

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

<https://doi.org/10.17113/ftb.64.04.26.9325>

original scientific paper

Evaluation of the Protective and Antioxidant Effects of Apilarnil Against Acute Exercise Induced Skeletal Muscle Damage in Rats

Running title: Apilarnil and Exercise-Induced Muscle Damage

Cemre Başol^{1*}, Meral Kekeçoğlu² and Sinem Kantarcioğlu Coşkun³

¹Duzce University Natural, Herbal and Cosmetic Products Doctoral Program, 81620 Duzce, Turkey

²Duzce University Faculty of Science, Department of Biology, 81620 Duzce, Turkey

³Duzce University Medical Faculty, Department of Pathology, 81620 Duzce, Turkey

Received: 17 August 2025

Accepted: 18 October 2026



Copyright © 2026 Authors retain copyright and grant the FTB journal the right of first publication under CC-BY 4.0 licence that allows others to share the work with an acknowledgement of the work's authorship and initial publication in the journal

SUMMARY

Research background. This study investigated the potential muscle-reparative effects of apilarnil, a drone bee-derived bioactive product, and its protective role against skeletal muscle damage induced by acute physical exercise. It aimed to compare the muscle repair mechanisms of apilarnil and whey protein in relation to oxidative stress and antioxidant status in male Sprague Dawley rats.

Experimental approach. Thirty-six male Sprague Dawley rats were randomly assigned to six experimental groups ($N=6$ per group): control (C), apilarnil only (AP), whey protein only (W), exercise only (E), exercise combined with whey protein (E+W), and exercise combined with apilarnil (E+AP). The exercise protocol included a two-week adaptation period followed by a single session of acute treadmill running. Apilarnil (0.8 g/kg) and whey protein (2 g/kg) were administered orally as a single

*Corresponding author

Phone: +5541976217

E-mail: cemrebasol1@gmail.com

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

dose 30 minutes post-exercise. Blood samples were collected via cardiac puncture for biochemical analyses of muscle injury markers creatine kinase (CK) and lactate dehydrogenase (LDH) as well as antioxidant enzymes including catalase (CAT) and glutathione (GSH), alongside inflammatory parameters. Skeletal muscle tissue samples were also subjected to histopathological evaluation.

Results and conclusions. Acute exercise induced oxidative stress, accompanied by significant increases in CK and LDH levels. Apilarnil administration attenuated these increases and enhanced antioxidant enzyme levels, suggesting an improved antioxidant defense against exercise-induced oxidative stress. Although the present findings do not provide conclusive evidence regarding the involvement of the SIRT1 signaling pathway in these protective effects, further mechanistic studies are required to clarify its role. Overall, these findings support the potential of apilarnil as a natural functional dietary supplement for reducing exercise-induced oxidative stress and promoting muscle recovery following acute physical activity.

Novelty and scientific contribution. This study is the first to compare the muscle repair mechanisms of apilarnil and whey protein in male Sprague Dawley rats in relation to oxidative stress and antioxidant status. The findings highlight apilarnil's potential as a natural alternative to whey protein, one of the most widely used sports supplements worldwide.

Key words: apilarnil; natural product; exercise; muscle damage; oxidative stress

INTRODUCTION

Drone brood larvae (apilarnil), a relatively underexplored but increasingly important bee product, has garnered attention in recent years due to its broad applicability in health-related fields, including medicine, cosmetics, and nutritional supplementation. Especially in the context of traditional and complementary medicine, apilarnil has emerged as a promising natural substance. It is obtained from 3–7 days old male honey bee larvae prior to pupation and is characterized by a viscous, nutrient-rich composition [1]. Chemically, fresh apilarnil contains 65–75 % water, 1–2 % ash, 7.23–15.4 % protein, 6.92–12.2 % carbohydrates, 3.5–8.38 % fatty acids and lipids, as well as a variety of vitamins and minerals [2–4]. Furthermore, it possesses a notable hormonal profile, including testosterone (14.80 ng/g), progesterone (14.40 ng/g), estradiol (4.12 ng/g), and prolactin (1.2 ng/g) [5], which contributes to its androgenic properties and potential use in treating male reproductive disorders.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Previous studies have demonstrated that apilarnil may stimulate the immune system, reduce cholesterol levels, enhance antioxidant capacity, and support cognitive function [6].

In recent years, in addition to the health benefits of regular exercise, studies have shown that muscle damage, inflammation or some oxidant effects are observed in unusual and severe types of exercise. The skeletal muscle may be damaged due to trauma, sudden movement or unfamiliar exercise in the skeletal muscle, and some of the routine mobility may be lost. Skeletal muscle damage involves first the process of apoptosis, then myofiber necrosis (myonecrosis) and the formation of inflammatory cytokines by immune cells such as macrophages and neutrophils [7]. It is known that the production of free radicals and reactive oxygen species (ROS) increases after intense acute exercise [8]. Oxygen consumption and heart rate increase proportionally due to increased muscle contractions depending on the intensity of exercise. The increase in energy needs increases metabolic activity and oxygen is consumed up to 20 times in exercise and up to 200 times in the muscles compared to the resting state [9]. Enhanced mitochondrial respiration leads to insufficient reduction of oxygen to water and loss of electrons in the mitochondrial electron transport chain. Thus oxidative stress enhances by the increasing in ROS [10]. If the rising ROS levels during exercise exceeds the antioxidant defense capacity of the cells, lipid peroxidation occurs [11].

For protein synthesis to be enhanced and protein degradation to be suppressed in muscle tissue, a substantial supply of amino acids must be delivered to myocytes. Exogenous amino acids administered post-exercise have been shown to mitigate oxidative stress by promoting anabolic processes within skeletal muscle. Apilarnil has been reported to exhibit significant androgenic activity, influencing sex hormone levels in rats [12]. Androgens, particularly testosterone, exert potent anabolic effects on both skeletal muscle and bone, enhancing calcium binding to bone tissue, stimulating protein synthesis, and contributing to musculoskeletal development [13]. Apilarnil, known for its rich amino acid profile and pronounced androgenic properties, presents as a promising natural nutritional supplement for athletes. It may help reduce skeletal muscle damage and oxidative stress associated with high-intensity exercise [14]. Although limited, existing studies suggest that apilarnil may increase muscle mass and protect against exercise-induced muscle degradation. These effects are likely attributable to its comprehensive amino acid composition and high protein content. Furthermore, as a natural product with robust antioxidant properties, apilarnil may help restore oxidative balance disrupted by exercise by promoting oxidative metabolic processes, thus enhancing exercise performance [15,16]. In addition, apilarnil has been associated with increased muscle glycogen

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

utilization and demonstrated energizing effects [16]. Its ability to address amino acid deficiencies makes it a valuable ergogenic aid for improving performance [17].

However, despite growing interest in the chemical composition of apilarnil, evidence regarding its in vivo physiological effects remains limited. Therefore, the present study aimed to investigate the effects of apilarnil as an alternative protein source on exercise-induced skeletal muscle damage and to evaluate its in vivo efficacy through histological assessment and analysis of oxidant/antioxidant balance.

MATERIALS AND METHODS

Nutrient content analysis

Apilarnil utilized in this study was produced at the Duzce University Beekeeping Research, Development, and Application Center (DAGEM). The physicochemical properties of the lyophilized apilarnil were analyzed and are presented in Table 1. Additionally, the percentages of total protein, carbohydrate, lipid, and sugar contents were determined. Total protein content was quantified using the Lowry colorimetric assay, in which proteins react with the Folin–Ciocalteu reagent after complex formation with copper ions under alkaline conditions, and absorbance was measured spectrophotometrically [18]. Lipid content was determined by the Soxhlet extraction method using diethyl ether as the extraction solvent, allowing continuous extraction of lipids from the lyophilized samples [19]. Carbohydrate and individual sugar contents were analyzed by HPLC-RI (Hitachi, Chromaster software) after appropriate sample extraction, with chromatographic separation and quantification performed according to the method described by Sesta [20].

Animal experiments

A total of thirty-six male Sprague-Dawley rats (aged 8–10 weeks; body weight 250–400 g) were used in the experiments. All animals were housed under standard laboratory conditions with a 12/12-hour light/dark cycle, an ambient temperature of 23 °C, and a relative humidity of (60±5) %. Rats were provided with standard pellet chow and tap water *ad libitum* throughout the study period. Male rats were equally assigned to six experimental groups: control group (C, *N*=6), apilarnil group (AP, *N*=6) receiving apilarnil supplementation, whey group (W, *N*=6) receiving whey protein supplementation, exercise group (E, *N*=6) subjected to acute exercise, exercise plus whey group (E+W, *N*=6) subjected to acute exercise and supplemented with whey protein, and exercise plus apilarnil group (E+AP, *N*=6) subjected to acute exercise and supplemented with apilarnil (Fig. 1).

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Exercise protocol

Prior to the acute exercise intervention, all rats in the exercise groups underwent a treadmill adaptation protocol using a motor-driven rodent treadmill (MAY/TME, Turkey) for a duration of two weeks. During the first week, animals were subjected to familiarization sessions consisting of treadmill running at a speed of 15 m/min, for 10 min per day, three days per week, on a 0 % incline. In the second week, the exercise intensity was increased to 20 m/min for 15 min per day, again three days per week, maintaining the same 0 % incline. 48 h following the completion of the adaptation period, an acute exercise protocol was applied in three sequential phases. In the first phase, rats ran for 5 min at 15 m/min on a 0 % incline. The second phase consisted of 10 minutes of running at 20 m/min on a 0 % incline. In the third and final phase, the protocol was completed with a 60-minute running session at 25 m/min on a 5 % incline [21].

Administration of apilarnil and whey protein

Powdered apilarnil was prepared by dissolving it in distilled water at a dose of 0.8 g/kg [22]. The solution was freshly prepared prior to each administration and delivered to the AP and E+AP groups via oral gastric gavage, 30 min after each acute exercise session throughout the experimental period. Similarly, isolated whey protein was prepared by dissolving it in distilled water at a dose of 2 g/kg [23]. The freshly prepared solution was administered to the designated groups by oral gastric gavage at the same time point 30 min following each exercise session. Following the final exercise session, oral supplement administration, and body mass measurements, all animals were anesthetized via intraperitoneal injection of a ketamine (90 mg/kg) and xylazine (10 mg/kg) combination. Blood samples were collected via cardiac puncture under anesthesia. Immediately after decapitation, gastrocnemius and biceps muscle tissues were excised from each rat for subsequent biochemical and histological analyses.

Histological analysis

Following euthanasia, gastrocnemius and biceps muscle tissue samples were harvested, carefully dissected to remove surrounding connective tissues, and subsequently weighed. The specimens were fixed in 10 % neutral buffered formaldehyde for 24 h. Transverse and longitudinal sections were prepared from each tissue sample. After routine tissue processing, samples were embedded in paraffin blocks using a tissue processor (Sakura Tissue-Tek, Japan), sectioned with a rotary microtome (Leica RM2245, China), and stained with hematoxylin and eosin (H&E) for histopathological examination.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Histological evaluation was performed by a board-certified pathologist using a light microscope (Nikon Eclipse, Japan). Multiple randomly selected non-overlapping microscopic fields from each section were examined at 10, 20 and 40× magnification. Tissue sections were assessed using a semiquantitative histological scoring system (H-score), where 0=normal, 1=mild, 2=moderate, and 3=severe damage. The parameters evaluated included myonecrosis, formation of immature muscle fibers, edema, perifascicular fat infiltration, and chronic inflammation.

Measurement of biochemical parameters

Blood samples were centrifuged at 4000 rpm for 15 min to separate the serum. The concentrations of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), creatine kinase (CK), lactate dehydrogenase (LDH), sirtuin-1 (SIRT1), interleukin-6 (IL-6), and interleukin-10 (IL-10) were quantitatively determined using commercially available colorimetric assay kits according to the manufacturer's instructions: Rat SOD ELISA Kit (Cat. No. 201-11-0169-96T), Rat CAT ELISA Kit (Cat. No. 201-11-5106-96T), Rat GSH ELISA Kit (Cat. No. 201-11-5134-96T), Rat MDA ELISA Kit (Cat. No. 201-11-0157-96T), Rat CK ELISA Kit (Cat. No. 201-11-0905-96T), Rat LDH ELISA Kit (Cat. No. 201-11-0531-96T), Rat SIRT1 ELISA Kit (Cat. No. 201-11-1498-96T), Rat IL-6 ELISA Kit (Cat. No. 201-11-0136-96T), and Rat IL-10 ELISA Kit (Cat. No. 201-11-0109-96T) (SunRed Bio, Shanghai, China).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics v. 27.0 [24] and GraphPad Prism v.10.0 Software [25]. Data are presented as mean±standard deviation. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). When a significant overall effect was detected, pairwise comparisons were conducted using the least significant difference (LSD) post hoc test. A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Changes in body and muscle mass

Body mass of all experimental groups was monitored periodically throughout the exercise intervention. A statistically significant difference was observed in the body mass of rats within each group when comparing measurements taken at the beginning and at the end of the study. The lowest body mass gain was observed in the AP and E groups, respectively, while the highest body mass gain was observed in the C group (Table 2).

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

At the conclusion of the experiment, the mass of the gastrocnemius and biceps muscle tissues was measured. An increase in gastrocnemius muscle mass was noted in the AP group relative to the C group (Fig. 2a). Although differences in biceps muscle mass (Fig. 2b) was not statistically significant, the AP group exhibited higher values than the other groups. These findings are in agreement with previous research [26]. Consistent with the present results, Yokoyama *et al.* reported that protein derived from bee larvae prevented muscle damage and muscle mass loss, and reduced levels of 3-methylhistidine, a biomarker of muscle protein breakdown [17]. Additionally, Kogalniceanu-Odobescu *et al.* demonstrated that apilarnil supplementation in rats undergoing acute swimming exercise stimulated oxidative pathways involved in energy metabolism. Their study indicated that apilarnil acts as a potent bioenergetic agent by enhancing energy metabolism and improving exercise performance while reducing fatigue [16]. Similarly, in the present study, although overall body mass gain in the apilarnil-supplemented group was modest, a significant increase in gastrocnemius muscle mass was observed. This finding suggests that apilarnil supplementation may exert favorable effects on muscle tissue and supports its potential to promote lean muscle development, although further studies are needed to determine whether these effects extend to overall skeletal muscle mass.

Biochemical parameters

Exercise, known to induce a wide range of physiological and biochemical changes, significantly increases oxygen consumption in the body, particularly within skeletal muscle [27]. This elevated oxygen demand leads to a marked increase in ROS production. While ROS generation occurs during physical activity, the concomitant upregulation of endogenous antioxidant defense mechanisms serves to mitigate potential oxidative damage. However, the extent of this compensatory response is influenced by the type, intensity, and duration of the exercise performed [28]. During high-intensity exercise, when oxygen consumption rises sharply, the antioxidant defense system may fail to match the pace of ROS production, thereby resulting in oxidative stress [29]. The majority of evidence from both human and animal studies suggests that MDA, a well-established marker of oxidative stress, does not change during acute bouts of exercise [30]. Nonetheless, several studies have demonstrated that MDA levels may increase in response to elevated metabolic activity [31–33]. In the present study, a statistically significant difference in MDA concentration was observed among the groups ($p < 0.001$). Specifically, the E group exhibited a significant increase in MDA levels compared to the C group ($p = 0.017$) (Table 3). The significant increase in MDA levels observed in the E group supports the notion that the applied acute exercise protocol induced oxidative stress.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

The literature consistently highlights the importance of mitigating exercise-induced oxidative stress through the use of antioxidant-rich supplements. Antioxidant supplementation has been shown to offer protective effects against the formation of free radicals generated during exercise and to counteract their deleterious impacts [34]. Apilarnil has emerged as a promising therapeutic and dietary supplement due to its rich biochemical composition and the positive outcomes reported in previous studies. As a bee derived product, apilarnil possesses notable antioxidant properties and exhibits protective effects against muscle damage, primarily attributed to its high content of proteins, amino acids, vitamins, and minerals. In the present study, although a reduction in MDA concentrations was observed in the E+AP group compared to the increase seen in the E group, this decrease did not reach statistical significance. The W group exhibited a pronounced increase in MDA levels compared with the C group ($p=0.003$) (Table 3). Notably, the highest MDA levels were detected in the W and E+W groups, suggesting that whey protein may be more prone to promoting lipid peroxidation via free radical generation than apilarnil. It is well established that exercise-induced increases in MDA are associated with enhanced lipid peroxidation, which can disrupt ion homeostasis and impair cell membrane integrity [35]. In such cases, inadequate superoxide dismutation due to SOD deficiency leads to excessive superoxide radical accumulation, potentially resulting in hydrogen peroxide formation or peroxynitrite generation via reaction with nitric oxide. When SOD activity is insufficient, CAT is activated to degrade the accumulating hydrogen peroxide, forming part of the enzymatic antioxidant defense system that helps maintain cellular redox balance and suppress MDA levels elevated during acute exercise. In parallel, GSH, functioning as a non-enzymatic antioxidant, contributes to hydrogen peroxide detoxification [36].

Previous findings regarding the antioxidant response to exercise remain inconsistent, largely depending on exercise modality and intensity. For instance, Çakar *et al.* reported that intense exercise increased CAT, SOD, and GSH activities [14]. Tauler *et al.* observed increased CAT and GSH activities in response to prolonged intense exercise, while SOD activity remained unchanged [37]. In contrast, Thirumalai *et al.* demonstrated that chronic intensive exercise elevated oxidative stress while reducing the activities of SOD, CAT, and GSH in rats [38]. Similarly, other studies have documented reductions in neutrophil antioxidant activity following high intensity exercise, suggesting that oxidative stress may suppress endogenous antioxidant capacity [39,40]. In the current study, while the acute exercise protocol elicited oxidative stress, it did not produce significant alterations in the concentrations of superoxide dismutase (SOD), catalase (CAT), or glutathione (GSH), which are essential elements of the antioxidant defense system. These findings, in line with previous research,

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

indicate that antioxidant activity may decrease in response to higher exercise intensities. Thus, it is plausible that the intensity or duration of the exercise regimen implemented in this study was insufficient to impair the body's antioxidant defense mechanisms.

Statistical analysis of CAT and GSH levels among the experimental groups revealed significant differences ($p=0.016$ and $p=0.026$, respectively). In terms of antioxidant enzyme levels, both the AP ($p=0.012$) and W ($p=0.016$) groups showed significantly higher CAT levels compared with the C group. Similarly significant increases in GSH were observed in the AP ($p=0.006$) and W ($p=0.018$) groups relative to the control. Notably, in the non-exercised groups, both AP and W demonstrated elevated CAT and GSH concentrations compared with the control, indicating an enhanced antioxidative response. Moreover, a significant elevation in CAT level was observed in the E+AP ($p=0.040$) and E+W ($p=0.031$) groups relative to the group subjected to acute exercise alone. GSH level was significantly elevated in the E+AP group compared to the E group ($p=0.047$) (Table 3). These findings suggest that apilarnil supplementation enhances antioxidant defense in response to exercise, in line with previous studies reporting that both apilarnil and whey protein exert antioxidant effects by upregulating GSH and CAT levels [22,41]. For instance, Doğanyığıt *et al.* reported that apilarnil supplementation reversed increases in MDA and reductions in SOD and CAT levels in the brain and liver tissues of LPS-induced rats [22]. Collectively, the results of the present study reinforce the notion that apilarnil acts as a natural antioxidant supplement. Its ability to enhance CAT and GSH levels highlights its role in scavenging hydrogen peroxide and contributing to cellular antioxidant defense, particularly under oxidative stress conditions such as acute exercise.

It is well established that structural deterioration of the muscle membrane occurs following exercise, especially after unaccustomed, eccentric, or high-intensity acute exercise [42]. Muscle damage results in the leakage of intracellular enzymes, primarily creatine kinase (CK) and lactate dehydrogenase (LDH), into the bloodstream. In the present study, significant differences were observed in CK and LDH concentration among the groups ($p=0.005$ and $p=0.027$, respectively). Specifically, plasma CK concentration was significantly elevated in the E group compared to the C group ($p=0.003$). Similarly, LDH concentration was significantly higher in the E group relative to controls ($p=0.02$). These findings are consistent with previous studies; for instance, Viña *et al.* demonstrated increased plasma CK and LDH concentration following intense exercise in both rats and humans [43]. Likewise, Malaguti *et al.* reported elevated levels of these enzymes in rats subjected to acute exercise protocols [44]. In the present study, this outcome confirms that the applied exercise

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

protocol effectively induced muscle damage, consistent with previous studies employing similar models.

Following exercise-induced muscle damage, an increase in protein turnover occurs, characterized by enhanced protein degradation followed by compensatory protein synthesis, initiating muscle repair within approximately 48 h [45,46]. In the current study, in order to evaluate the immediate effects of apilarnil supplementation, animals were sacrificed 30 min after the completion of the exercise protocol and supplement administration, and their CK, LDH and SIRT1 levels were determined.

CK concentrations were significantly reduced in the E+W ($p<0.001$) and E+AP ($p=0.012$) groups compared to the E group (Fig. 3a). Additionally, LDH concentration was significantly higher in the E+AP ($p=0.014$) and E+W ($p=0.008$) groups compared to the E group (Fig. 3b). These results suggest that apilarnil and whey protein supplementation may attenuate exercise-induced alterations in CK and LDH concentrations, which are commonly used as indicators of muscle damage.

Based on the obtained data, a statistically significant difference was observed among the groups in terms of IL-6 concentration ($p=0.01$). Specifically, the W group showed significantly elevated IL-6 concentrations compared to the C group ($p=0.011$) (Table 3). It should be noted that exercise-induced IL-6 release is not exclusively associated with an inflammatory response. Skeletal muscle-derived IL-6 functions as a myokine and plays important roles in exercise adaptation, including the regulation of energy metabolism through the activation of glycogenolysis [47]. Moreover, IL-6 can exhibit both pro-inflammatory and anti-inflammatory properties depending on the physiological context and cellular environment. Previous studies have reported conflicting effects of whey protein on inflammatory cytokines, with findings indicating reductions [48], no change [49] or increases [50] in inflammatory marker concentrations. Therefore, the elevated IL-6 concentration observed in the W group should not necessarily be interpreted as an indicator of enhanced inflammation, but may also reflect alterations in metabolic signaling or exercise-related adaptation. In the present study, this elevation is likely influenced by the dosage and duration of whey protein supplementation, highlighting the complex and multifaceted regulatory role of IL-6 in inflammatory processes.

SIRT1 is a nicotinamide adenine dinucleotide (NAD^+)-dependent class III histone deacetylase that functions as a central regulator of cellular metabolism, oxidative stress, inflammation, apoptosis, and aging. SIRT1 exerts its biological effects through the deacetylation of multiple transcription factors and signaling molecules, including p53, nuclear factor-kappa B (NF- κ B), and peroxisome proliferator-

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

activated receptor gamma coactivator-1 alpha (PGC-1 α), thereby contributing to the maintenance of metabolic homeostasis and cellular adaptation to stress [51]. Reduced SIRT1 expression or activity has been associated with aging, metabolic disorders, cardiovascular diseases, neurodegenerative diseases, and other pathological conditions characterized by oxidative stress and chronic inflammation. Previous studies have demonstrated that exercise-induced activation of SIRT1 promotes PGC-1 α deacetylation and interacts with AMP-activated protein kinase (AMPK), thereby facilitating mitochondrial biogenesis, enhancing antioxidant defense, and supporting metabolic adaptations in skeletal muscle [52]. In addition, antioxidant supplementation has been reported to modulate exercise-induced physiological responses through the upregulation of SIRT1 activation or gene expression [53]. For instance, Yokoyama *et al.* demonstrated that mice supplemented with bee larva-derived protein exhibited increased SIRT1 protein expression [17]. Similarly, Lensu *et al.* reported elevated SIRT1 and SIRT3 expression in rats subjected to jogging combined with whey protein supplementation compared to controls [54]. In the present study, however, no significant differences were observed in plasma SIRT1 concentrations among the experimental groups (Fig. 3c). The apparent discrepancy between these findings may, at least in part, be attributed to differences in the biological endpoints assessed, as the aforementioned studies evaluated SIRT1 expression in skeletal muscle tissue, whereas the present study quantified circulating SIRT1 concentrations. Moreover, variations in supplementation regimen, exercise protocol, and timing of sample collection may have further contributed to the divergent findings. Accordingly, future studies incorporating direct assessment of SIRT1 expression and enzymatic activity in skeletal muscle tissue are warranted to clarify the potential contribution of this signaling pathway to the protective effects of apilarnil supplementation.

Histopathological evaluation

Skeletal muscle damage typically involves apoptosis followed by myofiber necrosis (myonecrosis) and subsequent production of inflammatory cytokines by immune cells such as macrophages and neutrophils [7]. Histologically, myonecrosis is characterized by swollen, eosinophilic, and homogeneous myofibers with vacuolation and fragmented pyknotic nuclei [55]. In the present study, a higher number of muscle fibers exhibiting myonecrosis was observed in the gastrocnemius and biceps muscles across nearly all groups, compared with other types of tissue damage. Assessment of muscle damage revealed that degenerative changes were more pronounced in the gastrocnemius muscle than in the biceps muscle. The observation of myonecrosis and degeneration with similar histopathological scores across all groups in both muscle tissues, including

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

the control group, suggests that pre-existing stress or trauma may have contributed to baseline alterations in muscle tissue.

Following myonecrosis, immature muscle fibers and interstitial edema were observed, in that order (Fig. 4). Muscle edema is commonly observed in a spectrum of non-infectious, immune-mediated inflammatory conditions affecting skeletal muscle and typically occurs secondary to necrosis and inflammation [56]. In the present study, edema was identified histologically based on the presence of increased interstitial spaces, separation of muscle fibers, and expansion of the connective tissue compartment within the muscle sections. These findings were considered indicative of fluid accumulation within the interstitial space and were evaluated according to the semiquantitative scoring system. Immature muscle fibers, which comprise muscle precursor cells and serve as indicators of regenerative activity, are characterized by their small size and presence of centralized nuclei [57]. Semiquantitative evaluation indicated no significant differences in immature muscle fiber prevalence across the experimental groups. However, a consistently higher abundance of immature fibers was observed in the gastrocnemius muscle relative to the biceps muscle across all groups.

In the current study, perifascicular fat accumulation was mild, and no evidence of chronic inflammation was detected. Intramuscular fat accumulation, defined as adipocyte infiltration within skeletal muscle, has been associated with decreased muscle strength, impaired insulin sensitivity, and increased mortality, particularly in the aging population [58]. In the present histological analyses, only minimal perifascicular fat accumulation was detected, exclusively in the exercise and whey groups. The absence of statistically significant differences may reflect the insufficient stimulus of the employed exercise model to induce fat deposition in muscle tissue. Moreover, the short duration between nutritional supplementation and sacrifice approximately thirty minutes may have limited the development of detectable fat accumulation.

Based on the findings of the present study, apilarnil appears to have the potential to support increases in gastrocnemius muscle mass and enhance exercise performance. These results suggest that apilarnil may be a promising natural supplement for athletes aiming to promote muscle development during physical activity. Its high protein content, combined with relatively low carbohydrate and lipid levels, further underscores its suitability as a dietary aid in sports nutrition. The acute exercise protocol and two-week supplementation period employed in this study were sufficient to elicit cellular-level changes in rats; however, no significant effects were observed at the tissue level. Future studies involving longer intervention periods, varied dosing regimens, or more intense exercise

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

models may reveal chronic tissue damage in the exercise groups, thereby enabling a more comprehensive assessment of the reparative potential of nutritional supplements such as apilarnil. Furthermore, the use of immunohistochemical techniques may provide more detailed insights into the mechanisms of muscle regeneration.

CONCLUSIONS

In conclusion, the findings of this study suggest that apilarnil may contribute to the attenuation of exercise-induced oxidative stress, as indicated by the observed changes in biochemical parameters associated with antioxidant status (CAT and GSH) and muscle injury (CK and LDH). Although no significant histopathological differences were detected among the experimental groups, the overall biochemical findings support the potential of apilarnil to promote recovery following acute physical exercise. Although no definitive evidence was obtained to confirm the involvement of the SIRT1 signaling pathway in the observed protective effects, its potential role cannot be ruled out and should be explored in future mechanistic studies. Overall, apilarnil appears to be a promising natural functional supplement for alleviating exercise-induced oxidative stress and supporting skeletal muscle recovery.

STUDY LIMITATIONS

The present study has several limitations. First, the findings were obtained using an experimental rat model and therefore cannot be directly extrapolated to humans. Second, the relatively small sample size ($N=6$ per group) may limit the statistical power of the study. Third, only the effects of a single dose of apilarnil administered after an acute exercise protocol were evaluated; therefore, the long-term effects, dose-response relationship, and efficacy under chronic exercise conditions remain unclear. Another limitation is that the lack of pre-exercise blood sampling prevented within-animal comparison of biochemical parameters before and after exercise. Furthermore, although histopathological alterations were evaluated, no significant differences were observed among the experimental groups. This may be attributable, at least in part, to the relatively short duration of the exercise protocol and supplementation period. Accordingly, future studies employing longer exercise interventions and prolonged supplementation regimens are warranted to better characterize the histopathological effects of apilarnil on skeletal muscle. Finally, although the results suggest that the protective effects of apilarnil are associated with enhanced antioxidant capacity and appear to be independent of SIRT1 signaling, additional molecular studies investigating other signaling pathways are required to further elucidate the underlying mechanisms.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

FUNDING

No external funding was received for the study.

ETHICS APPROVAL

This study was approved by the Duzce University Animal Experiments Local Ethics Committee (HAYDEK) (Protocol No. 2022/12/02). All animal procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals.

ACKNOWLEDGEMENTS

This study was conducted as part of a doctoral dissertation. The authors gratefully acknowledge the support of the Duzce University Scientific Research Projects Commission under the project titled 'Investigation of the Effect of Apilarnil Supplement on Muscle Damage in Rats Exposed to Acute Exercise' (Project No: 2023.05.01.1396). Ethical approval for the study was obtained from the Ethics Committee of Duzce University (02/12/2022).

CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

AUTHORS' CONTRIBUTIONS

C.B. contributed to the conceptualization of the study and the writing of the original draft, M.K. contributed to the review, editing, and validation of the manuscript, and S.K.C. performed the histological analysis. All authors have read and agreed to the published version of the manuscript.

ORCID ID

C. Başol <https://orcid.org/0000-0001-5683-5814>

M. Kekeçoğlu <https://orcid.org/0000-0002-2564-8343>

S. Kantarcıoğlu Coşkun <https://orcid.org/0000-0002-8133-8665>

REFERENCES

1. Sawczuk R, Karpinska J, Milyk W. What do we need to know about drone brood homogenate and what is known. J Ethnopharmacol. 2019;245:111581.
<https://doi.org/10.1016/j.jep.2018.10.042>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

2. Isidorov VA, Bakier S, Stocki M. GC-MS investigation of the chemical composition of honeybee drone and queen larvae homogenate. *J Apic Sci.* 2016;60(2):111–20.
<https://doi.org/10.1515/jas-2016-0011>
3. Finke MD. Nutrient composition of bee brood and its potential as human food. *Ecol Food Nutr.* 2005;44(4):257–70.
<https://doi.org/10.1080/03670240500187278>
4. Silici S. Chemical content and bioactive properties of drone larvae (apilarnil). *Mellifera.* 2019;19:14–22.
5. Yücel B, Açıkgöz Z, Bayraktar H, Seremet C. The effects of apilarnil (drone bee larvae) administration on growth performance and secondary sex characteristics of male broilers. *J Anim Vet Adv.* 2011;10(17):1163–2266.
<https://doi.org/10.3923/javaa.2011.2263.2266>
6. Shoinbayeva KB, Omirzak T, Bigara T, Abubakirova A, Dauylbay A. Biologically active preparation and reproductive function of stud rams. *Asian J Pharm.* 2017;11:184–91.
7. Forcina L, Cosentino M, Musarò A. Mechanisms regulating muscle regeneration: Insights into the interrelated and time-dependent phases of tissue healing. *Cells.* 2020;9(5):1297.
<https://doi.org/10.3390/cells9051297>
8. Oláh A, Németh BT, Mátyás C, Horváth EM, Hidi L, Birtalan E, *et al.* Cardiac effects of acute exhaustive exercise in a rat model. *Int J Cardiol.* 2015;182:258–66.
<https://doi.org/10.1016/j.ijcard.2014.12.045>
9. Child RB, Wilkinson DM, Fallowfield JL, Donnelly AE. Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. *Med Sci Sports Exerc.* 1998;30(11):1603–7.
<https://doi.org/10.1097/00005768-199811000-00008>
10. Chevion S, Moran DS, Heled Y, Shani Y, Regev G, Abbou B, *et al.* Plasma antioxidant status and cell injury after severe physical exercise *Proc Natl Acad Sci U S A.* 2003;100(9):5119–5123.
<https://doi.org/10.1073/pnas.0831097100>
11. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology.* 2003;189(1-2):41–54.
[https://doi.org/10.1016/S0300-483X\(03\)00151-3](https://doi.org/10.1016/S0300-483X(03)00151-3)
12. Seres A. Sexual hormone effects of honeybee (*Apis mellifera*) drone milk in male and female rats [PhD Thesis]. Szeged, Hungary: University of Szeged; 2014.
<https://doi.org/10.14232/phd.2476>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

13. Kelly DM, Jones TH. Testosterone: a metabolic hormone in health and disease. *J Endocrinol.* 2013;217(3):R25–45.
<https://doi.org/10.1530/JOE-12-0455>
14. Çakar F, Şimşek H, Kaya E, Ulucan A, Bengü AŞ, Sever A. Protective role of apilarnil against intense exercise-induced liver injury in rats: serological and histopathological evidence. *Vet Res Forum.* 2025;16(5):421–30.
<https://doi.org/10.30466/vrf.2025.2048266.4580>
15. Topal E, Strant M, Yücel B, Kösoğlu M, Margaoan R, Dayıoğlu M. Biochemical properties and apitherapeutic usage of queen bee and drone larvae. *J Anim Prod. Hayvansal Uretim;* 2018;59(2):77–82. (in Turkish)
<https://doi.org/10.29185/hayuretim.455478>
16. Kogalniceanu-Odobescu S, Goldiş V, Kogalniceanu S, Lancrajan I, Ardelean G. Changes of the glucidic metabolism determined by the physical effort of the treatment with aslavital and apilarnil. *J Med Arad.* 2010;13(3):33–41.
17. Yokoyama Y, Shinohara K, Kitamura N, Nakamura A, Onoue A, Tanaka K, *et al.* Metabolic effects of bee larva-derived protein in mice: assessment of an alternative protein source. *Foods.* 2021;10(11):2642.
<https://doi.org/10.3390/foods10112642>
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265–75.
19. Mărgăoan R, Al Mărghițaș L, Dezmirean DS, Bobiș O, Bonta V, Cătană C, *et al.* Comparative study on quality parameters of royal jelly, apilarnil and queen bee larvae triturate. *Bull UASVM Anim Sci Biotechnol.* 2017;74(2):1843–536.
<https://doi.org/10.15835/buasvmcn-asb>
20. Sesta G. Determination of sugars in royal jelly by HPLC. *Apidologie.* 2006;37(1):84–90.
<https://doi.org/10.1051/apido:2005061>
21. Wonders KY, Hydock DS, Schneider CM, Hayward R. Acute exercise protects against doxorubicin cardiotoxicity. *Integr Cancer Ther.* 2008;7(3):147–54.
<https://doi.org/10.1177/1534735408322848>
22. Doğanyığıt Z, Okan A, Kaymak E, Pandır D, Silici S. Investigation of protective effects of apilarnil against lipopolysaccharide induced liver injury in rats via TLR 4/ HMGB-1/ NF-κB pathway. *Biomed Pharmacother.* 2020;125.
<https://doi.org/10.1016/j.biopha.2020.109967>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

23. Shimizu Y, Hara H, Hira T. Glucagon-like peptide-1 response to whey protein is less diminished by dipeptidyl peptidase-4 in comparison with responses to dextrin, a lipid and casein in rats. *Br J Nutr*. 2021;125(4):398–407.
<https://doi.org/10.1017/S0007114520002834>
24. IBM SPSS Statistics, v. 27.0, IBM Corp, Armonk, NY, USA; 2020. Available from:
<https://www.ibm.com/docs/en/spss-statistics/27.0.0>
25. GraphPad Prism, v. 10.0. GraphPad Software. Boston, MA, USA; 2023. Available from:
<https://www.graphpad.com/>
26. Özkök A, Erdem B. Can food supplement produced from apilarnil be an alternative to testosterone replacement therapy. *Hacettepe J Biol Chem*. 2017;45(4):635–8.
<https://doi.org/10.15671/hjbc.2018.207>
27. Whyte JJ, Harold Laughlin M. The effects of acute and chronic exercise on the vasculature. *Acta Physiol (Oxf)*. 2010;199(4):441–450.
<https://doi.org/10.1111/j.1748-1716.2010.02127.x>
28. Aguiló A, Tauler P, Pilar Guix M, Villa G, Córdova A, Tur JA, *et al*. Effect of exercise intensity and training on antioxidants and cholesterol profile in cyclists. *J Nutr Biochem*. 2003;14(6):319–25.
[https://doi.org/10.1016/s0955-2863\(03\)00052-4](https://doi.org/10.1016/s0955-2863(03)00052-4)
29. Civan A, Kececi T, Cakmakci E. The effects of ginseng and exercise applications in sedentary individuals in women athletes on lipid hydroperoxide and nitric oxide. *Ovidius Univ Ann Ser Phys Educ Sport Sci Mov Health*. 2010;10(2):774–777
30. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: A 30 year history. *Dyn Med*. 2009;8:1.
<https://doi.org/10.1186/1476-5918-8-1>
31. Tozzi-Ciancarelli MG, Penco M, Di Massimo C. Influence of acute exercise on human platelet responsiveness: Possible involvement of exercise-induced oxidative stress. *Eur J Appl Physiol*. 2002;86(3):266–72.
32. <https://doi.org/10.1007/s00421-001-0542-8>
33. Algul S, Ugras S, Kara M. Comparative evaluation of mda levels during aerobic exercise in young trained and sedentary male subjects. *East J Med*. 2018;23(2):98–101.
<https://doi.org/10.5505/ejm.2018.40469>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

34. Bailey DM, Davies B, Young IS. Intermittent hypoxic training: Implications for lipid peroxidation induced by acute normoxic exercise in active men. *Clin Sci (Lond)*. 2001;101(5):465–75.
<https://doi.org/10.1042/CS20010112>
35. He F, Li J, Liu Z, Chuang CC, Yang W, Zuo L. Redox mechanism of reactive oxygen species in exercise. *Front. Physiol*. 2016;7:486.
<https://doi.org/10.3389/fphys.2016.00486>
36. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014:360438.
<https://doi.org/10.1155/2014/360438>
37. Aoyama K, Nakaki T. Inhibition of GTRAP3-18 may increase neuroprotective glutathione (GSH) synthesis. *Int J Mol Sci*. 2012;13(9):12017–35.
<https://doi.org/10.3390/ijms130912017>
38. Tauler P, Gimeno I, Aguiló A, Guix MP, Pons A. Regulation of erythrocyte antioxidant enzyme activities in athletes during competition and short-term recovery. *Pflugers Arch*. 1999;438(6):782–7.
<https://doi.org/10.1007/s004249900125>
39. Thirumalai T, Therasa SV, Elumalai E, David E. Intense and exhaustive exercise induce oxidative stress in skeletal muscle. *Asian Pac J Trop Dis*. 2011;1(1):63–6.
[https://doi.org/10.1016/S2222-1808\(11\)60016-9](https://doi.org/10.1016/S2222-1808(11)60016-9)
40. Tauler P, Aguiló A, Gimeno I, Guix P, Tur JA, Pons A. Different effects of exercise tests on the antioxidant enzyme activities in lymphocytes and neutrophils. *J Nutr Biochem*. 2004;15(8):479–84.
<https://doi.org/10.1016/j.jnutbio.2004.03.002>
41. Ferrer MD, Tauler P, Sureda A, Tur JA, Pons A. Antioxidant regulatory mechanisms in neutrophils and lymphocytes after intense exercise. *J Sports Sci*. 2009;27(1):49–58.
<https://doi.org/10.1080/02640410802409683>
42. Xu R, Liu N, Xu X, Kong B. Antioxidative effects of whey protein on peroxide-induced cytotoxicity. *J Dairy Sci*. 2011;94(8):3739–46.
<https://doi.org/10.3168/jds.2010-3891>
43. Wadley GD, Nicolas MA, Hiam DS, McConell GK. Xanthine oxidase inhibition attenuates skeletal muscle signaling following acute exercise but does not impair mitochondrial

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

adaptations to endurance training. *Am J Physiol Endocrinol Metab.* 2013;304(8):E853-62.

<https://doi.org/10.1152/ajpendo.00568.2012>

44. Viña J, Gimeno A, Sastre J, Desco C, Asensi M, Pallardó F V, *et al.* Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. *IUBMB Life.* 2000;49(6):539–44.

<https://doi.org/10.1080/15216540050167098>

45. Malaguti M, Angeloni C, Garatachea N, Baldini M, Leoncini E, Collado PS, *et al.* Sulforaphane treatment protects skeletal muscle against damage induced by exhaustive exercise in rats. *J Appl Physiol (1985).* 2009;107(4):1028–36.

<https://doi.org/10.1152/japplphysiol.00293.2009>

46. Ji LL, Gomez-Cabrera MC, Steinhafel N, Viña J. Acute exercise activates nuclear factor (NF)-KB signaling pathway in rat skeletal muscle. *The FASEB J.* 2004;18(13):1499–506.

<https://doi.org/10.1096/fj.04-1846com>

47. Tipton KD, Wolfe RR. Exercise-induced changes in protein metabolism. *Acta Physiol Scand.* 1998;162(3):377–87.

<https://doi.org/10.1046/j.1365-201X.1998.00306.x>

48. Kistner TM, Pedersen BK, Lieberman DE. Interleukin 6 as an energy allocator in muscle tissue. *Nat Metab.* 2022;4(2):170–9.

<https://doi.org/10.1038/s42255-022-00538-4>

49. Prokopidis K, Mazidi M, Sankaranarayanan R, Tajik B, McArdle A, Isanejad M. Effects of whey and soy protein supplementation on inflammatory cytokines in older adults: A systematic review and meta-analysis. *Br J Nutr.* 2023;129(5):759–70.

<https://doi.org/10.1017/S0007114522001787>

50. Adler S, Olsen W, Rackerby B, Spencer R, Dallas DC. Effects of whey protein supplementation on inflammatory marker concentrations in older adults. *Nutrients.* 2023;15(18):4081.

<https://doi.org/10.3390/nu15184081>

51. McKinlay BJ, Theocharidis A, Adebero T, Kurgan N, Fajardo VA, Roy BD, *et al.* Effects of post-exercise whey protein consumption on recovery indices in adolescent swimmers. *Int J Environ Res Public Health.* 2020;17(21):7761.

<https://doi.org/10.3390/ijerph17217761>

52. Imai S, Guarente L. NAD⁺ and sirtuins in aging and disease. *Trends Cell Biol.* 2014;24(8):464–71.

<https://doi.org/10.1016/j.tcb.2014.04.002>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

53. Huang C-C, Wang T, Tung Y-T, Lin W-T. Effect of exercise training on skeletal muscle SIRT1 and PGC-1 α expression levels in rats of different age. *Int J Med Sci.* 2016;13(4):260–70.
<https://doi.org/10.7150/ijms.14586>
54. Hart N, Sarga L, Csende Z, Koltai E, Koch LG, Britton SL, *et al.* Resveratrol enhances exercise training responses in rats selectively bred for high running performance. *Food Chem Toxicol.* 2013;61:53–9.
<https://doi.org/10.1016/j.fct.2013.01.051>
55. Lensu S, Pekkala SP, Mäkinen A, Karstunen N, Turpeinen AT, Hulmi JJ, *et al.* Beneficial effects of running and milk protein supplements on Sirtuins and risk factors of metabolic disorders in rats with low aerobic capacity. *Metabol Open.* 2019;4:100019.
<https://doi.org/10.1016/j.metop.2019.100019>
56. Manor D, Sadeh M. Muscle fibre necrosis induced by intramuscular injection of drugs. *Br J Exp Pathol.* 1989;70(4):457–62.
57. Greaves P, Chouinard L, Ernst H, Mecklenburg L, Pruimboom-Brees IM, Rinke M, *et al.* Proliferative and non-proliferative lesions of the rat and mouse soft tissue, skeletal muscle and mesothelium. *J Toxicol Pathol.* 2013;26:1S-26S.
<https://doi.org/10.1293/tox.26.1S>
58. McCarthy JJ, Mula J, Miyazaki M, Erfani R, Garrison K, Farooqui AB, *et al.* Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. *Development.* 2011;138(17):3657–66.
<https://doi.org/10.1242/dev.068858>
59. Hamrick MW, McGee-Lawrence ME, Frechette DM. Fatty infiltration of skeletal muscle: mechanisms and comparisons with bone marrow adiposity. *Front Endocrinol (Lausanne).* 2016;7:69.
<https://doi.org/10.3389/fendo.2016.00069>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 1. Physico-chemical and nutrient content analysis of three samples of lyophilized apilarnil

Repetition	Physico-chemical properties			
	w/%			E/kcal
	Ash	Moisture	Cholesterol	
1	3.14	3.90	2.28	1935
2	2.90	4.20	2.01	1915
3	3.10	4.09	2.11	1930
Mean	(3.05±0.13)	(4.06±0.15)	(2.13±0.14)	(1926.67±10.41)

Repetition	Nutrient composition						
	w/%						
	Protein	Carbohydrate	Lipid	Glucose	Fructose	Sucrose	Maltose
1	47.95	26.89	10.68	2.10	1.25	0.08	0.78
2	47.39	21.99	15.40	2.11	1.22	0.08	0.76
3	49.34	25.17	14.50	2.14	1.25	0.08	0.76
Mean	(48.23±1.00)	(24.68±2.49)	(13.53±2.51)	(2.12±0.02)	(1.24±0.017)	(0.08±0.00)	(0.77±0.012)

Values represent means of three independent determinations±S.D.

Table 2. Significance level of the difference between the mass of rats before and after the experiment

Group	m/g			p
	First day mass	Last day mass	Mass difference	
C	324	374	50	(0.001)***
AP	345	370	25	(0.005)**
W	326	368	42	(0.011)*
E	322	349	27	(0.013)*
E+W	333	373	40	(0.003)**
E+AP	323	355	32	(0.004)**

Dependent sample *t*-test was used to analyze the changes in mass before and after the experiment. **p*<0.05, ***p*<0.01, ****p*<0.001. C=control, AP=apilarnil, W=isolated whey protein, E=acute exercise, E+W=acute exercise+whey protein, E+AP=acute exercise+apilarnil

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 3. Effects of apilarnil and whey protein supplementations on plasma parameters of rats after acute exercise

Parameter	C	AP	W	E	E+W	E+AP
(n(MDA)/V(blood))/(nmol/L)	(5.45±1.01)	(5.34±0.72)	(7.52±1.26) _{a,b}	(6.97±0.68) _{a,b}	(8.11±1.59) _{a,b}	(6.88±0.58) _{a,b}
γ(CAT)/(ng/mL)	(13.11±4.11)	(30.95±17.41) _a	(30.99±15.69) _a	(18.90±7.24)	(34.02±9.58) _{a,d}	(33.24±10.68) _{a,d}
γ(GSH)/(mg/L)	(106.88±19.05)	(141.96±34.85) _{a,d}	(137.59±11) _{a,d}	(111.87±12.61)	(124.76±22.93)	(136.18±4.98) _{a,d}
γ(SOD)/(ng/mL)	(9.99±2.05)	(10.64±2.73)	(9.59±1.44)	(9.83±2.33)	(9.93±2.17)	(11.03±2.21)
γ(IL-6)/(pg/mL)	(85.81±6.60)	(90.20±5.62)	(97.18±8.93) _a	(91.43±4.39)	(99.98±8.71) _{a,b}	(85.57±3.97) _{c,e}
γ(IL-10)/(pg/mL)	(72.76±7.67)	(80.95±7.43)	(78.76±14.07)	(75.45±14.62)	(82.57±9.26)	(85.53±11.20)

Values represent means±SD. (N=6). ^a<p<0.05 vs C, ^b<p<0.05 vs AP, ^c<p<0.05 vs W, ^d<p<0.05 vs E, ^e<p<0.05 vs EW. C=control, AP=apilarnil, W=isolated whey protein, E=acute exercise, E+W=acute exercise+whey protein, E+AP=acute exercise+apilarnil, MDA=malondialdehyde, CAT=catalase, GSH=glutathione, SOD=superoxide dismutase, IL-6=interleukine-6, IL-10=interleukine-10

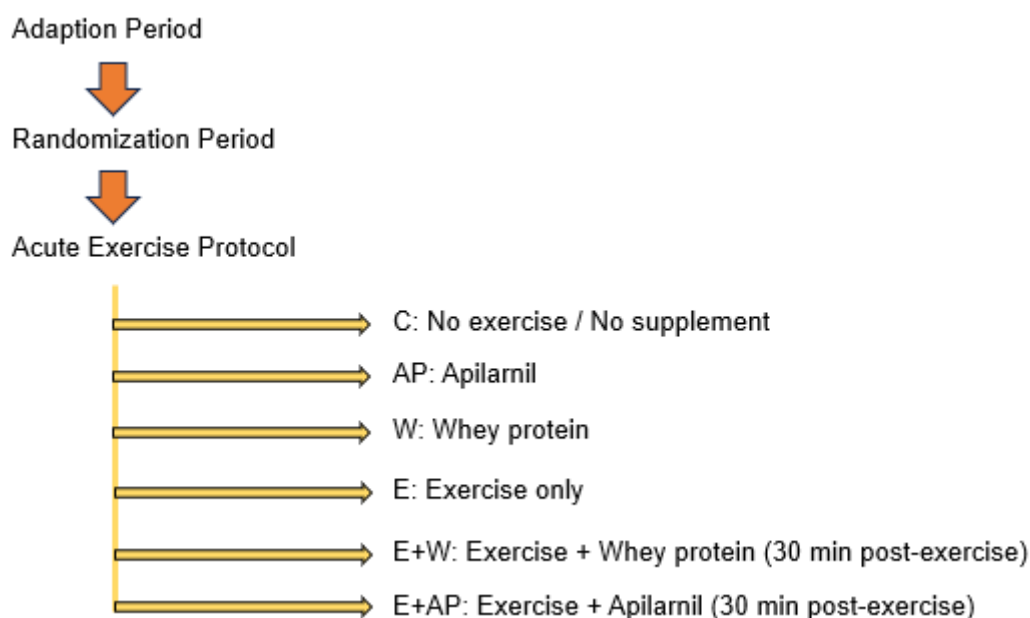


Fig. 1. Schematic representation of the experimental protocol. Rats were randomly assigned into six groups: control (C), apilarnil (AP), whey protein (W), exercise (E), exercise+apilarnil (E+AP), and exercise+whey protein (E+W). Apilarnil and whey protein supplements were administered by oral gavage 30 min after each acute exercise session. Control and non-exercised supplementation groups did not undergo exercise protocol

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

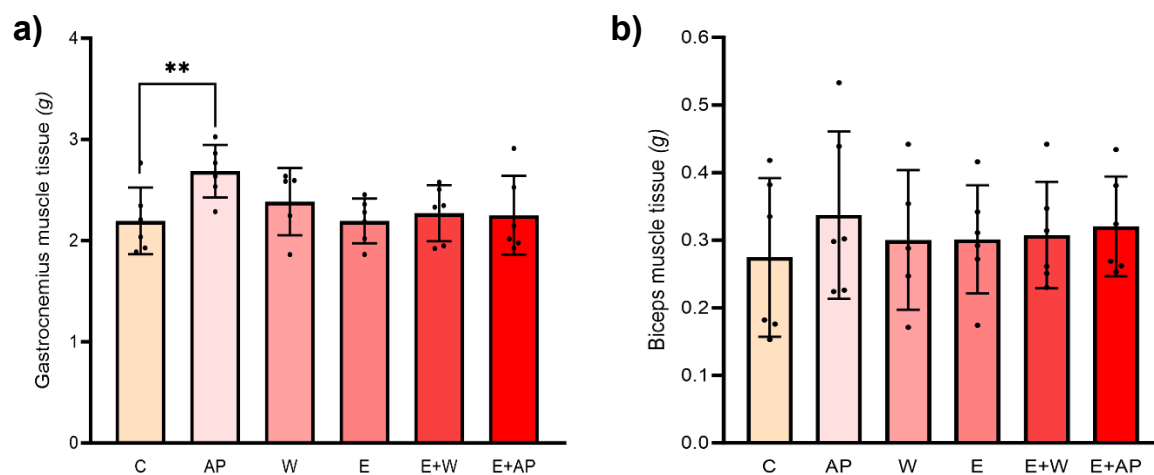
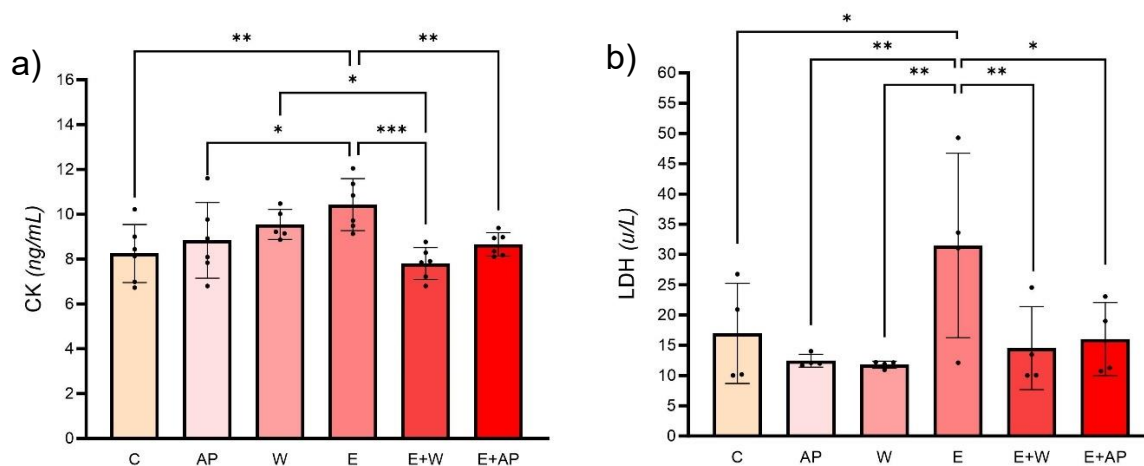


Fig. 2. a) Gastrocnemius muscle tissue mass of rats at the end of the experiment, b) biceps muscle tissue mass of rats at the end of the experiment. ** $p < 0.01$. C=control, AP=apilarnil, W=isolated whey protein, E=acute exercise, E+W=acute exercise+whey protein, E+AP=acute exercise+apilarnil



Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

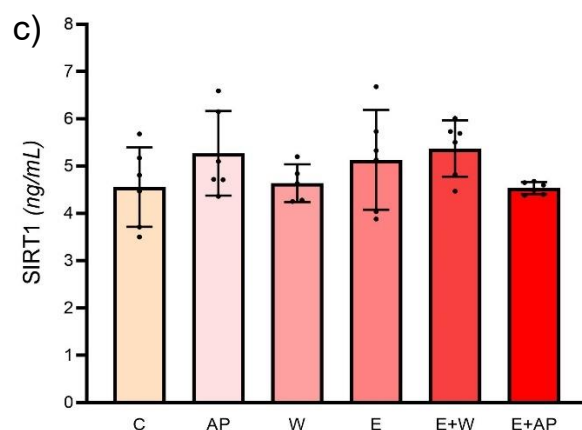
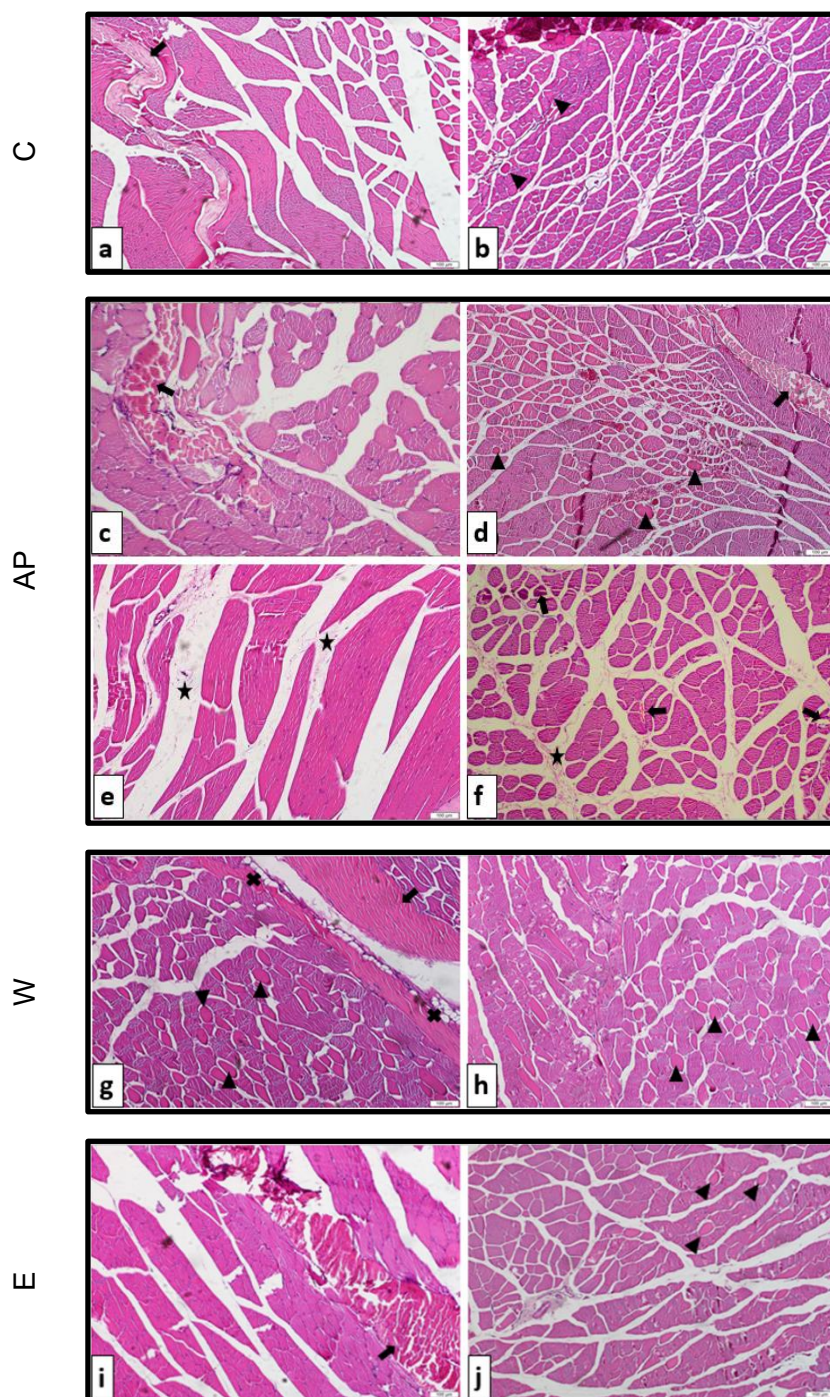
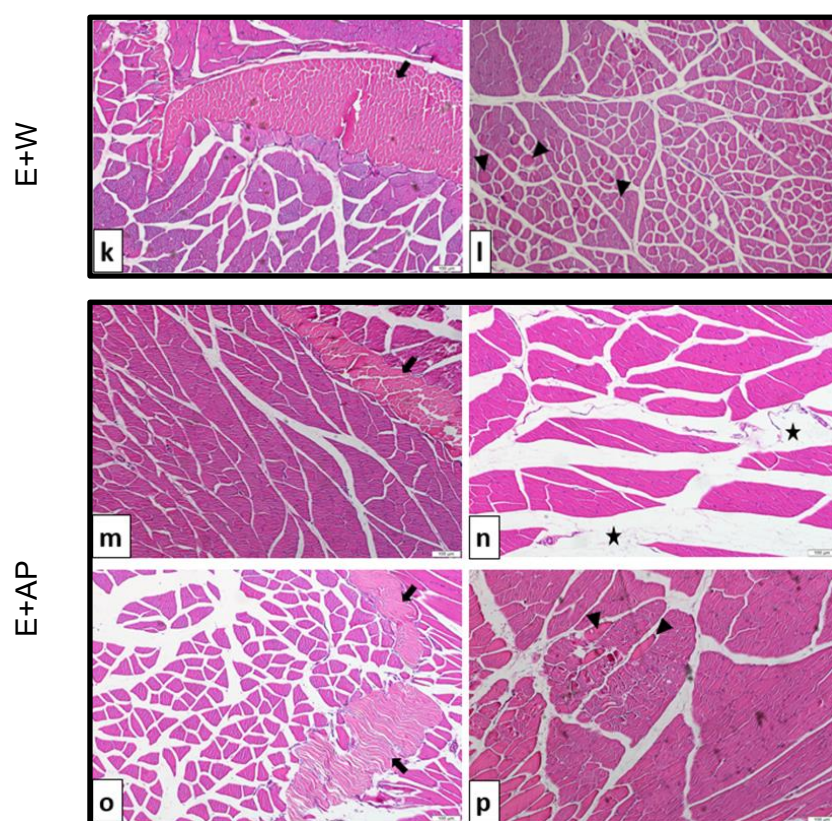


Fig. 3. a) CK level, b) LDH level and c) SIRT1 level. Data shown on histogram graph are expressed as mean $\pm$ S.D. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. C=control, AP= apilarnil, W=isolated whey protein, E=acute exercise, E+W=acute exercise+whey protein, E+AP=acute exercise+apilarnil

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.



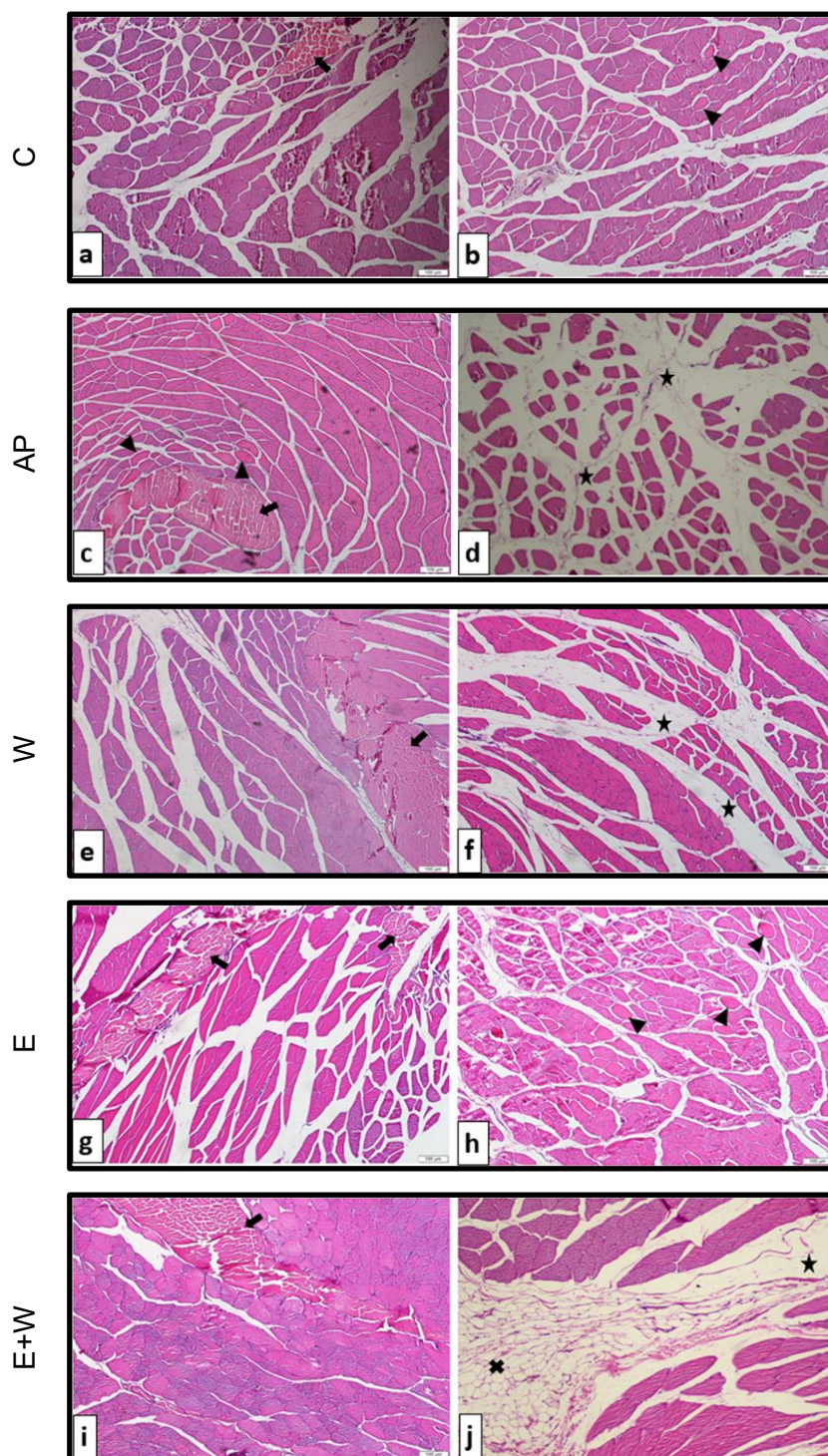
Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.



➡: myonecrosis, ►: immature muscle fiber, ★: edema, ✖: perifascicular fat accumulation

Fig. 4. Pathological changes seen in the gastrocnemius muscle tissue of rats stained with (H&E). a) C group, myonecrosis, (H&E), X10, b) C group, immature muscle fibre, (H&E), X10, c) AP group, myonecrosis, (H&E), X40, d) AP group myonecrosis and immature muscle fibre, (H&E), X10, e) AP group, edema, (H&E), X10, f) AP group, myonecrosis and edema, (H&E), X10, g) W group, myonecrosis, immature muscle fibre and perifascicular fat accumulation, (H&E), X10, h) W group, immature muscle fibre, (H&E), X10, i) E group myonecrosis, (H&E), X10, j) E group, immature muscle fibre, (H&E), X10, k) E+W group, myonecrosis, (H&E), X10, l) E+W group, immature muscle fibre, (H&E), X10, m) E+AP group, myonecrosis, (H&E), X10, n) E+AP group, edema, (H&E), X10, o) E+AP group, myonecrosis, (H&E), X10, p) E+AP group, immature muscle fibre, (H&E), X10. C=control, AP=apilarnil, W=isolated whey protein, E=acute exercise, E+W=acute exercise+whey protein, E+AP=acute exercise+apilarnil

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.



Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

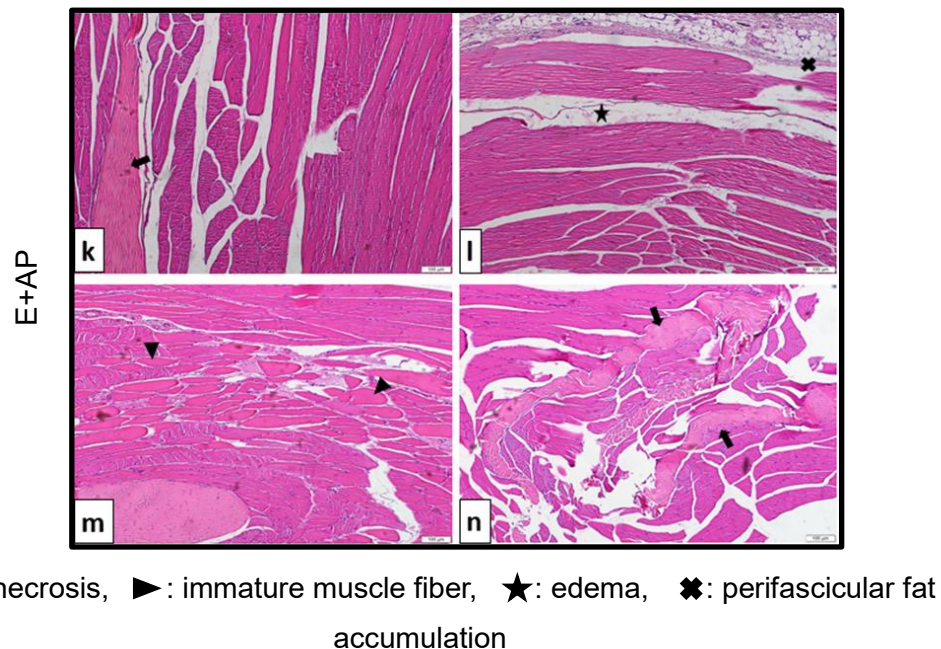


Fig. 5. Pathological changes seen in the biceps muscle tissue of rats stained with (H&E). a) C group, myonecrosis, (H&E), X10, b) C group, immature muscle fiber, (H&E), X10, c) AP group, myonecrosis and immature muscle fiber, (H&E), X10, d) AP group, edema, (H&E), X20, e) W group, myonecrosis, (H&E), X10, f) W group, edema, (H&E), X10, g) E group, myonecrosis, (H&E), X10, h) E group, myonecrosis and immature muscle fiber, (H&E), X10, i) E+W group, myonecrosis, (H&E), X10, j) E+W group, perifascicular fat accumulation and edema, (H&E), X10, k) E+AP group, myonecrosis, (H&E), X10, l) E+AP group, edema, (H&E), X10, m) E+AP group, immature muscle fiber, (H&E), X10, n) E+AP group, myonecrosis, (H&E), X10