WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 12, 214-224 Research Article ISSN 2278 – 4357

COMBINED TREATMENT WITH ALPHA- TOCOPHEROL PREVENTS SIMVASTATIN - INDUCED SKELETAL MUSCLE INJURY IN RATS

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Article Received on
12 October 2017,

Revised on 03 Nov. 2017,
Accepted on 24 Nov. 2017,

DOI: 10.20959/wjpps201712-10586

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ABSTRACT

Statins are widely used regardless of their myopathic side effects that ranging from weak myalgia to serious rhabdomyolysis. Simvastatin is one of the most common used of all statins anti-hyperlipidemic drugs despite the fact that there is much controversy about its safety. Alpha-tocopherol has antioxidant and anti- inflammatory properties. The aim of this study is to elucidate the myopathic effect of simvastatin and protective role of alpha- tocopherol in adult male rat skeletal muscle via biochemical, histological and immunohistochemical studies. Control group received 0.5 ml of olive oil; alpha- tocopherol group was treated with 100mg/ kg b.w, intramuscularly; simvastatin group was treated with 40mg/ kg b.w, orally; simvastatin and alpha-tocopherol group and a group pretreated with alpha- tocopherol then simvastatin associated with alpha- tocopherol daily for 2 weeks. The treatment with simvastatin caused significant deviations of myotoxicity biomarkers associated with histopathological and immunohistochemical variations. Pre-treatment and co- administration of alpha- tocopherol led to an enhancement in myotoxicity indexes.

KEYWORDS: simvastatin, α- tocopherol, myotoxicity, lactic acid dehydrogenase, collagen fibers, caspase.

INTRODUCTION

Muscle tissue is principally susceptible to drug- associated toxicity because of its relative plenty, excessive blood flow and metabolic level. Hyperlipidemic state spread among adults

according to their consuming large amounts of fast food. Statins are widely used to lower cholesterol levels,[1] so used for prevention of cardiovascular events. However, this usage accompanied by different grades of myopathy, ranging from mild myalgia to fatal rhabdomyolysis has been reported.[2] Simvastatin (Simva) is one of these drugs, used to decrease total cholesterol, low density lipoprotein (LDL) and triglycerides levels in the blood.[3] Hypercholesterolmic patients may occasionally guide to numerous dietary elements and natural products to adjust serum lipid concentrations due to these suspicions about the safety of statins.

Antioxidants reduce lipid peroxidation and its supplementations provide protective effects against hyperlipidemia.[4] Alpha- tocopherol (α-TP) (a main component of vitamin E) is considered to be the most effective lipid soluble antioxidant found in the human biological system. As it interacts with free radicals, the chain reaction of these radicals starts, avoiding lipid peroxidation; a process that produces stuffs that are destructive to cells, also has anti-inflammatory and anti-apoptotic effects.[5,6] Therefore, the principal object of the current study is to evaluate the efficacy role of alpha- tocopherol against simvastatin- induced myotoxicity in adult male albino rats.

MATERIALS AND METHODS

Chemicals

Zocor (simvastatin) 40mg are brick red, oval film coated tablets was purchased from Global Napi Pharmaceuticals Co. "GNP", Egypt. Alpha- tocopherol was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and solvents used were of the highest available commercial grade.

Animals

A total of twenty-five adult male albino rats of Wistar strain (weighing 150 ± 10 g) were obtained from animal house in Medical Research Center (MRC), Faculty of Medicine, Ain Shams University. The animals were acclimatized to the laboratory circumstances for a period of 2 weeks. They were housed and preserved at an ambient temperature of 25 ± 2°C, 50 ± 20% relative humidity and 12-h light/12-h dark cycle and were given a standard rat feed and water *ad libitum*. The strategy of the experiments was conducted in accordance with the guidelines required by the Experimental Animal Laboratory and permitted with the Animal Care and Use Committee of our institution of Ain Shams University, Cairo, Egypt.

Experimental design

All animals were allocated into five groups, each of five rats in this manner:

Group (1): Functioned as control group, rats received (0.5ml/ kg b.w) olive oil.

Group (2): Animals were administrated 100mg/ kg b.w of α-TP dissolved in 0.5 ml of olive oil.

Group (3): Rats were administrated with 40mg/ kg b.w of Simva dissolved in 0.5 ml distilled water.

Group (4): Rats were treated with Simva and α-TP together.

Group (5): Rats were treated with α-TP for 7 days prior the experiment then treated with Simva alone for 7days then associated with α-TP for another 7 days.

All rats administrated Simva and α-TP orally and intramuscularly once daily for 2 weeks, respectively. Towards the end of the experimental period, the experienced animal groups were sacrificed after 24 h of the last dose of different administrations. Tibialis Anterior (TA) skeletal muscles were instantly excised cleared of adhering connective tissue and weighed. These muscles were used for biochemical and microscopical examinations.

Determination of body and TA weights

The body weight of each animal of the control and treated groups was assessed once weekly during the experiment and once on the day of sacrifice. After scarification, the Tibialis Anterior muscles of all groups were quickly isolated, rinsed in 0.9% saline, blotted with a filter paper piece and weighed.

Biochemical study

The serum creatinine level was determined by the routine colorimetric methods using the commercial kits. Determination of the serum level of lactic acid dehydrogenase (LDH), Potassium and muscular tissue MDA were accomplished by standard spectrophotometric analysis using diagnostic kits.[7,8]

Histological analysis

For qualitative analysis of TA muscular histology, tissue samples were immediately fixed in 10% neutral formalin and processed routinely for paraffin embedding technique. These sections of 5-7µm thick were made using a rotary microtome and stained with hematoxylin and eosin.[9]

Histochemical analysis

Correspondingly, these sections were histochemically stained with Masson trichrome to clarify general collagen fibers.[10]

Immunohistochemical analysis

Caspase is one of the proteins stimulated during cell apoptosis and necrosis**.** This done by incubation of muscle sections with monoclonal antibodies against caspase.[11]

Statistical analysis

Data were expressed as mean values± SEM and statistical analysis was executed and exposed to one-way analysis of variance (ANOVA) to evaluate significant differences among treatment groups. P values less than 0.05 were considered significant. All statistical analyses were performed using SPSS statistical version 17 software package (SPSS® Inc., USA).

RESULTS

The results indicated a highly significant decrease in body and TA weights in the group (3) when compared to the control. The decrement ratio reached -16.9% and -15.6%, respectively. While the utilization of α-TP in groups (4) and (5) exposed a significant and highly significant increase in both weights in comparison to group (3). The increment ratio reached -13.4%, -8.4% and -6.5%, -2.2%, respectively. (Table 1).

Table 1: The mean body and TA weights (g) of control and treated groups.

Parameters / Groups	Body weight (g)	% of Change	TA weight (g)	% of Change
Group (1)	200.3±5.7	----	0.358±0.007	----
Group (2)	195.7±4.6	-2.3	0.345±0.006	-3.6
Group (3)	166.5±2.3^{a2}	-16.9	0.302±0.005 ^{a2}	-15.6
Group (4)	173.5±3.5^{a1b1}	-13.4	0.328±0.005 ^{a1b1}	-8.4
Group (5)	187.2±4.2^{b2}	-6.5	0.350±0.007 ^{b2}	-2.2

Data are expressed as means ± S.E. (n=5 in each group).

a=compared to control group (G1); b=compared to Simva group (G3).

1=significant change at $p<0.05$; 2=highly significant change at $p<0.01$.

Biochemical study

Table (2) presented that there was a statistical significant increase in the values of creatinine, LDH, potassium and MDA in group (3) in comparison to the control, while these values were significant and marked decrease in groups (4) and (5), respectively.

Table 2: Comparison between the effect of Simva alone or with α-TP (Mean± SD) on biochemical parameters.

Parameters / Groups	Creatinine (mg/dl)	LDH (U/L)	Potassium (meq/L)	MDA (nmol/ g tissue)
Group (1)	0.76±0.1	145.5±37	3.9±0.4	19.7±1
Group (2)	0.73±0.1	134.5±40	3.5±.04	20.5±2
Group (3)	0.97±0.2 ^{a3}	643.6±60 ^{a3}	5.2±0.2 ^{a3}	60.2±3 ^{a3}
Group (4)	0.85±0.1 ^{b1}	320.7±30 ^{b2}	4.5±0.1 ^{b1}	28.7±2 ^{b2}
Group (5)	0.66±0.1 ^{b3}	224.7±11 ^{b3}	3.8±0.3 ^{b2}	21.2±2 ^{b3}

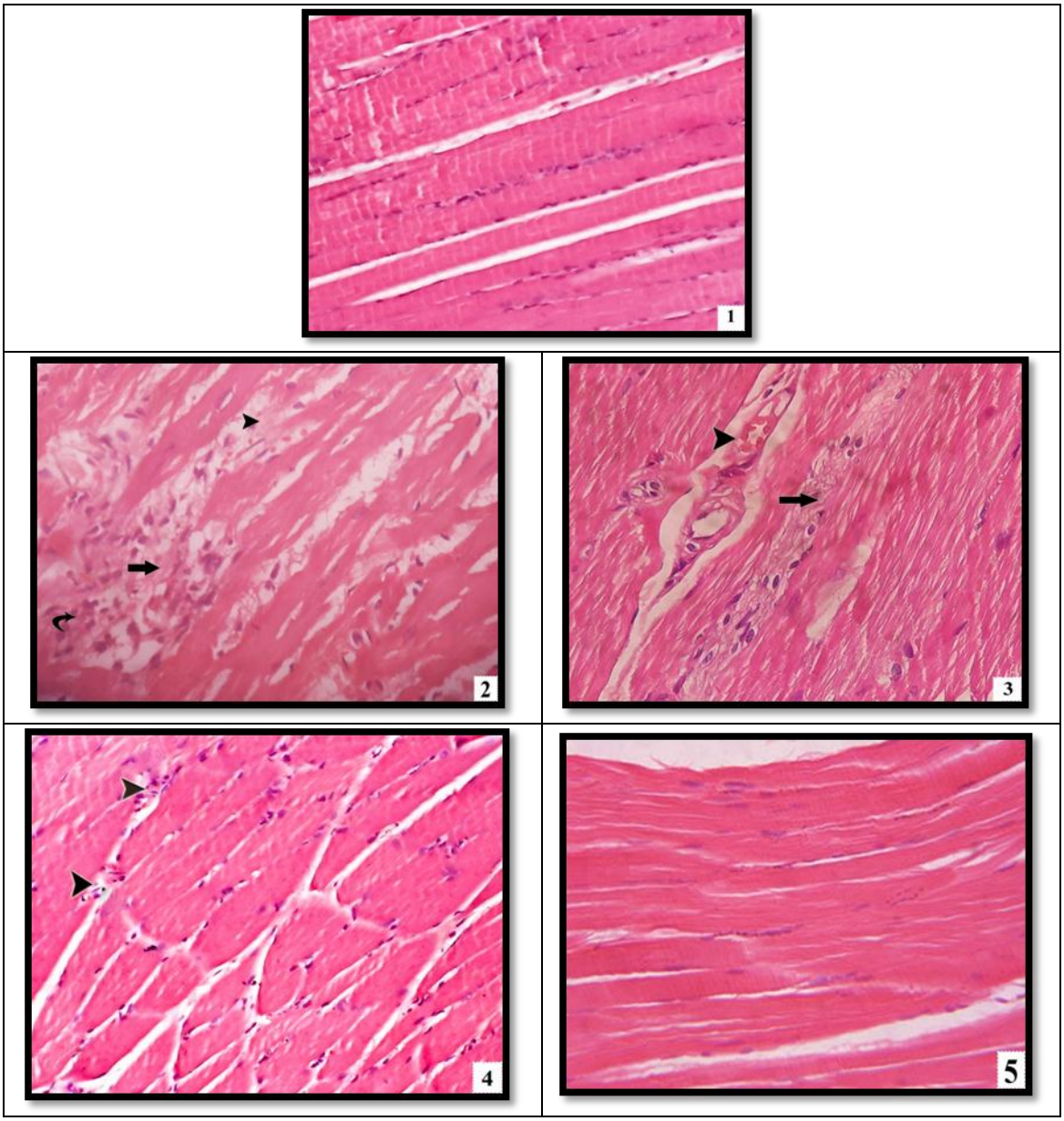
Data are expressed as means ± S.E. (n=5 in each group).

a=compared to control group (G1); b=compared to Simva group (G3).

1=significant change at $p<0.05$; 2=highly significant change at $p<0.01$; 3= very highly significant change at $p< 0.001$.

Histological study

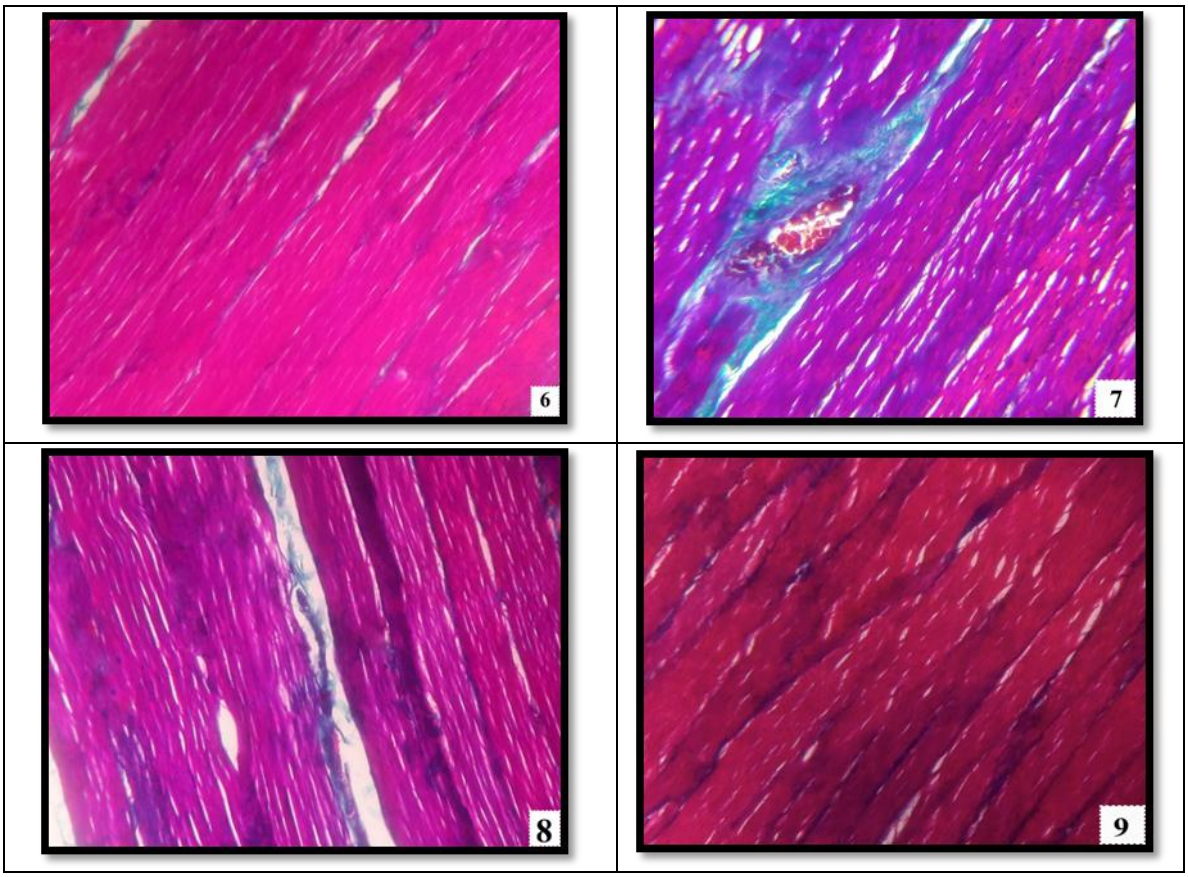
The inspection of the longitudinal sections of TA muscle in the rats of the control group displayed typical structure with unbranched cylindrical myofibers, flattened nuclei and cross striations (Fig. 1). TA muscle of the group (3) showed muscle injury where muscle fiber degeneration, necrosis, interstitial blood vessel hemorrhage, and neutrophil infiltration were detected (Figs.2&3). However, group (4) co- administration with α-TP exposed a reduction in damaged muscle fibers which was accompanied by scattered neutrophil infiltration (Fig. 4). Also, in group (5) prior treatment with α-TP showed an injury improvement and normal appearance of muscle fibers simulating the control (Fig. 5).



Figures (1-5): embraces photomicrographs of TA skeletal muscle longitudinal sections stained with H&E (X 400). (Fig.1) control group showing normal architecture of myofibers with flattened nuclei and cross striations. (Fig.2) simva- treated group showing replacement of some muscular components by remnants of coagulated clumps (arrow) intermixed with inflammatory cells (curved arrow) and remnants of myolytic myofibrils (arrow head). (Fig.3) showing congestion (arrow head) and degenerated necrotic myofibers (arrow) (Fig.4) group 4 showing normal structure with few myofibers suffered from slight inflammation (arrow heads). (Fig.5) group 5 showing nearly normal restoration of myofibers.

Histochemical study

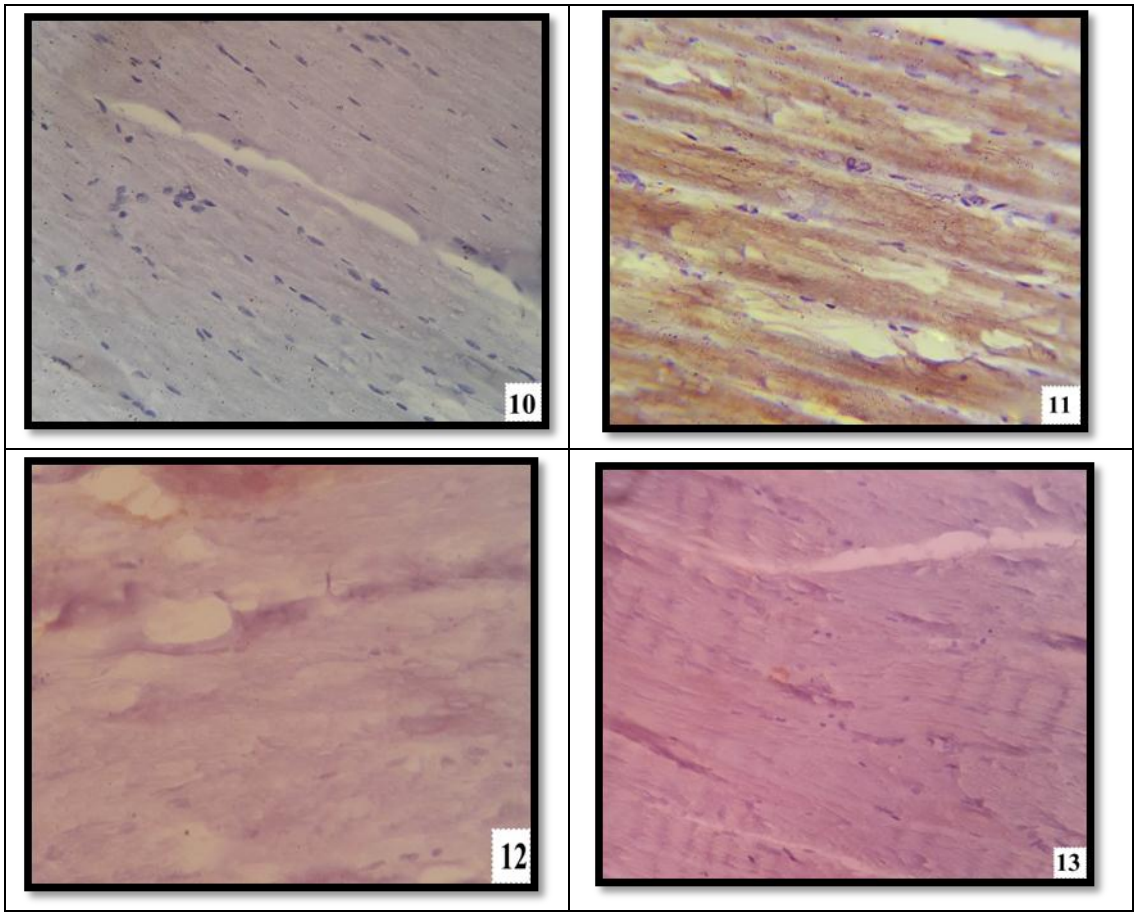
Evaluation of the control TA longitudinal sections stained with Masson's trichrome exhibited a uniform distribution of collagen fibers (Fig.6). Group (3) administrated Simva showed distinct increase in collagenous fibers around the affected myofibers (Fig. 7). Groups (4&5) presented mild and marked reduction in collagenous fibers as seen in (Figs.8&9).



Figures (6-9): includes photomicrographs of longitudinal sections of TA skeletal muscle stained with Masson trichrome (X400). (Fig. 6) control section showing fine collagen fibers within myofibers. (Fig.7) longitudinal section in TA of simva treated group showing extensive collagen fibers deposition within myofibers. (Fig. 8) simva associated α-TP group showing slight to moderate collagen fibers deposition between and within myofibers. (Fig.9) pre- treated with α-TP group showing almost normal distribution of collagen fibers.

Immunohistochemical Study

Assessment of the control TA longitudinal sections stained with caspase presented a deficiency of caspase immunoreactivity within the myofibers (Fig.10). Group (3) positive caspase immunoreactivity detected within the damaged myofibers (Fig.11). Groups (4&5) presented mild and marked pale caspase immunoreactivity as seen in (Figs.12&13).



Figures (10-13): contains longitudinal sections of TA skeletal muscle stained with caspase, (X400). (Fig.10) control section showing lack of caspase immunoreactivity within myofibers. (Fig.11) section in TA of simva treated group showing positive caspase immunoreactivity within the myofibers and aggregation of inflammatory cells appeared. (Fig.12) simva associated α-TP group showing faint caspase immunoreactivity within myofibers. (Fig.13) pre- treated with α-TP group Showing weak or absence of caspase immunoreactivity.

DISCUSSION

Myotoxicity is a conventional simvastatin with several undesirable effects that has been detected in the recent years. The data showed that simvastatin administration caused body weight loss that may be assigned to the gastrointestinal disorders (indigestion) and due to loss of injured skeletal muscles as reported before.[12] That also may be attributed to oxidative damage occurred by simvastatin especially for macromolecules as proteins, DNA and carbohydrates so, led to loss muscle weight.

The results in this study displayed a statistically significant increase in the levels of myotoxicity biomarkers in the simvastatin group.[13] The significant increase of LDH level

counted on the degree of muscle injury that could lead to renal failure which caused an increase of creatinine and potassium degrees that may be accredited to the damage of Na-K channel creating permanent cell damage.[14] Also, creatinine was always increased in patients with inflammatory myopathy.[15] Simvastatin caused an increase in MDA level that could advance muscle atrophy via imbalance between reactive oxygen species (ROS) production and scavenging, rising in the accumulation of ROS in the mitochondria in harmony with the earlier studies.[13]

The present study demonstrated histopathological, histochemical and immunohistochemical changes in TA skeletal muscle. The simvastatin group showed myofibrils degeneration, sarcoplasm fragmentation, pkynotic nuclei, collagen fibers deposition and cellular infiltration. These alterations may be attributed to the oxidative damage occurred by simvastatin in agreement with preceding studies.[16,2,17]

The current study showed that the pre- treatment and concurrent administration α-TP with simvastatin led to a marked improvement of all biochemical, histological, histochemical and immunohistochemical alterations. α-TP prevented the progression of muscle atrophy and improved its mass and function. In this study, α-TP treatment led to decrease TA muscle weight that may be attributed to decrease MDA level and increase the antioxidative properties so preventing macromolecules from damage as protein which is the basic unit of the muscle building and retain body and muscle weights in their normal range. Also, α-TP had the ability for creatinine clearance.[18,19]

Treatment with α-TP obstructed H_2O_2- induced reduction of heavy myosin chain (MyHC) or muscle protein and decrease LDH level (enzyme marker for muscle injury) via its restriction with the lipid peroxidation chain reaction.[20,6] According to the decrease of the muscle damage via antioxidant possessions. Also, it helped in the decrease of collagen fibers deposition as previous reported.[21,5] Interesting to note that α- tocopherol has been suggested to act as an antioxidant not only via its ability to scavenge radicals, but also by preventing the accumulation of neutrophils within ischemia/reperfusion (I/R) tissue.[18,6] α-TP is a substance with potent anti-inflammatory, antioxidant and anti-apoptotic effects.[6] Results of this study show that prophylactic administration of alpha-tocopherol prevents muscle injuries by eliminating oxygen radicals and inhibiting lipid peroxidation.

CONCLUSION

From the biochemical and histological perspectives, pretreatment with alpha- tocopherol has more protective effect and synergistic antioxidant effect on muscular injury and could help simvastatin to decrease hyperlipidemia syndrome and in rats.

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