



## Original Work

### The possible protective effect of *Panax ginseng* on experimentally induced colchicine myopathy in adult male albino rats: a histological study

Aiman Al-Maathadi<sup>1</sup>, Sinan Farhan<sup>2</sup>, Jihad Alzyoud<sup>3</sup>, Aiman Al-Qtaitata<sup>w1</sup>

<sup>1</sup>Department of Anatomy and Histology, Faculty of Medicine, Mutah University, Jordan

<sup>2</sup>Department of Pharmacy, Al-Rafidain University College, Baghdad, Iraq

<sup>3</sup>Faculty of Applied Health Sciences, The Hashemite University, Zarqa, Jordan

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**ABSTRACT:** Myopathy is one of the side effects of colchicine, manifested as pain, dysfunction, and weakness of muscles. Ginseng extract antioxidant and anti-inflammatory activities have been reported in muscular tissue. This study was conducted to investigate whether administration of ginseng ameliorates colchicine-induced skeletal muscles damage in adult male albino rats. Forty adult male albino rats were randomly divided into four groups (ten/group) – Group I (control group): rats received normal diet and orally given normal saline, Group II: ginseng extract was administered via oral gavage daily for one month at the doses of 300 mg/kg, Group III (Colchicine treated group): rats were given colchicine (50 µg/kg/day) via oral gavage for one month, and Group IV: concomitant administration of Ginseng and colchicine for one month. Serum creatine kinase (CK) levels were measured, and skeletal muscles specimens were processed for light and electron microscopic examination. Administration of colchicine (group III) showed elevated serum CK, and histologically myofibrillar disarray foci together with cellular infiltration and edema. Group IV showed reduction in most of those manifestations. Concomitant administration of ginseng with colchicine ameliorated most of the symptoms related to colchicine induced-myopathy.

**KEY WORDS:** *Panax ginseng*; *Colchicine*; *Myopathy*; *Skeletal muscle*

#### INTRODUCTION

Myopathy is a term used to describe muscle diseases characterized by abnormal muscle tissue, pain and weakness, which result in physical disability<sup>1</sup>. Consequently, this burdens the health system, and the quality of life of individuals, particularly athletes. Muscle pathologies include a wide range of conditions that are attributed to genetic factors, inflammatory conditions, dietary supplements and drugs. Muscular Dystrophies are the example of genetic related myopathies and myasthenia gravis, an immune related disorder in which antibodies attack acetylcholine receptors at the neuromuscular junction<sup>2</sup>. Interestingly, drug injections into muscles can often cause damage to

muscles<sup>3</sup>. Drug induced myopathy is a side effect of several drugs used to treat several medical conditions, for example, colchicine for gout, chloroquine for malaria, statins as anti-cholesterol, interferon-α for hepatitis, and emetine for vomiting<sup>4</sup>. Other medications could cause myopathy primary or secondary to a neuropathy such as recreational drugs and reverse-transcriptase inhibitors (RTIs) for HIV. AIDS drug-induced myopathy could result from alteration in the muscle tissue metabolism such as malfunctioning mitochondria and respiratory exchange ratio (RER) in case of statin, microtubules synthesis in case of colchicine, or interfere with the phosphorylation status as in RTIs<sup>5</sup>.

Colchicine is an alkaloid medication extracted from the corms or seeds of *Colchicum autumnale* and prescribed for patients suffering from gout and familial Mediterranean fever<sup>6</sup>. Colchicine is known for its effects on muscle microtubule synthesis by

<sup>w</sup>Correspondence at: Department of Anatomy and Histology, Faculty of Medicine, Mutah University, PO box 7, Karak, Jordan. Email: [aimanaq@mutah.edu.jo](mailto:aimanaq@mutah.edu.jo)

interference with the polymerization of tubulin, resulting in a myopathy characterized by pain and dysfunction especially at long term uses<sup>7</sup>. Creatine kinase (CK) is an enzyme important as a catalyzer for the reversible phosphorylation of creatine (Cr) by adenosine triphosphate (ATP) in muscle and brain tissue<sup>8</sup>. Increased serum level of CK is an indicator of myopathy as in myocardial infarction (MI). Clinical findings of colchicine-induced myopathy include muscle weakness, increased CK, alteration in electromyography, and histological vacuolar myopathy<sup>4,5</sup>. Stopping the use of the drug relieves symptoms, although prolonged high CK levels are seen in some patients<sup>8</sup>.

Panax Ginseng is a perennial plant belonging to the Panax genus of the Araliaceae family and considered as one of the most highly valued natural dietary supplements worldwide<sup>9</sup>. The plant roots are collected, processed, and formulated into different medicinal supplements. Panax Ginseng contains a number of chemicals that are considered as the active constituents responsible for its therapeutic potentials, including Ginsenosides or ginseng saponins<sup>9</sup>. Ginsenosides amphiphilic properties facilitate its action either through plasma membrane structures or their interaction with steroid receptors<sup>9-11</sup>. The beneficial value of the Panax Ginseng formulations was recognized in different pathologies and symptoms such as diabetes mellitus, tumors, pain, fever, and muscle weakness<sup>12</sup>. Early uses of Panax Ginseng were for the purpose of lowering the aging process and extending life<sup>9</sup>. Furthermore, different extracts of ginseng have demonstrated its beneficial effects against radiotherapy side effects in experimental studies, and these beneficial effects were explained by the improvement of the immune response and antioxidant abilities of ginseng constituents<sup>13</sup>. Ginseng remedies are recognized as an anti-inflammatory, and anti-oxidant factors in a model of myopathy<sup>14</sup>. Recently, ginseng supplements for one month have shown an increase in physical, biochemical and biomechanical properties of muscles in a mice model compared to controls<sup>15</sup>. Ginseng antioxidant effect was demonstrated in cardiac myopathy of rats subjected to hyperbaric oxygen by decreasing damage to the muscle and aortic endothelium<sup>16</sup>. The ability of ginseng supplements to eliminate reactive oxygen species is demonstrated in laboratory studies<sup>17</sup>. This ability of ginseng as an anti-oxidative stress is reported in studies on the skeletal muscles and liver of animal models following intense exercise<sup>18-20</sup>. On the other hand, ginseng supplements have been shown to modulate several enzymes in muscle cells such as creatine kinase, mitochondrial citrate synthase (antioxidant), 3-hydroxyacyl-CoA dehydrogenase (antioxidant), beta Glucuronidase (inflammatory), and glucose-6-phosphate dehydrogenase

(inflammatory) malondialdehyde (antioxidant)<sup>12,19</sup>. The purpose of this study was to investigate the protective effect of ginseng against colchicine-induced skeletal muscles damage in adult male albino rats.

## METHODOLOGY

This study was carried out on 40 adult male albino rats with an average body weight of 150-200g. Animals were allowed free access to food and water *ad libitum* and exposed to controlled conditions of temperature ( $20 \pm 3^\circ\text{C}$ ), and humidity (45-65%) with a 12-12 hrs. light-dark cycle throughout the experimental course. The experiments were conducted according to the ethical norms approved by the Faculty of Medicine Ethics Committee at Mutah University. Rats were accommodated in separate cages and after one-week accommodation, the rats were evenly and randomly divided into 4 groups, 10 rats each. Group I (control group): Rats were given normal diet and orally given normal saline for one month. Since there was no reference compound available, this group received only physiological saline solution, which was also the solution used to dissolve the extract. Group II (Ginseng group): Rats were given ginseng extract (100 mg/kg body weight) via oral gavage daily for one month (dose of 300 mg/kg body weight is within the recommended range<sup>20</sup>). Group III (Colchicine group): rats were given colchicine (50  $\mu\text{g}/\text{kg}$  body weight/day), via oral gavage, for one-month. Physiological saline solution was used to dissolve the extract<sup>21</sup>. Group IV (combined group): Rats were given Ginseng and colchicine as described above for one month. Colchicine 500 $\mu\text{g}$  tablet and Panax ginseng extract capsule 100 mg were purchased from a local pharmacy.

## Serum biochemistry

At the end of experiment, all animals were sacrificed by decapitation after being anesthetized by ether. Blood samples were collected from abdominal aorta in plain tubes for laboratory examination. Creatine kinase (CK) serum activity levels were quantitatively estimated by kinetic International Federation of Clinical Chemistry (IFCC) method using an automatic analyzer (Reckon Diagnostics, India). The values were expressed as mean  $\pm$  SE. The difference between groups was determined using ANOVA test, and  $p < 0.05$  values were considered significant.

## Histology Examination

Skeletal muscle tissue samples were harvested from soleus muscle of the hind limbs of rats. Skin was incised, muscle was exposed and two muscle

samples were immediately harvested and processed for each animal by two methods. The first muscle samples were prepared for light microscopic examination by immediately being fixed in 10% neutral buffered formalin and paraffin, and embedded sections were stained using H & E stain<sup>22</sup>. The second specimen was immediately fixed in 3% glutaraldehyde solution and was processed and examined using transmission electron microscope<sup>23</sup>.

## RESULT

### Biochemical results

Serum Creatine kinase (CK) level is considered an important indicator for skeletal muscle damage. CK levels were increased significantly in the Group III rats administered colchicine, ( $386.8 \pm 59.4$ u/L) compared to the normal control group, group I ( $167.1 \pm 21.4$ u/L). Group IV (combined Colchicine and ginseng) showed no significant increase levels of CK ( $226.8 \pm 76.4$ u/L). Adding ginseng only to food (group II) did not show significant increase on CK levels ( $186.4 \pm 22.3$ u/L).

### Histological Results

Examination of longitudinal sections of skeletal muscle under light microscope showed that, rats in control and ginseng groups (groups I & II), muscle fibers have normal striation, dark acidophilic sarcoplasm and multiple flattened nuclei at the periphery (**Figure 1a**). Group III (colchicine group) presented abnormal changes including, variation in the size of muscle fibers, loss of organization, mononuclear infiltration, focal necrosis and edema in the extra cellular matrix (**Figure 1b-d**). Concomitant administration of ginseng with colchicine ameliorates most of these changes in muscle tissue (**Figure 1e and f**).

### Electron microscopic results

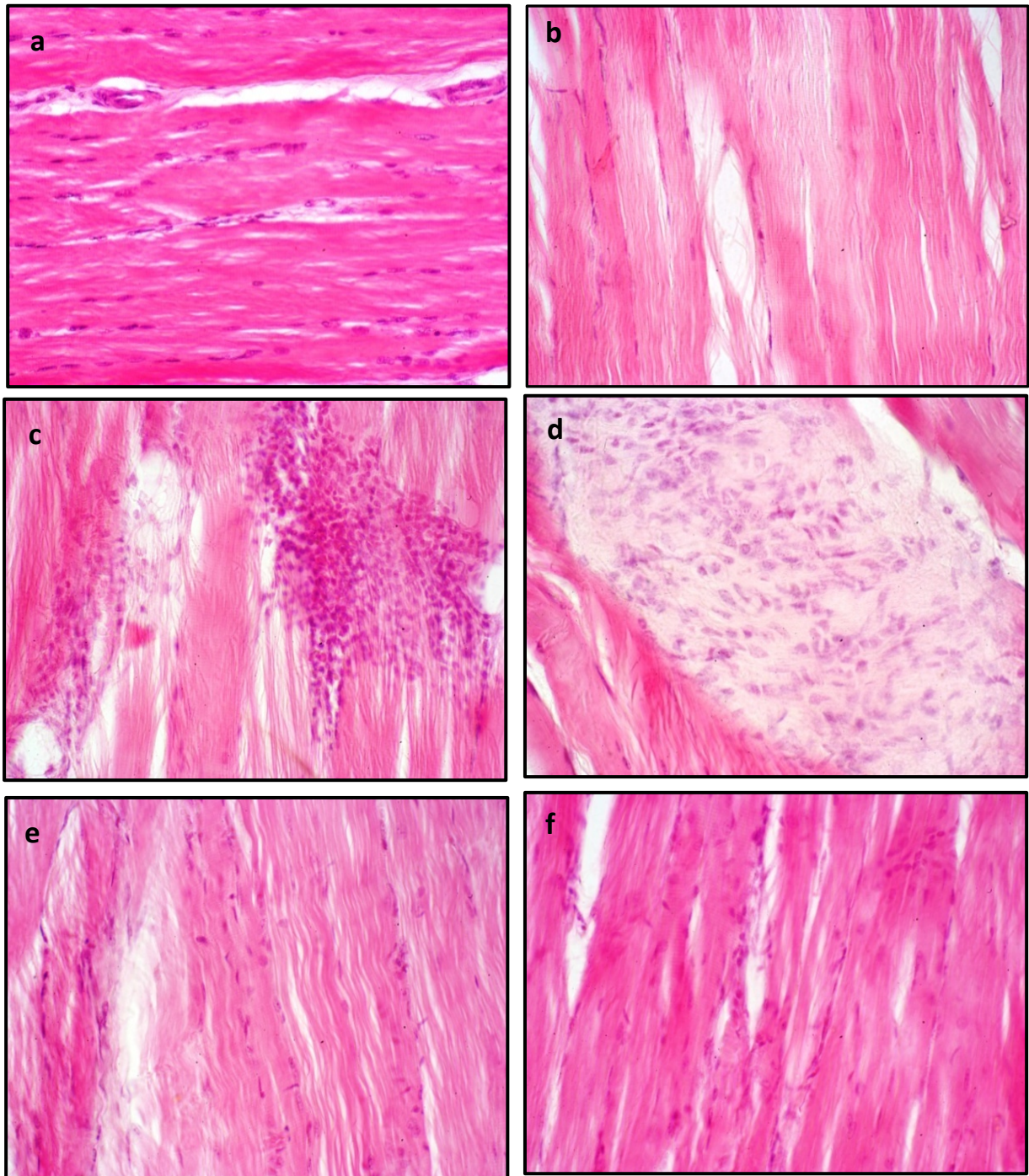
Muscle sections examined under transmission electron microscope (TEM) for rats in groups I and II showed normal organized muscle fibers with normal appearance and presence of mitochondria, no abnormality in the microstructure of myofibrils which includes the alternating dark and light bands, Z lines, A band, I band and H zone (**Figure 2a**). In the Colchicine group (group III), rats' muscle tissue demonstrated abnormality, which included cells with irregularly outlined and condensed nuclei, fragmented and discontinuous myofibrils (**Figure 2b**), abnormal shape and arrangement of the mitochondria (**Figure 2c**). Rats in the combined group (received concomitant colchicine and panax ginseng; group IV) showed that most of the

myofibrils demonstrated normal arrangement of A and I bands. However, myofibrils spacing and mitochondria polymorphs could be seen in certain locations of the muscle tissue (**Figure 2d and e**).

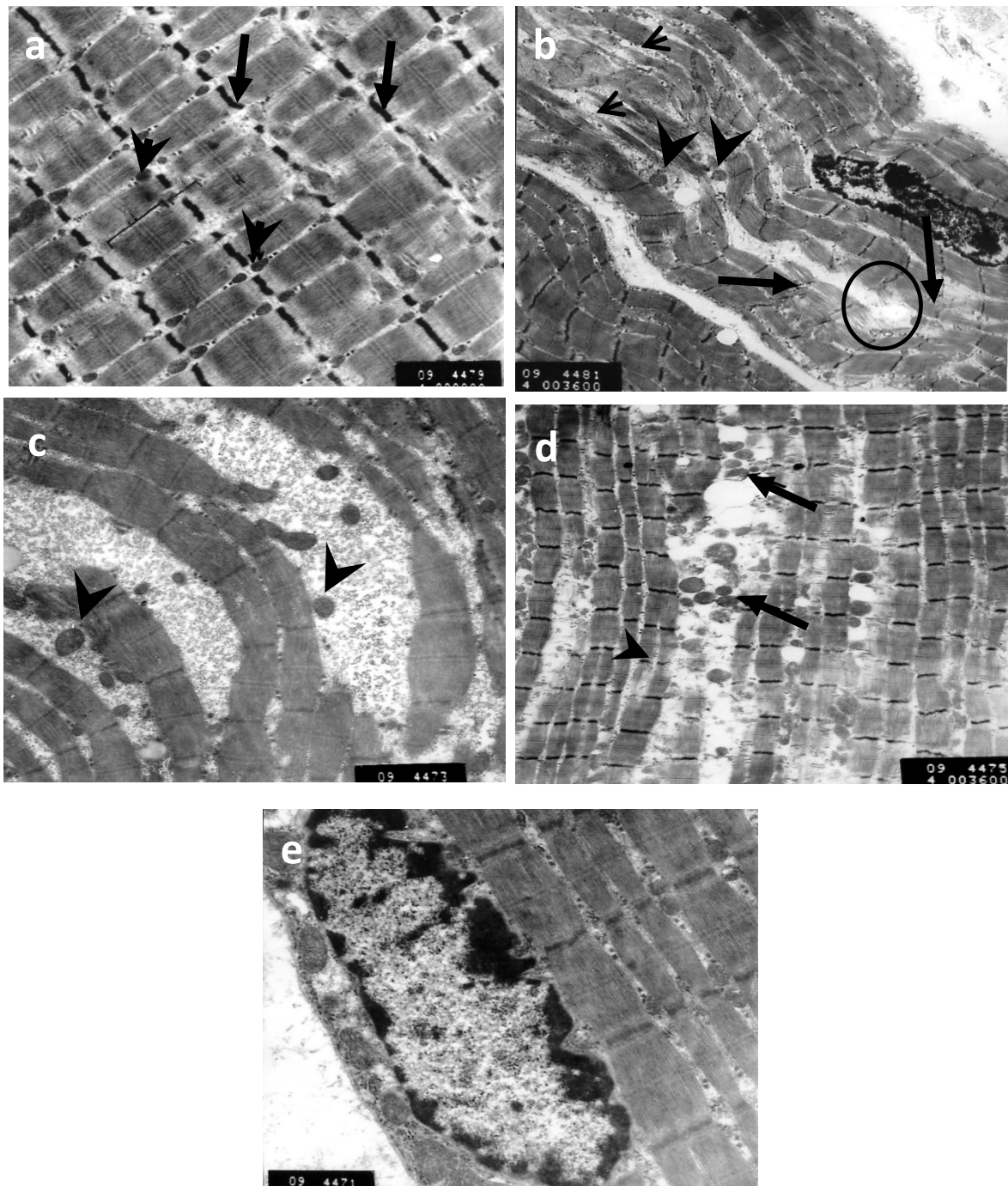
## DISCUSSION

This *in vivo* animal study appeared to prove colchicine-induced myopathy model in skeletal muscle supported by histological, and blood chemistry findings. In addition, histological, and biochemistry results support the use of Panax Ginseng as supplement to lessen the adverse effects of colchicine induced myopathy in skeletal muscle tissue. Initially, Serum Creatine kinase (CK), an indicator of muscle tissue injury, levels were increased in colchicine-treated group. Our findings were consistent with previous reported case study of patients with Familial Mediterranean Fever who received colchicine treatment with high levels of serum CK and inflammatory cells and disorganized myofibrils and formation of vacuoles<sup>24</sup>. Furthermore, our findings are in accordance with previous studies, which demonstrates a characteristic myopathic change in muscle in experimental animals<sup>25,26</sup>. Colchicine myopathy and polymyositis cases experience similar symptoms of weakness in their proximal muscles, elevated levels of serum CK and electromyography (EMG) profile<sup>27</sup>.

Colchicine may produce a neuromuscular disorder even when given in customary doses. Colchicine myoneuropathy was recognizable by the quick clinical and electrophysiologic improvement following drug withdrawal<sup>21,28</sup>. In colchicine toxic effects on muscle tissue, the histological profile showed disorganized myofibers, lack of striation, inflammatory cell infiltrate, edema and separation of fibers<sup>21,24</sup>. However, there are two mechanisms by which colchicine produce pathology profile: by inhibition of tubulin polymerization into microtubules, as well as by alteration in the expression of dopaminergic receptors<sup>2,29</sup>. Colchicine affects microtubules synthesis in cells by inhibiting its polymerization, therefore, affecting the transporting system within cells notably muscle and nerve fibers<sup>26,30</sup> and subsequently affecting cell division<sup>30,31</sup>. One potential explanation for the autopathic vacuoles seen in histological sections is the interference with lysosomes transportation within the microtubules<sup>21</sup>. Soleus muscle (primarily Type I skeletal muscle fibers) was found to have a 1.7-fold higher relative content of  $\alpha$ -tubulin compared with primarily Type IIb skeletal muscle fibers. The selective type I involvement is probably due to the higher tubulin amount in type I fibers<sup>14,32</sup>.



**Figure 1: Photomicrograph of longitudinal section of soleus muscle of rats stained with H&E X400. (a) Group I (control group) showing normal muscle features; (b-d) Group III (Colchicine group); (b) area of disturbance normal striation and pale acidophilic sarcoplasm and edema in the interstitium associated with focal area of muscle fiber necrosis; (c and d) a disarray of myofibers, loss of striation, increased space between fibers due to increased connective tissue and edema associated with mononuclear cellular infiltration; (e and f) Group IV (combined Colchicine and Ginseng) a mild loss of normal striation and mild cellular infiltration.**



**Figure 2: TEM images of longitudinal section of soleus muscle of rats. (a) Control group and ginseng group (group I & II), showing normal pattern of striation, A band, I band, Z line (arrowed), H zone and mitochondria (wide arrowheads) between the muscle fibers; (b and c) Colchicine group (group III) showing, fragmentation of myofibrils (arrowheads), in some areas the myofibrils are lost (encircled), the nucleus is irregular in shape and shows condensed chromatin. The Z-lines are seen to be interrupted (arrowed); Notice a small number of mitochondria (wide arrowheads); (d and e) Combined group (colchicine and panax ginseng; group IV) showing the protected effect of ginseng normal arrangement of A and I bands in (d) and mitochondria polymorphs (arrowed) in (e) and muscle fibers appeared similar to the control group.**

Colchicine myopathy treatment is simple and based on colchicine interruption. Our data suggested that *Panax ginseng* could reduce muscle damage and help prevent injuries. Changes induced by

colchicine in rats' muscle tissues were improved after concomitant administration of ginseng with colchicine<sup>20</sup>. *Ginseng* in different formulations has resulted in the decrease of serum CK levels,

peroxidation of lipids, and some inflammatory indicators in rats that experience treadmill<sup>14</sup>. The protective effect of ginseng on colchicine-induced toxic myopathy by lowering serum CK is hypothesized to be mediated by the antioxidant effect of its active ginsenoside ingredients<sup>16,33</sup>. Ginsenosides interactions with cell membrane will modify its fluidity and prevent membrane peroxidation<sup>34</sup>. Ginseng helps in rhinitis<sup>35</sup> and antioxidant activity<sup>20,36</sup>.

## Conclusion

The model used in the present study to induce myopathy was colchicine. The muscle showed abnormal changes. The administration of panax ginseng was able to improve colchicine-induced damage within skeletal muscle.

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