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Curcumin improves atorvastatin-induced myotoxicity in rats: Histopathological and biochemical evidence

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Abstract
Atorvastatin is considered to be one of the most commonly used of all statins anti-hyperlipidemic drugs despite the fact that there is much controversy about its safety. Its therapeutic use becomes severely limited by the hazards of inducing myotoxicity. Curcumin is one of the safe spices that have chemoprotection and cytoprotection effects against endogenous and exogenous noxious stimuli. This study investigates the effect of curcumin on atorvastatin sub-chronic use-induced myotoxicity in rats by the assessment of serum creatinine phosphokinase, lactic acid dehydrogenase, myoglobin, troponin, potassium, creatinine, and histopathological changes of skeletal, smooth, and cardiac muscles by light and electron microscope examination. Eighty adult albino rats were divided into four groups; each group consists of 20 rats. The control group received water, the second group received atorvastatin, the third group received curcumin, and the fourth group received curcumin with atorvastatin for 90 days by gastric gavage. The prolonged use of atorvastatin induced significant abnormalities of all myotoxicity biomarkers associated with histopathological and ultrastructural changes in the different types of the muscles. Co-administration of curcumin with sub-chronic use of atorvastatin led to an improvement in myotoxicity manifestations.

Keywords
atorvastatin, curcumin, myotoxicity

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Introduction

Atorvastatin is believed to be one of the statin drugs that decreases the total cholesterol, bad cholesterol (LDL), and triglycerides levels in the blood which cause coronary artery disease. Furthermore, atorvastatin also increases the useful cholesterol level (HDL) that protects against coronary artery disease.[1] However, the safety of atorvastatin is still controversial and its therapeutic use becomes severely limited due to the induced myopathy that ranges from myalgia to apoptosis and necrosis in the atorvastatin users, yet it was approved by FDA in December 1996.[2] In addition to this, there is no devised combination therapy with atorvastatin to diminish its possible side effects such as myotoxicity, granting the fact that the combination of atorvastatin with other drugs may be used to improve its therapeutic effect in decreasing the risk of heart diseases.[3]

Spices are commonly used as food additives to enhance the taste and the flavor of food. They have

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medicinal uses because of their discovered therapeutic effects. Curcumin is considered one of the safest spices that has been approved by the Food and Drug Administration (FDA) in the USA, the Natural Health Products Directorate of Canada, the Joint FAO/ WHO Expert Committee on Food Additives of the Food and Agriculture Organization/ World Health Organization.[4] It is a yellow-orange dye that is extracted from the turmeric spice which has anti-inflammatory, antioxidant, anti-microbial, and anti-carcinogenic effects beside its chemoprotection and cytoprotection effects against endogenous and exogenous noxious stimuli, and its efficacy for treating any renal, myocardium, and nervous tissue injury.[5]

Therefore, the current study aims to investigate the effect of curcumin on atorvastatin sub-chronic use-induced myotoxicity in rats by the assessment of the levels of creatinine phosphokinase, lactic acid dehydrogenase, troponin I, myoglobin, potassium, creatinine, oxidative stress parameters, and an evaluation for morphological and ultra-structural changes in cardiac, diaphragm, and extensor digitorum longus muscles.

Materials and methods

Eighty healthy adult albino rats weighing 200–300 g were obtained from the animal house of King Abdel Aziz University, Jeddah. The rats were exposed to 12 h day and night cycles and were fed with water and the standard rat pellets during the experimental period. They were divided into four groups, each group comprising of 20 rats. The first control group received distilled water (0.1 mL) while the second group received 50 mg/kg/day of atorvastatin (1% of LD50 "5000mg/kg") dissolved in distilled water.[6] The third group received 200–300 mg/kg/day of curcumin dissolved in distilled water,[7] while the fourth group received 50 mg/kg/day of atorvastatin dissolved in distilled water with 200–300 mg/kg/day of curcumin which was also dissolved in distilled water. The daily administration of the distilled water, atorvastatin, and curcumin were done by gastric gavage for 90 days. The atorvastatin was available in 40 mg tablet form that was manufactured by Egyptian International Pharmaceutical Industries Co. (EIPICO, 10th of Ramadan City, Egypt) while Curcumin was purchased from Sigma Chemical Co.

Blood sample collection

On the last day of the experiment, the rats were anaesthetized by diethyl ether. The blood samples were collected from the orbital sinus, using the covered test tubes. It was then left at room temperature for 15–30 min to clot. The samples were centrifuged at 2000 rpm at 4°C for 10 min to remove the clot and to separate the serum sample that was stored at −20°C until the assay. Measurements of troponin I and creatinine phosphokinase were done using an Elecsys Analyser by the troponin I STAT third generation and creatinine phosphokinase STAT methods (Roche Diagnostics, Tutzing, Germany). These assays were based on electrochemiluminescence immunoassay technology (ECLIA) using two mouse monoclonal antibodies in a sandwich format. It was a two-step assay. They were done on Elecsys 1010 and 2010 immunoassay analyzers according to the manufacturer's instructions (Roche Diagnostics, Tutzing, Germany). Myoglobin concentrations were determined using the respective Stratus fluorometric enzymeimmunoassay (Dade Behring, Newark, DE, USA).[8] The serum creatinine level was determined by the routine colorimetric methods using the commercial kits and quantified on the clinical biochemistry autoanalyzer.[9] Determination of the serum level of lactic acid dehydrogenase and potassium were performed by standard spectrophotometric analysis using diagnostic kits.[10]

Histopathological studies

After 24 h, following the last administration of atorvastatin and curcumin, the rats were sacrificed after being excessively anesthetized. Tissue samples of the extensor digitorum longus muscle, cardiac muscle, and diaphragm were dissected from the rats of the four groups and then it was fixed in 10% neutral buffered formalin. The fixed specimens were trimmed, washed, and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4–6 μm thickness, and stained by Mallory stain, hematoxylin and eosin (H&E). The muscular histological slides were examined and scored under a light microscope by a blinded pathologist to quantify the experimental groups for the extent of muscular histopathological changes for each muscle-damaged parameter

Table 1. Comparison between the effects of atorvastatin used alone or with curcumin on mean ± SD of different biomarkers levels of myotoxicity in the rats.

Group / parameter	First (mean ± SD)	Second (mean ± SD)	Third (mean ± SD)	Fourth (mean ± SD)	F
Troponin-I (ng/mL)	0.0025 ± 0.001	0.7620 ± 0.014*	0.0045 ± 0.005	0.1545 ± 0.016†	2.220
CPK (U/L)	269.1 ± 35.872	688.7 ± 86.937*	220.45 ± 9.512	335.80 ± 9.51†	398.839
Myoglobin (ng/ml)	127.2 ± 3.792	299.0 ± 7.145*	127.75 ± 4.025	147.5 ± 6.022†	467.74
Creatinine (mg/dl)	0.77 ± 0.114	0.94 ± 0.172*	0.80 ± 0.121	0.64 ± 0.081†	3.4350
LDH (U/L)	142.35 ± 36.552	843.4 ± 59.102*	135.45 ± 40.066	227.5 ± 10.008†	1415.83
Potassium (meq/L)	4.1 ± 0.361	4.9 ± 0.200*	3.8 ± 0.125	3.9 ± 0.323†	73.537

Number per group: 20.
CPK, creatinine phosphokinase; LDH, lactic acid dehydrogenase; SD, standard deviation.
The first group (control) received the distilled water. The second group received atorvastatin only. The third group received curcumin only. The fourth group received curcumin with atorvastatin.
*P <0.001 (significant difference in comparison with the first group).
†P <0.001 (significant difference in comparison with the second group).

(myofibers degeneration, cell infiltration, pyknotic nuclei, and collagen fibers distribution). This was done by using a scoring scale of 0–2 for each parameter that represented normal conditions (0), moderate damages (1), and severe muscular damages (2). Three photographs were taken of the three different parts of each muscle sample and analyzed individually to quantify each muscular histopathological parameter.[11]

Ultrastructure studies were performed by using the transmission electron microscope, the tissue specimens of the muscles were prepared by soaking and fixating the specimens in 2.7% glutaraldehyde solution at 0.1 M phosphate buffer for 1.5 h at 4°C. It was then washed in 0.15 M phosphate buffer (pH 7.2) and post-fixed in 2% osmic acid solution at 0.15 M phosphate buffer for 1 h at 4°C. Dehydration was done by using acetone and then by the inclusion which occurred in the epoxy soaked resin Epon 812. The blocks were cut with an ultramicrotome type LKB at 70 nm thickness. The sections were differentiated with the solutions of the uranyl acetate and lead citrate for the analysis by an electron microscope.[12]

Tissue preparation

A total of 500 mg of muscular tissue was homogenized in 4 mL of buffer solution of phosphate buffered saline at PH 7.4 whereby the homogenates were centrifuged at 10,000 × g for 15 min at 4°C. The resultant supernatant was used for oxidative stress parameters assay such as malondialdehyde, catalase, superoxide dismutase, glutathione peroxidase, and glutathione.[13]

Statistical analysis

Statistical analysis was performed using SPSS version 17. The data was expressed as mean ± SD and the analysis was performed by using one-way ANOVA and post-hoc multiple comparisons tests (TUKEY) to investigate the difference between the biomarker levels among the different groups where a P value of 0.05 was considered statistically significant.

Ethical considerations

The most appropriate animal species were chosen for this research. Promotion of high standard care and animal well-being were exercised at all times. An appropriate sample size of animals for the experiment was calculated for using the fewest number of animals to obtain the valid results statistically. Painful procedures were performed under anesthesia to avoid any distress and pain that could be inflicted on the animals. Our standards of animal care and administration are consistent with the requirements and standards of international laws and regulations.

Results

Biochemical findings

Table 1 represents mean ± SD values of biochemical markers in the rats. Mean ± SD values of Troponin I in the control group which received distilled water, in the second group which received atorvastatin only, in the third group which received curcumin, and in the fourth group which

Table 2. Comparison between the effects of atorvastatin used alone or with curcumin on mean ± SD of oxidative stress parameters in the rats.

Group / parameter	First (mean ± SD)	Second (mean ± SD)	Third (mean ± SD)	Fourth (mean ± SD)	F
Catalase	30.72 ± 1.55	11.42 ± 2.73*	35.18 ± 1.42	32.77 ± 2.51†	554.411
Peroxidase	12.41 ± 1.21	7.69 ± 1.15*	11.89 ± 1.12	13.33 ± 1.19†	154.754
GSH	90.22 ± 1.41	59.52 ± 3.85*	92.02 ± 3.13	91.42 ± 2.76†	437.557
MDA	20.71 ± 0.59	54.3 ± 2.57*	21.20 ± 2.47	22.65 ± 1.94†	1.011
Superoxide dismutase	24.31 ± 1.73	14.21 ± 0.92*	23.38 ± 2.57	23.53 ± 1.55†	186.217

Number per group: 20.
GSH, glutathione, MDA, malondialdehyde; SD, standard deviation.
The first group (control) received the distilled water. The second group received atorvastatin only. The third group received curcumin only. The fourth group received curcumin with atorvastatin.
*P <0.001 (significant difference in comparison with the first group).
†P <0.001 (significant difference in comparison with the second group).

received curcumin with atorvastatin are as follows: 0.0025 ± 0.001, 0.7620 ± 0.014, 0.0045 ± 0.005, and 0.1545 ± 0.016 ng/mL, respectively. The value of F indicates that the difference between the groups was 2.220 with statistical significance at P <0.001. Mean ± SD values of creatinine phosphokinase (CPK) in the control, second, third, and fourth groups were 269.1 ± 35.872, 688.7 ± 86.937, 220.45 ± 9.512, and 335.80 ± 9.51 U/L, respectively. The value of F indicates that the difference between the groups was 398.839 and statistical significance was at P <0.001. Mean ± SD values of myoglobin in the control, second, third, and fourth groups were 127.2 ± 3.792, 299.0 ± 7.145, 127.75 ± 4.025, and 147.5 ± 6.022 ng/mL, respectively. The value of F indicates the difference between the groups was 467.74 and statistical significance was at P <0.001. Mean ± SD values of creatinine in the control, second, third, and fourth groups were 0.77 ± 0.114, 0.94 ± 0.172, 0.80 ± 0.121, and 0.64 ± 0.081 mg/dl, respectively. The value of F indicates the difference between the groups was 3.4350 and statistical significance was at P <0.001. Mean ± SD values of lactic acid dehydrogenase (LDH) in the control, second, third, and fourth groups were 142.35 ± 36.552, 843.4 ± 59.102, 135.45 ± 40.066, and 227.5 ± 10.008 U/L, respectively. The value of F indicates the difference between the groups was 1415.83 and statistical significance was at P <0.001. Mean ± SD values of potassium (K) in the control, second, third, and fourth groups were 4.1 ± 0.361, 4.9 ± 0.200, 3.8 ± 0.125, and 3.9 ± 0.323 meq/L, respectively. The value of F indicates the difference between the groups was 73.537 while statistical significance was at P <0.001.

Oxidative stress parameters

Table 2 shows that there is a statistical significant decrease in the values of catalase, peroxidase, glutathione, and superoxide dismutase in the second group (atorvastatin) in comparison to the control group, while these values are increased statistically significantly in the fourth group (atorvastatin with curcumin) in comparison to the second group (atorvastatin). Conversely, the value of malondialdehyde (MDA) is increased statistically significantly in the second group (atorvastatin) in comparison with the control group and then it is decreased statistically significantly in the fourth group (atorvastatin with curcumin) in comparison to the second group (atorvastatin).

Histopathological findings

Skeletal muscle (extensor digitorum longus) examination by the light microscope. The examination of the longitudinal section of the extensor digitorum longus muscle in the rats of the first control group showed normal structure (Figure 1a) with normal collagen fiber distribution in the endomysium around the blood vessels (Figure 2a). The extensor digitorum longus muscle in the rats of the second group, which received atorvastatin only, showed splitting myofibers with fragmentation of its sarcoplasm, cellular infiltration, dense central nuclei, and necrotic nuclei remnants (Figure 1b) with excessive collagen fibers around the affected myofibers (Figure 2b). The longitudinal section of the extensor digitorum longus muscle in the rats of the third group, which received curcumin, showed normal cylindrical, parallel, and non-branching muscle

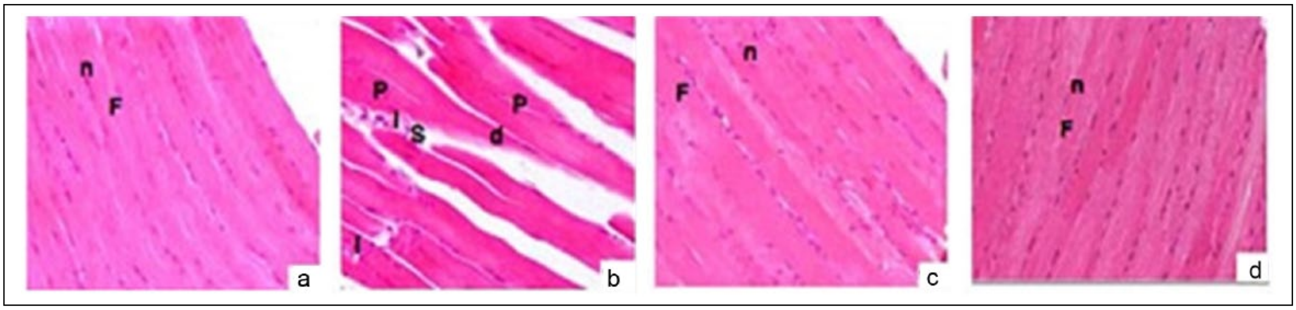

Figure 1. (a) A photomicrograph of the longitudinal section in the control rat extensor digitorum longus muscle shows normal non-branching muscle fibers (F) and multiple elongated vesicular peripheral nuclei (n). H&E ×400. (b) A photomicrograph of the longitudinal section in the second group rat extensor digitorum longus muscle shows degenerated muscle fibers (d) with myofibers splitting (s) and cellular infiltration (I), pyknotic nuclei (p), and the remnants of the degenerated nuclei (f). H&E ×400. (c) A photomicrograph of the longitudinal section in the third group rat extensor digitorum longus muscle shows non-branching muscle fibers (F) and multiple elongated vesicular peripheral nuclei (n). H&E ×400. (d) A photomicrograph of longitudinal section in the fourth group rat extensor digitorum longus muscle shows nearly the normal appearance of intact muscle fibers (F) and multiple nuclei (n). H&E ×400.

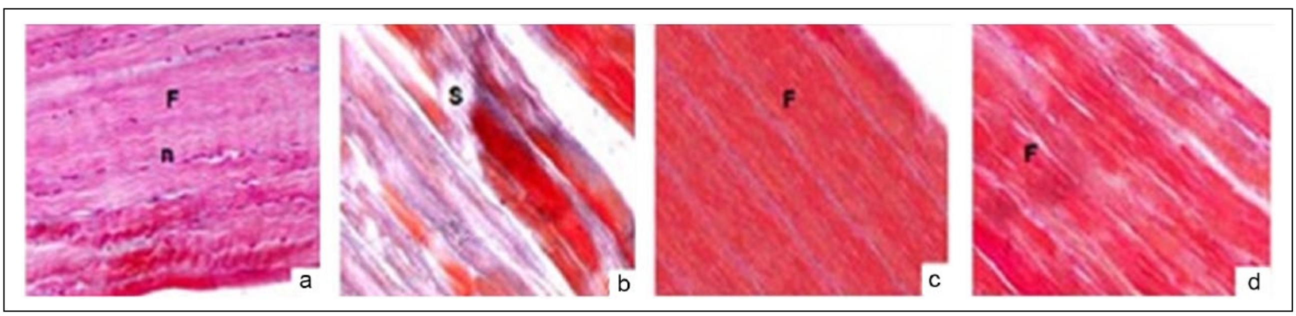

Figure 2. (a) A photomicrograph of the longitudinal section in the control rat extensor digitorum longus muscle shows normal distribution of blue-stained collagen fibers around non-branching muscle fibers (F). Mallory ×400. (b) A photomicrograph of the longitudinal section in the second group rat extensor digitorum longus muscle shows excessive blue collagen fibers around the splitting muscle fibers (s). Mallory ×400. (c) A photomicrograph of the longitudinal section in the third group rat extensor digitorum longus muscle shows few collagen muscle fibers (F). Mallory ×400. (d) A photomicrograph of the longitudinal section in the fourth group rat extensor digitorum longus muscle shows nearly few normal collagen muscle fibers (F). Mallory ×400.

fiber bundles and multiple peripheral nuclei (Figure 1c) with few collagen fibers in the endomysium around the blood vessels (Figure 2c). The extensor digitorum longus muscle of the fourth group of rats, which received curcumin with atorvastatin, showed an injury improvement and normal appearance of muscle fibers simulating the control. The third group showed evidence of small areas of cellular infiltration (Figure 1d) and few collagen fibers in the endomysium (Figure 2d).

Skeletal muscle (extensor digitorum longus) examination by the transmission electron microscope

The ultrastructure of the extensor digitorum longus muscle in the rats of the first control group showed normal sarcomeres, sarcolemma, oval nuclei, and mitochondria (Figure 3a). The extensor digitorum longus muscle in the rats of the second group, which received atorvastatin only, showed marked accumulation of mitochondria in the subsarcolemmal and intermyofibrillar spaces with unsystematic and degenerated parts of myofibrils with the partial loss of myofilaments and vacuolation in mitochondria (Figure 3b). The ultrastructure of the extensor digitorum longus muscle in the rats of the third group, which received curcumin, showed the normal appearance of the myofibril arrangement parallel to the long axis with normal sarcomeres, oval nuclei, and mitochondria in subsarcolemmal area (Figure 3c). The extensor digitorum longus muscle of the fourth group of rats, which received curcumin

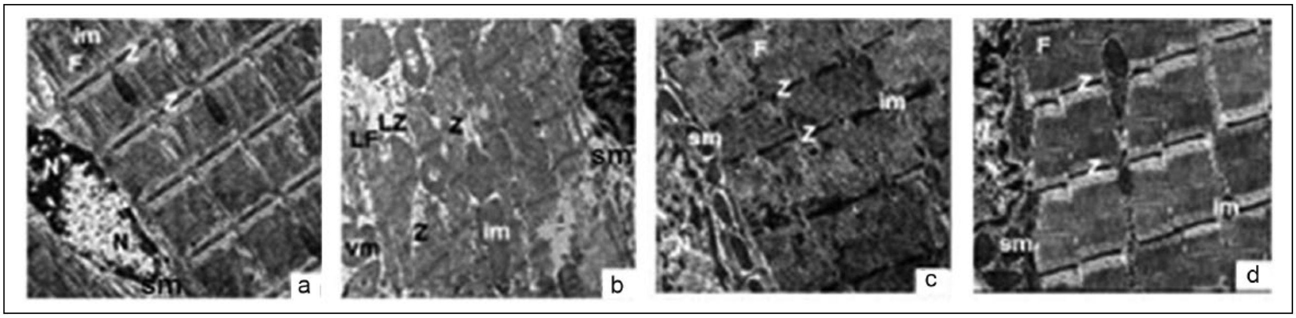

Figure 3. (a) An electron micrograph of longitudinal section in the control rat extensor digitorum longus muscle shows normal arrangement of myofibrils (F) with light and dark bands. Sarcomeres are found between two successive Z lines (Z) with oval nucleus (N) under the sarcolemma and subsarcolemmal mitochondria (sm) and intermyofibrillar mitochondria (im). TEM ×25,000. (b) An electron micrograph of longitudinal section in the second group rat extensor digitorum longus muscle shows disorganization and degeneration of myofibrils (LF), marked aggregation of mitochondria in subsarcolemmal space (sm), and intermyofibrillar space (im) with vacuolated mitochondria (vm) in the intermyofibrillar space and pyknotic nucleus (N). Disruption of Z line (Z) and loss of Z line (LZ). TEM ×25,000. (c) An electron micrograph of longitudinal section in the third group rat extensor digitorum longus muscle shows myofibrils (F), sarcomeres between two successive Z lines (Z), an oval nucleus (N) with subsarcolemmal mitochondria (sm) and intermyofibrillar mitochondria (im). TEM ×25,000. (d) An electron micrograph of longitudinal section in the fourth group rat extensor digitorum longus muscle shows a nearly normal appearance of myofibrils (F) and sarcomeres in-between two successive Z lines (Z) with normal oval nucleus (N), subsarcolemmal mitochondria (sm), and intermyofibrillar mitochondria (im). TEM ×25,000.

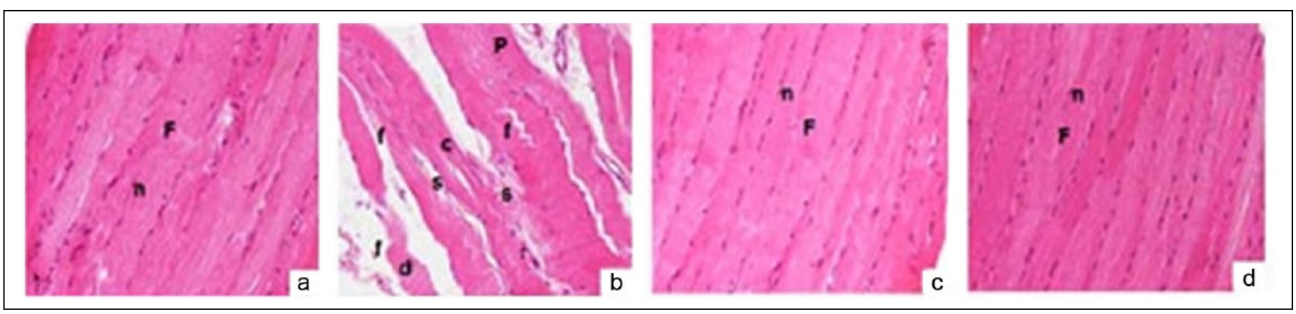

Figure 4. (a) A photomicrograph of the longitudinal section in the control rat diaphragm shows normal muscle fibers (F) and multiple vesicular nuclei (n). H&E ×400. (b) A photomicrograph of the longitudinal section in the second group rat diaphragm shows degenerated muscle fibers (d), myofibers splitting (s), pyknotic nuclei (p), and the remnants of the degenerated nuclei (f). H&E ×400. (c) A photomicrograph of the longitudinal section in the third group rat diaphragm shows normal muscle fibers (F) and multiple vesicular peripheral nuclei (n). H&E ×400. (d) A photomicrograph of the longitudinal section in the fourth group rat diaphragm shows nearly normal appearance of the intact muscle fibers (F) and multiple vesicular nuclei (n). H&E ×400.

with atorvastatin, displayed approximately the same ultrastructure of the first and third groups. The sarcomeres, nuclei, and mitochondria were approximately normal (Figure 3d) for these groups.

Smooth muscle (diaphragm) examination by the light microscope

The examination of the longitudinal section of the diaphragm in the rats of the first control group showed a normal structural appearance (Figure 4a) with normal collagen fiber distribution in the connective tissue (Figure 5a), but the diaphragm in the rats of the second group, which received atorvastatin only, showed splitting myofibers, focal areas of cellular infiltration, sarcoplasm fragmentation, and pyknotic nuclei (Figure 4b) with excessive collagen fiber distribution (Figure 5b). The longitudinal section of the diaphragm in the rats of the third group, which received curcumin, showed completely normal muscles fibers (Figure 4c) with few collagen fibers in the connective tissue (Figure 5c). The diaphragm of the fourth group of rats, which received curcumin with atorvastatin, showed an almost near to normal appearance of muscle fibers simulating the control and the third group with rare areas of cellular infiltration (Figure 4d) and few collagen fibers (Figure 5d).

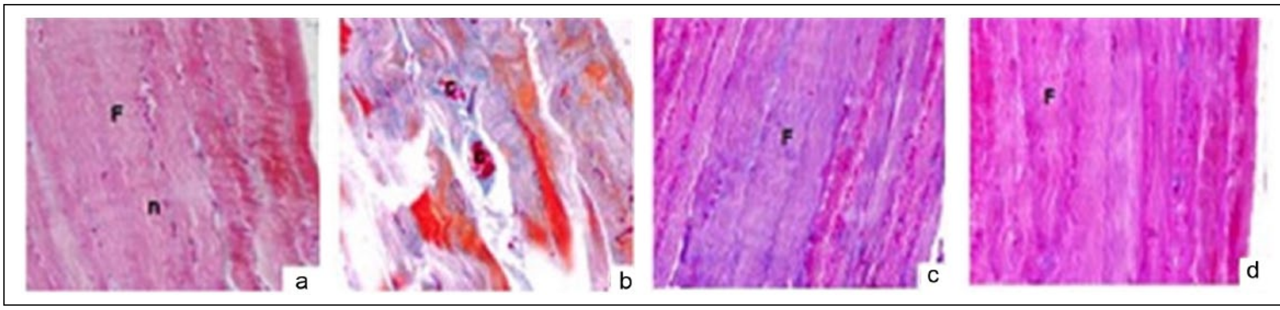

Figure 5. (a) A photomicrograph of the longitudinal section in the control rat diaphragm shows normal distribution of blue-stained collagen fibers around the muscle fibers (F) with many elongated peripheral nuclei (n). Mallory ×400. (b) A photomicrograph of the longitudinal section in the second group rat diaphragm shows excessive blue collagen fibers with blood vessels congestion (c). Mallory ×400. (c) A photomicrograph of longitudinal section in the third group rat diaphragm shows few collagen fibers (F). Mallory ×400. (d) A photomicrograph of longitudinal section in the fourth group rat diaphragm shows nearly normal few collagen fibers (F). Mallory ×400.

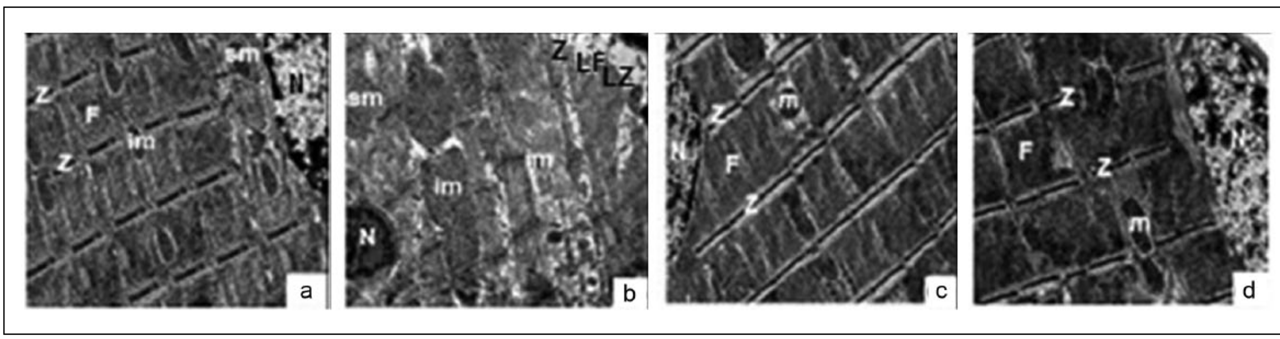

Figure 6. (a) An electron micrograph of the longitudinal section in the control rat diaphragm shows normal myofibrils (F) and sarcomeres, two successive Z lines (Z), oval nucleus (N) under the sarcolemma, subsarcolemmal mitochondria (sm), and intermyofibrillar mitochondria (im). TEM ×25,000. (b) An electron micrograph of the longitudinal section in the second group rat diaphragm shows marked degeneration of myofibrils (LF) with marked aggregation of mitochondria in subsarcolemmal space (sm) and inter myofibrillar space (im) with pyknotic nucleus (N), and Z line loss (LZ). TEM ×25,000. (c) An electron micrograph of longitudinal section in the third group rat diaphragm shows normal myofibrils (F), oval nucleus (N) with mitochondria (m), and sarcomeres in between two successive Z lines (Z). TEM ×25,000. (d) An electron micrograph of the longitudinal section in the fourth group rat diaphragm shows a nearly normal appearance of myofibrils (F), sarcomeres, oval nucleus (N), mitochondria (m) with two successive Z lines (Z). TEM ×25,000.

Smooth muscle (diaphragm) examination by the transmission electron microscope

The ultrastructure of the diaphragm in the rats of the first control group showed a normal appearance (Figure 6a). But the diaphragm in the rats of the second group, which received atorvastatin only, showed marked abnormal ultrastructures such as mitochondria with degenerated parts of myofibrils (Figure 6b). The ultrastructure of the diaphragm in the rats of the third group, which received curcumin, showed a normal appearance of myofibrils, sarcomeres, nuclei, and mitochondria (Figure 6c) while the diaphragm of the fourth group rats, which received curcumin with atorvastatin, showed nearly the same ultrastructure of the first group where sarcomeres and nuclei were approximately normal with a few mitochondria in the subsarcolemmal area (Figure 6d).

Cardiac muscle examination by the light microscope

The cardiac muscle examination in the rats of the first control group showed a normal appearance of muscle fiber (Figure 7a) with few collagen fibers (Figure 8a), but the cardiac muscle in the rats of the second group, which received atorvastatin only, showed a noticeable loss of structure, cardiomyocytes shrinkage, and vacuolated cytoplasm with small fragmented pyknotic nuclei (Figure 7b) with excessive collagen fibers (Figure 8b). The transverse section of cardiac muscle in the rats of the third group, which received curcumin, showed

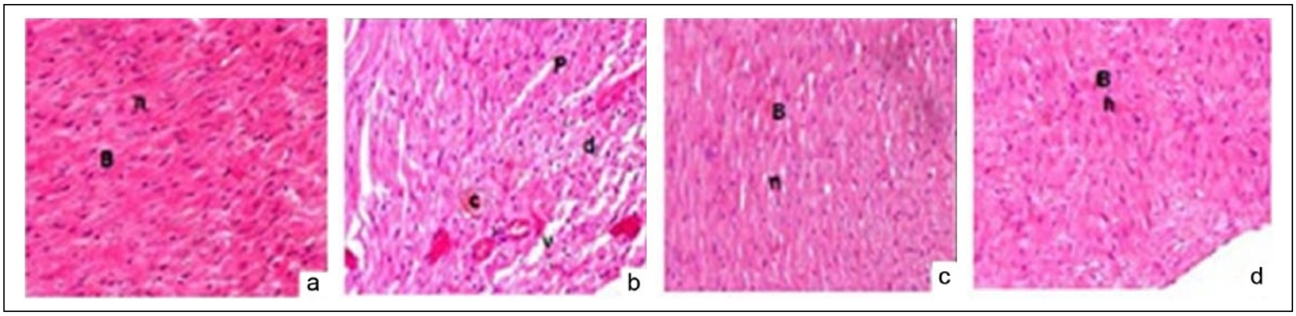

Figure 7. (a) A photomicrograph of the transverse section in the control rat cardiac muscle shows normal branching and anastomosis muscle fibers (B), acidophilic sarcoplasm, and central elongated vesicular nuclei (n). H&E ×400. (b) A photomicrograph of the transverse section in the second group rat cardiac muscle shows marked degeneration of fibers (d), acidophilic sarcoplasm, a huge number of pyknotic nuclei (p), and vacuoles (v) with blood vessels congestion (c). H&E ×400. (c) A photomicrograph of the transverse section in the third group rat cardiac muscle shows normal non branching muscle fibers (B), acidophilic sarcoplasm, and central elongated vesicular nuclei (n). H&E ×400. (d) A photomicrograph of the transverse section in the fourth group rat cardiac muscle shows nearly intact branching and anastomosis muscle fibers (B), acidophilic sarcoplasm, and central elongated vesicular nuclei (n). H&E ×400.

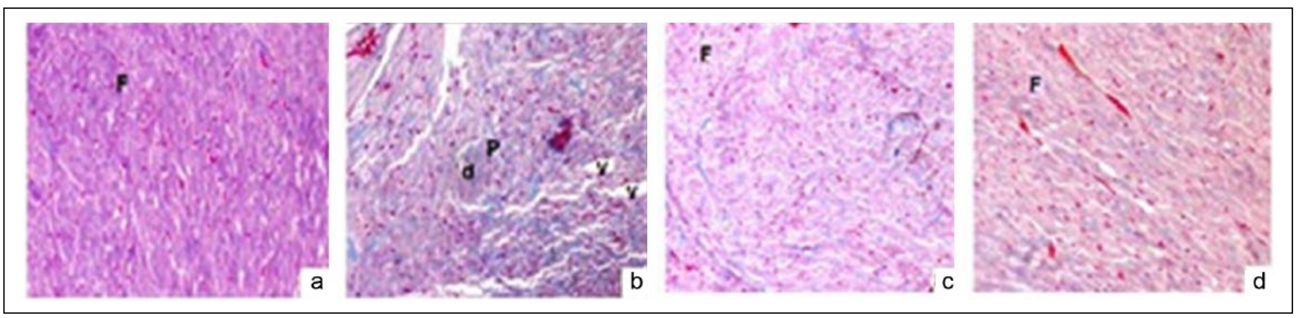

Figure 8. (a) A photomicrograph of the transverse section in the control rat cardiac muscle shows normal distribution of collagen fibers (F). Mallory ×400. (b) A photomicrograph of the transverse section in the second group rat cardiac muscle shows excess collagen fibers, cardiac muscle fibers degeneration (d), pyknotic nuclei (p), vacuoles (v), and blood vessels congestion. Mallory ×400. (c) A photomicrograph of the transverse section in the third group rat cardiac muscle shows few collagen muscle fibers (F). Mallory ×400. (d) A photomicrograph of transverse section in the fourth group rat cardiac muscle shows nearly normal few collagen muscle fibers (F). Mallory ×400.

entirely normal branching and anastomosis cardiac muscle fibers (Figure 7c) with few collagen muscle fibers (Figure 8c). The cardiac muscles of the fourth group of rats, which received curcumin with atorvastatin, showed a close to normal appearance of branching and anastomosis cardiac muscle fibers simulating the control and third groups (Figure 7d) and the normal few collagen fibers (Figure 8d).

Cardiac muscle examination by the transmission electron microscope

The ultrastructure of cardiac muscle in the rats of the first control group showed a normal appearance (Figure 9a), but the cardiac muscle in the rats of the second group, which received atorvastatin only, showed significant abnormal mitochondria, pyknotic nucleus, myofibrils degeneration, and vacuoles in the cytoplasm (Figure 9b). The ultrastructure of the cardiac muscles in the rats of the third group, which received curcumin, showed normal appearance of ultrastructures (Figure 9c) while the cardiac muscles of the fourth group rats, which received curcumin with atorvastatin, showed a near to normal appearance similar to the first and third groups where the ultrastructure was approximately normal with numerous mitochondria in the subsarcolemmal area (Figure 9d).

Quantification of histopathological changes in the different muscles of rats

Table 3 shows the quantification of histopathological changes in the different muscles of rats that were

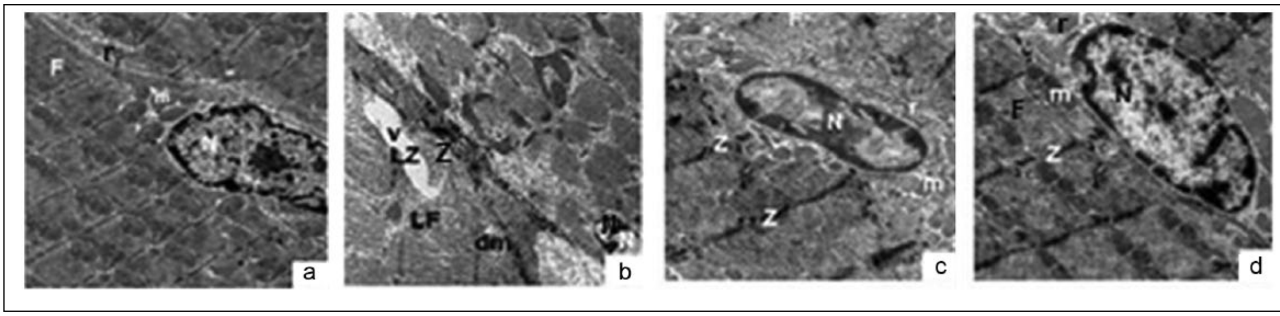

Figure 9. (a) An electron micrograph of the transverse section in the control rat cardiac muscle shows normal myofibrils (F) with sarcomeres in between two successive Z lines (Z), oval nucleus (N) under the sarcolemma with numerous mitochondria (m), and the reticular fibrils between the two cells (r). TEM ×25,000. (b) An electron micrograph of the transverse section in the second group rat cardiac muscle shows degeneration of myofibrils (LF), marked aggregation of mitochondria in subsarcolemmal space (sm) and intermyofibrillar space (im) with vacuolation (v), pyknotic nucleus (N), and Z line (LZ) disruption. TEM ×25,000. (c) An electron micrograph of the transverse section in the third group rat cardiac muscle shows myofibrils (F) and sarcomeres, two successive Z lines (Z), an oval elongated nucleus (N) with numerous mitochondria (m), and the reticular fibers in between the two cells (r). TEM ×25,000. (d) An electron micrograph of the transverse section in the fourth group rat cardiac muscle shows nearly normal myofibrils (F) and sarcomeres within two successive Z lines (Z), normal oval nucleus (N), numerous mitochondria (m), and the reticular fibers in between the two cells (r). TEM ×25,000.

Table 3. Comparison between the effects of atorvastatin used alone or with curcumin on the rats' muscles histopathological parameters.

Group / parameter	First (mean ± SD)	Second (mean ± SD)	Third (mean ± SD)	Fourth (mean ± SD)
Myofiber degeneration	0.1 ± 0.2	2.6 ± 0.2*	0.2 ± 0.5	0.4 ± 0.1†
Pyknotic nuclei	0.1 ± 0.7	2.2 ± 0.8*	0.2 ± 0.3	0.4 ± 0.5†
Cell infiltration	0.1 ± 0.7	3.1 ± 0.2*	0.1 ± 0.2	0.3 ± 0.4†
Collagen fiber distribution	0.1 ± 0.1	4.2 ± 0.5*	0.3 ± 0.3	0.4 ± 0.1†
Total score	0.4 ± 0.2	11.11 ± 0.2*	0.8 ± 0.1	0.15 ± 0.1†

Number per group: 20.
SD, standard deviation.
The first group (control) received the distilled water. The second group received atorvastatin only. The third group received curcumin only. The fourth group received curcumin with atorvastatin.
*P <0.001 (significant difference in comparison with the first group).
†P <0.001 (significant difference in comparison with the second group).

stained by the Mallory stain and H&E where a statistical significant difference in the overall histopathological parameters of the muscle damage (myofibers degeneration, cell infiltration, pyknotic nuclei, and collagen fibers distribution) was observed in the second group (atorvastatin) in comparison to the control group and in the fourth group (atorvastatin and curcumin) in comparison to the second group.

Discussion

Myotoxicity is a common atorvastatin with numerous adverse effects which has been observed in recent years. This study evaluates the sub-chronic use of atorvastatin induced myotoxicity and assesses the curcumin effect on the modulation of atorvastatin toxicity on the different types of the muscles in rats.

Our results showed a statistically significant increase in the levels of myotoxicity biomarkers in the second group, which received atorvastatin only, in agreement with Abdel Haleem and Elsayed[14] and Pierno et al.[15] The rise of creatinine phosphokinase levels is a sign of myositis and the constant biomarker for any muscle affliction according to the literature;[16,17] it is the key biomarker of the myopathy diagnosis in the skeletal and cardiac muscles because it generates adenosine triphosphate via adenosine diphosphate phosphorylation where its level is increased after the muscle cell membrane damage, with the subsequent leakage into the circulation according to Westwood et al.[18]

in agreement with Jeremias and Gibson[19] and O'Brien[20] that showed that troponin I and creatinine phosphokinase are highly specific for myocardium and the gold standard for acute myocardial damage detection. Furthermore, the significant increase of CPK, lactic acid dehydrogenase, and myoglobin depend mainly on the degree of muscle damage that may lead to myoglobinuria and renal failure which causes an increase in the levels of creatinine and potassium that may also be attributed to impairment in Na-K channel causing irreversible cell damage.[21]

The present study demonstrated histopathological and ultrastructural changes in the different types of muscles of the second group (atorvastatin only) such as myofibrils degeneration, partial loss of myofilaments, sarcoplasm fragmentation, cellular infiltration, abnormal aggregation of mitochondria in the subsarcolemmal and intermyofibrillar spaces, vacuolation in mitochondria and cytoplasm, pyknotic nucleus, and excessive collagen fibers distribution. Our results are consistent with Radcliffe and Campbell,[22] that of the referred histological changes which are often non-specific despite the vacuolation which is considered a characteristic morphological abnormality finding in atorvastatin-induced myotoxicity in the animals and the human muscles leading to an intracellular membrane vesicle transport disturbance in the myofibers.[23] There are many mechanisms that explain atorvastatin induced myotoxicity.[24] This experiment showed that the mechanism may be due to the atorvastatin effect on the gene expression via transcription and protein degradation by the ubiquitin proteasome pathway while Pierno et al.[25] referred to apoptosis induction, secondary metabolic intermediates, depletion, and chloride channel conductance alterations. In the other context, Liu et al.[26] showed that atorvastatin causes mevalonate deficiency that affects oxidative phosphorylation and mitochondrial adenosine triphosphate production leading to the muscular metabolism impairment inducing oxidative stress causing myotoxicity.

The current study shows that the concurrent use of curcumin with atorvastatin leads to a marked improvement of all biochemical, histopathological, and ultrastructural abnormalities. Curcumin is an anti-inflammatory and an antioxidant agent which modulates the oxidative stress manifestations of atorvastatin in different types of muscles which are consistent with results of Aggarwal and Harikumar,[5] which showed that curcumin has inhibitory actions on any tissue injury mediated by the inflammatory transcription factors, protein kinases, oxidative stress, and inflammation. In accordance with Wei et al.,[27] our results confirmed that curcumin can reduce the myocardial enzymes and inflammatory factors preventing cardiac injury. Furthermore, curcumin benefits depend on the decrease of the reactive oxygen species generation, phosphorylation of c-Jun N-terminal kinase, p38 MAP kinase, signal transducer, and activator of transcription (STAT)-3 in TNF-a-stimulated cells according to Avci et al.[28] In addition, Manjunatha and Srinivasan[29] reported that curcumin has an antioxidant effect based on lipid peroxidation modulation and the increase of antioxidant enzymes activity because it reverses glutathione depletion which is consistent with our results. It should also be noted that the antioxidant effects of curcumin increases the serum MMP-13 levels that are responsible for the restoration of normal collagen distribution in the muscles in agreement with Pinlaor et al.[30] In conclusion, sub-chronic use of atorvastatin may lead to myotoxicity that is manifested by biochemical abnormalities and histopathological and ultrastructural changes in the different types of rat muscles. Curcumin as an antioxidant may modulate atorvastatin- induced myotoxicity. Further research in humans is recommended in order to verify our results.

Declaration of conflicting interests

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