Curcumin modulates myotoxicity associated with sub-chronic use of atorvastatin in rats: A histopathological and biochemical analysis

Said Said Elshama\textsuperscript{a}, Ayman El-Meghawry El-Kenawy\textsuperscript{b,*}, Hosam-Eldin Hussein Osman\textsuperscript{c}

\textsuperscript{a}Department of Forensic Medicine and Clinical Toxicology, College of Medicine, Taif University, Suez Canal University, Egypt
\textsuperscript{b}Department of Pathology, College of Medicine, Taif University, GEBRI, University of Sadat City, Egypt
\textsuperscript{c}Department of Anatomy, College of Medicine, Taif University, Al-Azhar University, KSA

Received 2 January 2016; accepted 7 October 2016

Abstract Atorvastatin is considered to be one of the most commonly used of all statins antihyperlipidemic 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 twenty rats. The control group received water, the second group received atorvastatin, 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.

© 2016 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Atorvastatin is believed to be one of the statin drugs that decreases the total cholesterol, bad cholesterol (LDL) and triglyceride levels in the blood which cause coronary artery disease. Furthermore, atorvastatin also increases the useful cholesterol level (HDL) that protects against coronary artery...
disease (Shetty et al., 2008). 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 (Rodine et al., 2010). 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 (Yiannis et al., 2010).

Spices are commonly used as food additives to enhance the taste and the flavor of the food. It has medicinal uses because of its 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 (Srivastava and Mehta, 2009). It is a yellow orange dye that is extracted from the turmeric spice which has anti-inflammatory, antioxidant, anti-microbial and anti-amyloid effects (Cole et al., 2007) beside its chemoprotection and cytoprotection effects against endogenous and exogenous noxious stimuli, and its efficacy for treating any renal, myocardium and nervous tissue injury (Aggarwal and Harikumar, 2009).

Recently, Curcumin has been found to possess anti-carcinogenic activities because of its anti-proliferative effect in multiple cancers and its effect on mutagenesis, cell cycle regulation, apoptosis and tumor growth (Wilken et al., 2011; Aggarwal et al., 2003).

Therefore, the current study aimed to investigate the effect of curcumin on atorvastatin sub-chronic use - induced myotoxicity in rats by the assessment of the levels of creatinine

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison between the effects of atorvastatin used alone or with curcumin on Mean ± SD of different biomarkers levels of myotoxicity in the rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Troponin-1 (ng/ml)</td>
<td>0.0025 ± 0.001</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>269.1 ± 35.872</td>
</tr>
<tr>
<td>Myoglobin (ng/ml)</td>
<td>127.2 ± 3.792</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.77 ± 0.114</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>142.35 ± 36.552</td>
</tr>
<tr>
<td>Potassium (meq/L)</td>
<td>4.1 ± 0.361</td>
</tr>
</tbody>
</table>

Number per group: 20.
SD: standard deviation.
CPK: Creatinine phosphokinase.
LDH: lactic acid dehydrogenase.
First group (control) received distilled water.
Second group received atorvastatin only.
Third group received curcumin only.
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).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison between the effects of atorvastatin used alone or with curcumin on Mean ± SD of oxidative stress parameters in the rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Catalase</td>
<td>30.72 ± 1.55</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>12.41 ± 1.21</td>
</tr>
<tr>
<td>GSH</td>
<td>90.22 ± 1.41</td>
</tr>
<tr>
<td>MDA</td>
<td>20.71 ± 0.59</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>24.31 ± 1.73</td>
</tr>
</tbody>
</table>

Number per group: 20.
SD: standard deviation.
GSH: Glutathione.
MDA: Malondialdehyde.
First group (control) received the distilled water.
Second group received atorvastatin only.
Third group received curcumin only.
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).
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.

2. Material and methods

Eighty healthy adult albino rats weighing 200–300 g were obtained from the animal house of King Abdel Aziz University-Jeddah. 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 the four groups: each group comprising of twenty 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 “5000 mg/kg”), dissolved in distilled water (Ronald et al., 2009). The third group received 200–300 mg/kg/day of curcumin, dissolved in distilled water (Farombi et al., 2008) while the fourth group received 50 mg/kg/day of atorvastatin, dissolved in distilled water with 200–300 mg/kg/day of curcumin, dissolved in distilled water. The daily administration of the distilled water, atorvastatin and curcumin was done by the gastric gavage for 90 days. The atorvastatin was available in 40 mg tablet form that was manufactured by Egyptian International Pharmaceutical Industries Co. (E.I.P.I.CO.), 10th of Ramadan City, Egypt, while Curcumin was purchased from Sigma Chemical Co.

2.1. Blood sample collection

On the last day of the experiment, the rats were anesthetized 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 analyzer by the troponin I STAT third generation and creatinine phosphokinase STAT methods (Roche Diagnostics, Tutzing, Germany). These assays were based on electrochemiluminescence immunoassay (ECLIA) technology 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 enzyme immunoassay (Dade Behring, Newark, Delaware, USA) (Wu et al., 1999). Serum creatinine level was determined by the routine colorimetric methods using the commercial kits and quantified on clinical biochemistry autoanalyzer (Alexander and Griffith, 1992). Determination of the serum level of lactic acid dehydrogenase and potassium was performed by standard spectrophotometric analysis by using diagnostic kits (McKenzie, 2010).
2.2. 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. 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 (myofibers degeneration, cell infiltration, pyknotic nuclei and collagen fibers distribution). This was done by using a scoring scale of 0 to 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 (Bancroft and Gamble, 2002).

Ultrastructure studies were performed by using the transmission electron microscope, and 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 one hour 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 (Graham and Orenstein, 2007).

2.3. Tissue preparation

500 mg of muscular tissues were 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 (Davalos et al., 2003).

2.4. Statistical analysis

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

2.5. Ethical considerations

The most appropriate animal species were chosen for this research. Promotion of high standard care and animal well-being was exercised at all times. An appropriate sample size

Figure 2  (a) A photomicrograph of longitudinal section in the control rat extensor digitorum longus muscle shows normal distribution of blue stained collagen fibers around non branching muscle fibers (F). (b) A photomicrograph of longitudinal section in the second group rat extensor digitorum longus muscle shows excessive blue collagen fibers around the splitting muscle fibers (s). (c) A photomicrograph of longitudinal section in the third group rat extensor digitorum longus muscle shows few collagen muscle fibers (F). (d) A photomicrograph of longitudinal section in the fourth group rat extensor digitorum longus muscle shows nearly normal few collagen muscle fibers (F).
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. The rats were sacrificed after being excessively anesthetized via ether. Our standards of animal care and administration are consistent with the requirements and standards of international laws and regulations.

3. Results

3.1. 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, the second group which received atorvastatin only, the third group which received curcumin, the fourth group that received curcumin with atorvastatin were 0.0025 ± .001; 0.7620 ± 0.014; 0.0045 ± 0.005 and 0.1545 ± 0.016 respectively. Value of $F$ which indicates the difference between groups, was 2.220 with statistically significant at $P < 0.001$. Mean ± SD values of Creatinine phosphokinase (CPK) in the control, second, third and the fourth groups were 269.1 ± 35.872; 688.7 ± 86.937; 220.45 ± 9.512 and 335.80 ± 9.51 respectively. Value of $F$ which indicates the difference between groups, was 398.839 and statistically significant whereas $p < 0.001$. Mean ± SD values of Myoglobin in the control, second, third and the fourth groups were 127.2 ± 3.792; 299.0 ± 7.145; 127.75 ± 4.025 and 147.5 ± 6.022 respectively. Value of $F$ which indicates the difference between groups, was 467.74 and statistically significant whereas $p < 0.001$. Mean ± SD values of creatinine in the

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). (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, pyknotic nucleus (N). Disruption of Z line (Z) and loss of Z line (LZ). (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). (d) An electron micrograph of longitudinal section in the fourth group rat extensor digitorum longus muscle shows 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).
Figure 4  (a) A photomicrograph of longitudinal section in the control rat diaphragm shows normal muscle fibers (F) and multiple vesicular nuclei (n). (b) A photomicrograph of 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). (c) A photomicrograph of longitudinal section in the third group rat diaphragm shows normal muscle fibers (F) and multiple vesicular peripheral nuclei (n). (d) A photomicrograph of longitudinal section in the fourth group rat diaphragm shows nearly normal appearance of the intact muscle fibers (F) and multiple vesicular nuclei (n).

Figure 5  (a) A photomicrograph of 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). (b) A photomicrograph of longitudinal section in the second group rat diaphragm shows excessive blue collagen fibers with blood vessels congestion (c). (c) A photomicrograph of longitudinal section in the third group rat diaphragm shows few collagen fibers (F). (d) A photomicrograph of longitudinal section in the fourth group rat diaphragm shows nearly normal few collagen fibers (F).
The values of $F$, which indicates the difference between groups, were 3.4550 and statistically significant whereas $p < 0.001$. Mean ± SD values of lactate dehydrogenase (LDH) in the control, second, third and the fourth groups were 142.35 ± 36.552, 843.4 ± 59.102, 135.45 ± 40.066 and 227.5 ± 10.008 respectively. The value of $F$ which indicates the difference between groups, was 1415.83 and statistically significant at $p < 0.001$. Mean ± SD values of potassium (K) in the control, second, third and the fourth groups were 4.1 ± 0.361, 4.9 ± 0.200, 3.8 ± 0.125 and 3.9 ± 0.323 respectively. The value of $F$ which indicates the difference between groups, was 73.537 while statistically significant was at $p < 0.001$.

### 3.2. Oxidative stress parameters

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

### 3.3. Histopathological findings

#### 3.3.1. Skeletal muscle (extensor digitorum longus) by the light microscope

Examination of longitudinal section of extensor digitorum longus muscle in the rats of the first control group showed normal structure (Fig. 1a) with normal collagen fiber distribution in endomysium and around the blood vessels (Fig. 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.
(Fig. 1b) with excessive collagen fibers around the affected myofibers (Fig. 2b). Longitudinal section of extensor digitorum longus muscle in the rats of the third group which received curcumin showed normal cylindrical, parallel and non-branching muscle fiber bundles and multiple peripheral nuclei (Fig. 1c) with few collagen fibers in endomysium and around the blood vessels (Fig. 2c). The extensor digitorum longus muscle of the fourth group rats that received curcumin with atorvastatin showed injury improvement and normal appearance of muscle fibers simulating the control and third group showed evidence of small areas of cellular infiltration (Fig. 1d) and few collagen fibers in the endomysium (Fig. 2d).

3.3.2. Skeletal muscle (extensor digitorum longus) 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 (Fig. 3a), and 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 (Fig. 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. (Fig. 3c) The extensor digitorum longus muscle of the fourth group of rats that received curcumin with atorvastatin, displayed approximately the same ultrastructure of the first and third groups. The sarcomeres, nuclei and mitochondria were approximately normal (Fig. 3d) for these groups.

3.3.3. Smooth muscle (diaphragm) by the light microscope

Examination of longitudinal section of diaphragm in the rats of the first control group showed normal structural appearance (Fig. 4a) with normal collagen fibers distribution in the connective tissues (Fig. 5a). But 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 (Fig. 4b) with excessive collagen fibers distribution (Fig. 5b). Longitudinal section of the diaphragm in the rats of the third group which received curcumin showed completely normal muscles fibers (Fig. 4c) with few collagen fibers in connective tissue (Fig. 5c). The diaphragm of the fourth group rats that received curcumin with atorvastatin showed an almost near to normal appearance of muscle fibers simulating the control and third group with rare areas of cellular infiltration (Fig. 4d) and few collagen fibers (Fig. 5d).

3.3.4. Smooth muscle (diaphragm) by the transmission electron microscope

The ultrastructure of diaphragm in the rats of the first control group showed normal appearance (Fig. 6a). But 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 (Fig. 6b) with excessive collagen fibers distribution (Fig. 6c). Longitudinal section of the diaphragm in the rats of the third group which received curcumin showed nearly intact branching and anastomosis muscle fibers (Fig. 6d).
showed marked abnormal ultrastructures such as mitochondria with degenerated parts of myofibrils (Fig. 6b). The ultrastructure of diaphragm in the rats of the third group which received curcumin showed normal appearance of myofibrils, sarcomeres, nuclei and mitochondria (Fig. 6c) while diaphragm of the fourth group rats that 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 (Fig. 6d).

3.3.5. Cardiac muscle by the light microscope
Examination of cardiac muscle in the rats of the first control group showed normal appearance of muscle fibers (Fig. 7a) with normal few collagen fibers (Fig. 8a). But cardiac muscle in the rats of the second group which received atorvastatin only, showed noticeable loss of architecture, cardiomyocytes shrinkage and vacuolated cytoplasm with small fragmented pyknotic nuclei (Fig. 7b) with excessive collagen fibers (Fig. 8b). Transverse section of cardiac muscle in the rats of the third group which received curcumin showed entirely normal branching and anastomosis cardiac muscle fibers (Fig. 7c) with little collagen muscle fibers (Fig. 8c). Cardiac muscles of the fourth group rats that received curcumin with atorvastatin showed close to normal appearance of branching and anastomosis cardiac muscle fibers simulating the control and third groups (Fig. 7d) and normal few collagen fibers (Fig. 8d).

3.3.6. Cardiac muscle by the transmission electron microscope
The ultrastructure of cardiac muscle in the rats of the first control group showed normal appearance (Fig. 9a). But cardiac muscle in the rats of the second group which received atorvastatin only, showed significant abnormal mitochondria with pyknotic nucleus and myofibrils degeneration with vacuoles in the cytoplasm (Fig. 9b). The ultrastructure of cardiac muscle in the rats of the third group which received curcumin showed normal appearance of ultrastructures (Fig. 9c) while cardiac muscle of the fourth group rats that received curcumin with atorvastatin showed near to normal appearance similar to the first and third group whereas ultrastructures were approximately normal with numerous mitochondria in the subsarcolemmal area (Fig. 9d).

3.3.7. 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 stained by the Mallory stain, hematoxylin and eosin where a statistically 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 with the control group and in the fourth group (atorvastatin and curcumin) in comparison with the second group.
4. Discussion

Myotoxicity is a common atorvastatin adverse effect which has been observed in the 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. The rise of creatinine phosphokinase levels is a sign of myositis and the constant biomarker for any muscle affliction according to Phillips et al., 2002 and Chapman and Carrie, 2005; 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. (2005), in agreement with Jeremias and Gibson, 2005 and O'Brien, 2008 that showed that troponin I and creatinine phosphokinase are highly specific for myocardium and the gold standard for acute myocardial damage detection. Furthermore, Thompson et al. (2003) showed that the significant increase of creatinine phosphokinase, lactic acid dehydrogenase and myoglobin depends 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.

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...
Curcumin modulates myotoxicity associated with use of atorvastatin

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>First M ± SD</th>
<th>Second M ± SD</th>
<th>Third M ± SD</th>
<th>Fourth M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myofibers degeneration</td>
<td>0.1 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>0.2 ± 0.5</td>
<td>0.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Pyknotic nuclei</td>
<td>0.1 ± 0.7</td>
<td>2.2 ± 0.8</td>
<td>0.2 ± 0.3</td>
<td>0.4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Cell infiltration</td>
<td>0.1 ± 0.7</td>
<td>3.1 ± 0.2</td>
<td>0.1 ± 0.2</td>
<td>0.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Collagen fibers distribution</td>
<td>0.1 ± 0.1</td>
<td>4.2 ± 0.5</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>0.4 ± 0.2</td>
<td>11.11 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>0.15 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Number per group: 20.
M ± SD: Mean ± Standard deviation.
First group (control) received the distilled water.
Second group received atorvastatin only.
Third group received curcumin only.
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).

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 (2009), 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. (2012), 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. (2012). In addition, Manjunatha and Srinivasan (2007), 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 increase the serum MMP-13 levels that are responsible for the restoration of normal collagen distribution in the muscles in agreement with Pinlaor et al. (2010).

5. Conclusion

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.

References


O’Brien, P.J., 2008. Cardiac troponin is the most effective translational safety biomarker for myocardial infarction in cardiotoxicity. Toxicology 245 (3), 206–218.


