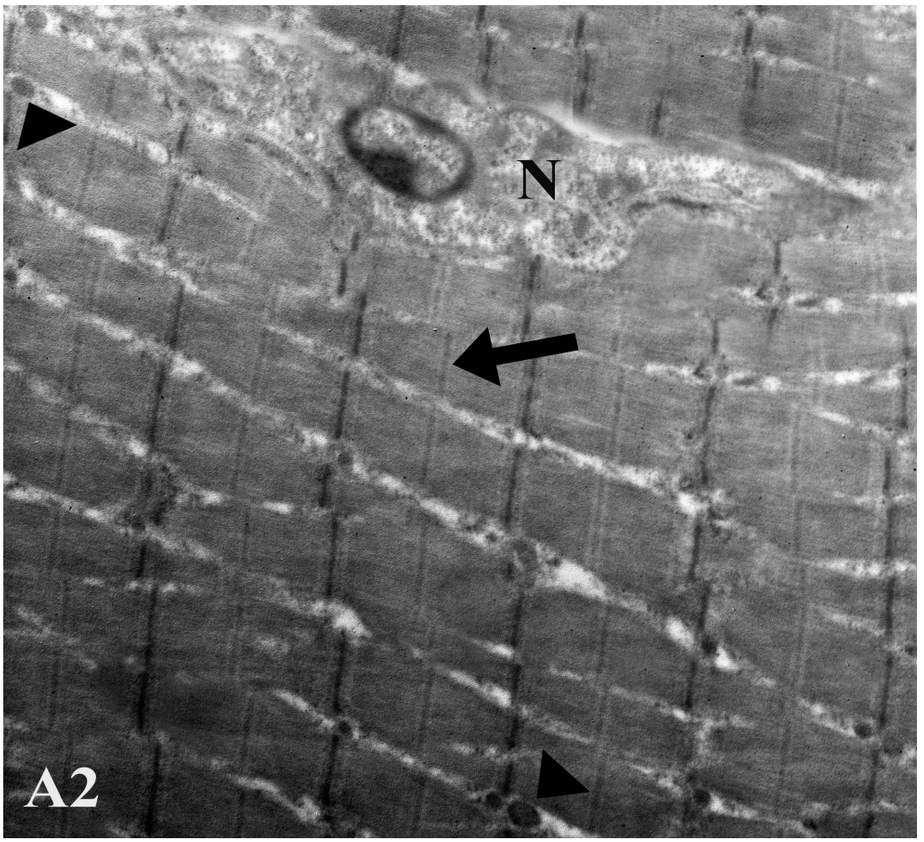

Fig. 9A2: Effect of morin on fluoxetine (Prozac)-induced EM changes of the mice skeletal muscle of the control group showed, H zone bisected by M line (→), mitochondria (►), and flat euchromatic nucleus (N). (x 2000).

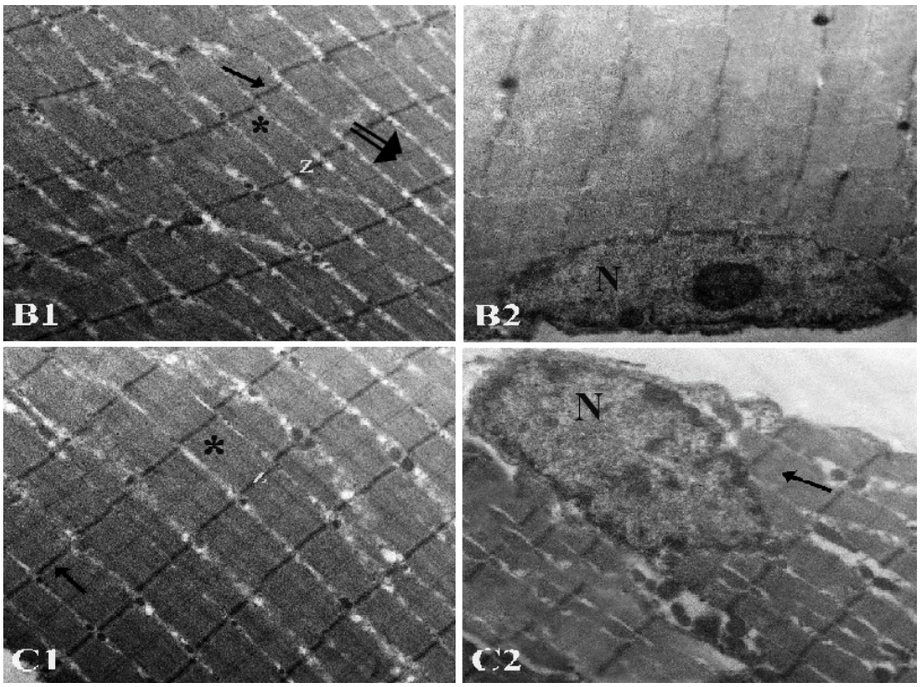

Fig. 10: Effect of morin on fluoxetine (Prozac)-induced EM changes of the mice skeletal muscle (x2000).
B1) MLD group: myofibrils situated in the sarcoplasm parallel to the long axis of the myofibers with Z-line at the middle of the light band (Z), alternating light (→) and dark (*) bands and H zone bisected by M line (double arrow). B2) MLD group: flat euchromatic peripheral nucleus (N). C1) MHD group: myofibrils situated in the sarcoplasm parallel to the long axis of the myofibers with Z-line (Z), alternating light (→) and dark (*) bands. C2) MHD group: H zone bisected by M line (→), and euchromatic peripheral nucleus (N).

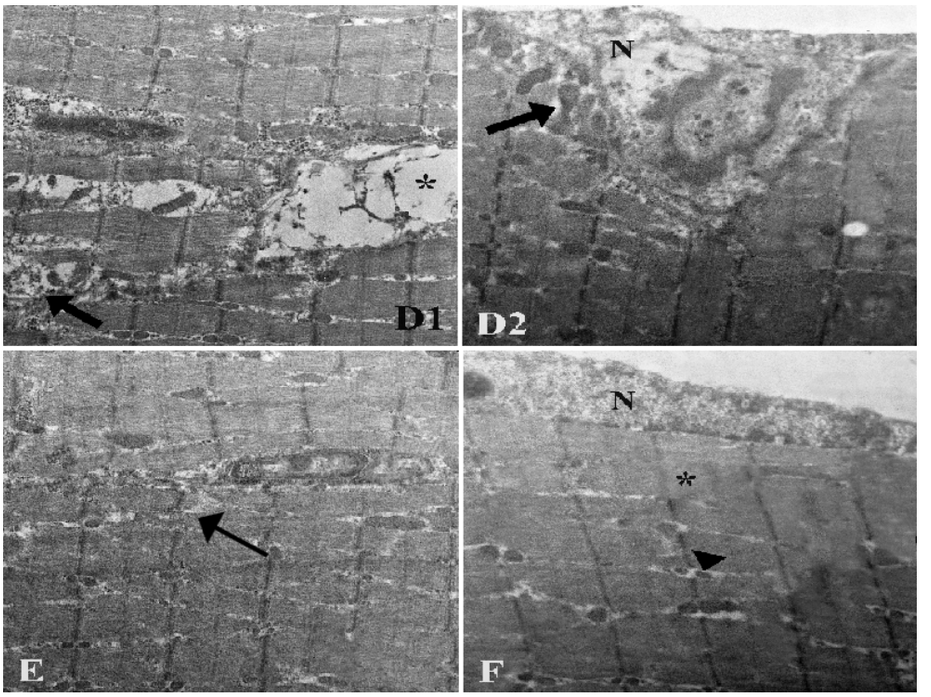

Fig. 11: Effect of morin on fluoxetine (Prozac)-induced EM changes of the mice skeletal muscle (x2000).
D1) PR group: disarranged myofibrils (→) with wide separation (*). D2) PR group: accumulations of mitochondria of different sizes and shapes (→), and degenerated nucleolar membrane and vacuolated nucleoplasm (N). E) PR+MLD group: mild improvement with disarranged fibrils (→). F) PR+MHD Group: nearly normal myofibrils (*), mitochondria (►), and nucleus (N).

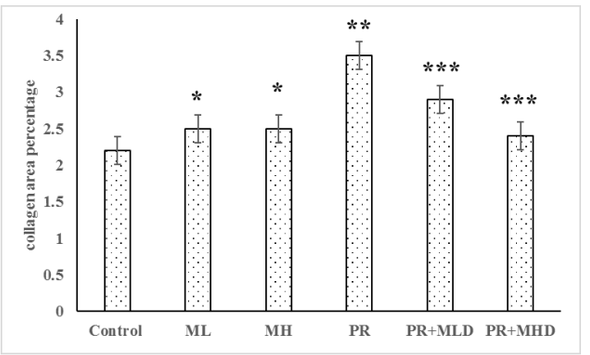

Fig. 12: Effect of morin on fluoxetine (Prozac)-induced changes on the collagen area percentage of mice skeletal muscle.
Values presented as means ± SD. Control, ML (morin low dose) & MH (morin high dose) (* $P > 0.05$ compared to the control group). PR (Prozac group) (** $P < 0.05$ compared to control). PR+MLD & PR+MHD (*** $P < 0.05$ compared to PR group).

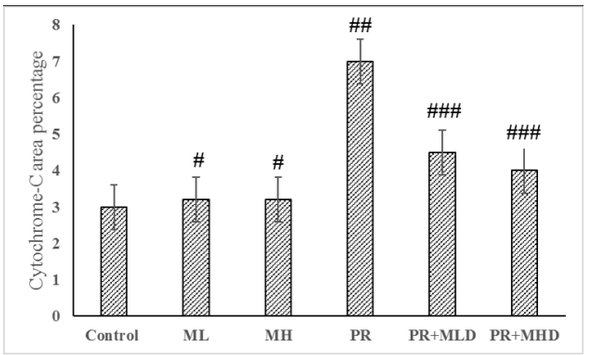

Fig 13: Effect of morin on fluoxetine (Prozac)-induced changes on the area percentage of cytochrome-c immunostaining of mice skeletal muscle. Values presented as means ± SD. Control, ML (morin low dose) & MH (morin high dose) (# $P > 0.05$ compared to the control group). PR (Prozac group) (## $P < 0.05$ compared to control). PR+MLD & PR+MHD (### $P < 0.05$ compared to PR group).

DISCUSSION

Fluoxetine (Prozac) has been used for the treatment of depression. It was believed that it was safe without inducing any organ side effects. Also, different researches proved the adverse effect of fluoxetine on the cardiovascular system as well as blood vessels. This was through its effect on the Na+/K+ voltage gated channels as well as the level of the intracellular Ca+. On the other hand, the present study revealed that fluoxetine (PR) has an effect on the skeletal muscle with different structural changes proved by light and electron microscopic study[15].

The light microscopic results of the Prozac (PR) group at the present research revealed muscle fiber vacuolations, splitting, mononuclear cell infiltrations and small, dark shaped central nuclei at H&E sections, decreased PAS +ve reaction with faint distribution in some skeletal muscle fibers, increased collagen fibers as well as increased cytochrome- C cytoplasmic immunohistochemical reactions. In addition, the morphometric results revealed significant increase in the collagen area percentage, besides the significant increase in the area percentage of Cytochrome-C immunostaining.

The free radical-mediated actions of Prozac together with the increased oxidative phosphorylation were reported to be a cause for its degenerative effects. These could affect the production of ATP from mitochondria so, the energy needed for the different intracellular activities will be affected like electron transport as well as the metabolism of lipids, carbohydrates and proteins. Consequently, muscle fiber disarrangement and the decreased PAS +ve reaction[16]. Additionally, PR could lead to oxidative stress with the damage of carbohydrates, lipids, DNA and proteins and it has been postulated that oxidative stress initiates different inflammatory conditions[16].

This because protein oxidations lead to the release of different inflammatory signals molecules and that recognized as an inflammatory signal. These inflammatory stimuli lead to the release of PRDX2, a ubiquitous redox-active intracellular enzyme[17]. After its releasing, it acts as a redox-dependent inflammatory mediator which in turn stimulates macrophages for the production and the release of different cytokines like TNF-α triggering the inflammatory response with mononuclear cellular infiltrations at H&E stained sections[18].

The present research revealed significantly increased collagen fibers which could be explained by the fact that TNF-α induce tissue fibrosis by activating the NF-κB signaling pathways which is also has been proved by previous study made on the liver by yang *et al*., (2015)[19]. Moreover, the decrease in PAS +ve reaction with faint distribution in some skeletal muscle fibers in the PR group might be attributed to the defect in lipid as well as glycogen content[20].

Cytochrome-C is a small hemeprotein release from the mitochondria initiating apoptosis. The significantly increased cytoplasmic reaction for Cytochrome-C in the present work was attributed to the released TNF-α[20]. Additionally, the released free radicals lead to the initiation of oxidative stress consequently, an important components of the cell will be oxidized losing their ability to function normally. Also, this oxidative damage could lead to cell death[16].

The EM results of PR group of the present research showed disarranged myofibrils with wide separation, accumulations of mitochondria of different sizes and shapes, and degenerated nucleolar membrane with vacuolations at the nucleoplasm.

The accumulations of mitochondria of different sizes and shapes that was observed as an EM changes in the present research could be attributed to the down-regulation of PR to the Na+/Ca+2 exchange level leading to increased Ca+2 concentration at the sarcoplasmic reticulum as well as cytoplasm with an overload-induced Ca+2 in the mitochondria. Consequently, swollen mitochondria with different sizes and shapes[21].

Moreover, the degenerated nucleolar membrane could be attributed to the PR- associated free radical release with oxidative membrane damage as well as lipid peroxidation[16].

Flavonoids are natural products found in fruits, herbs, and nuts. They are regularly taken as a part of daily human diet. They gained attention recently due to their broad pharmacological actions. Morin is a natural flavonoid present in Moraceae and can also be found in white berry, cranberry branches, guava leaves, onion, and apple. It is also widely distributed in tea, cereal grains and a variety of fruits and vegetables[22].

The present research proved the protective effect of morin against fluoxetine induced skeletal muscle degenerative changes. This was proved by the nearly normal histological structure at H&E stained sections, significantly decreased collagen fibers and its mean area percentage as well as cytochrome C reaction to be like control. Also, near normal PAS +ve reaction besides the nearly normal EM findings.

It was reported that morin could inhibit the production of ROS at tissues and reduced the released apoptotic factors from mitochondria, increases Bcl2 gene expression and antioxidant genes, and thus could protect the tissue cells from injury, dysfunction and apoptosis[23].

Moreover, morin has various pharmacological properties such as antioxidant, anti-inflammatory and anti-stress effects. It inhibits the release of the inflammatory markers like TNF-α and IL-1β with the inhibited NF-κB stimulation so, prevent tissue inflammation and apoptosis[10].

The antioxidant action of morin could be due to its beneficial role on the mitochondria with the generation of ATP[24]. It also scavenges the free radicals through rapid

donation of hydrogen atom to radicals and form complexes with iron ions with the formation of metal ion chelates. Additionally, it scavenges superoxide, H_2O_2 beside the restoration of the antioxidant levels. Consequently, restoration of the cell integrity and functions[25].

CONCLUSION

From the previously mentioned data, it could be concluded that fluoxetine induced degenerative changes on the mice skeletal muscle. Moreover, morin dose dependently could improve these changes.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير مورين علي التغيرات التركيبية التى يسببها فلوكستين (بروزاك) للعضلات الهيكلية فى الجرذان: دراسه مجهرية ضوئيه وإلكترونيه

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مقدمة: فلوكستين(بروزاك)هو دواء مضاد للإكتئاب له تأثير سلبي على الأنسجة مع إستخدامه لفترات طويلة. المورين هو مادة البوليفينول الطبيعية ذات الفوائد الصحية المختلفة بسبب تأثيراتها المضادة للأكسدة والمضادة للإلتهابات.

الهدف: صمم هذا العمل لتقييم تأثير المورين على التغيرات التركيبية التي يسببها فلوكستين (بروزاك) للعضلات الهيكلية للجرذان عن طريق الدراسة المجهرية الضوئية والإلكترونية.

المواد والطرق: تم إستخدام ٣٥ جرذ (٥٠-٧٠ جم) لمدة ٣ أسابيع وقسمت إلى ٦ مجموعات (٥ جرذان لكل مجموعة) ؛ المجموعة ١: مجموعة التحكم ،المجموعة ٢: مجموعة جرعة منخفضة من مورين مورين ٣٠ مغ / كغ / يوم مذاب شفوياً في الماء المقطر ،المجموعة ٣: مجموعة مورين ذات الجرعة العالية : مورين ١٠٠ مجم / كجم / يوم مذاب في الماء المقطر ، المجموعة ٤: مجموعة بروزاك : الحقن داخل الصفاق ٠,٠٦ مجم بروزاك/جرذ/اليوم في محلول ملحي معقم. المجموعة ٥: جرعة منخفضة من مورين ٣٠ مغ / كغ / يوم ومعالجة سابقاً قبل البروزاك, المجموعة ٦: جرعة عالية من لمورين ٣٠ مجم / كجم / يوم عن طريق الفم قبل البروزاك. هذا وقد تم أخذ الجرعات بالفم عم طريق أنبوبة داخل المعدة.

النتائج: أظهرت مجموعة بروزاك وجود فجوات ، وإرتشاح خلوى إلتهابى وأنوية صغيرة داكنة النواة ومركزية فى شرائح الهيماتوكسلين والإيوسين. وإنخفاض فى تفاعل PAS , ، كذلك زيادة ملحوظة في ألياف الكولاجين ، وزيادة ملحوظة في التفاعل السيتوبلازمى للسيتوكروم-س ، فصل واسع فى الليفات العضلية ، وتراكمات من الميتوكوندريا بأحجام وأشكال مختلفة ، ووجود غشاء نووي متحلل وفجوات في سيتوبلازم النواة في الفحص المجهري الإلكتروني. أيضًا تم تحسين هذه التغييرات بالجرعات المختلفة للمورين.

الخلاصة: مورين بجرعاتها المختلفة يمكن أن تحمى التغيرات التركيبية التى يسببها فلوكستين (بروزاك) للعضلات الهيكلية في الجرذان.