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Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 31, Number 2, Pages 165-176 (2022)
ISSN 1303-6025 E-ISSN 2651-3749
DOI: 10.53447/communc.1178452

Research Article; Received: September 21, 2022; Accepted: October 20, 2022

THE EFFECT OF MELATONIN ON RAT SOLEUS MUSCLE TREATED WITH CARBON TETRACHLORIDE

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ABSTRACT. Antioxidants are known to restrains various tissue damage caused by the administration of carbon tetrachloride (CCl_4). This study examined whether melatonin (MEL), a molecule known to have antioxidant properties, has a protective effect on the rat soleus muscle, where toxic damage is caused by the application of CCl_4. In the study, eighteen albino-type male Wistar rats were used and divided into three groups as Control Group (group 1), CCl_4 group (group 2) and CCl_4 + MEL group (group 3). End of the 12 weeks, blood samples were taken as intracardiac from the rats under ketamine/rompun anesthesia, and the soleus muscles of the rats were removed. Tissue samples were subjected to routine preparation procedures for light microscopy. Sections 5 µm thick taken and stained with hematoxylin-eosin (HE) for histopathological examinations and Masson's Trichrome stain for fibrosis formations. In conclusion, the CCl_4 group displayed muscular hypertrophy, fiber orientation dysfunction and atrophy in some areas. In addition, fibrosis was spotted around the venous and nerve plexuses. In contrast to the CCl_4 group, the melatonin group displayed no fibrosis and maintained tissue integrity. Therefore, when comparing CCl_4+MEL and CCl_4 groups, it was observed that melatonin had a stabilizing or even curative effect on the injuries.

Keywords. Skeletal muscle, soleus muscle, melatonin, CCl_4
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1. INTRODUCTION

Carbon tetrachloride (CCl_4) is a volatile, non-flammable and colorless liquid chemical that has been known to cause a variety of tissue damage via its free radical, trichloromethyl, which is produced in the liver [1]. It is a well-known model and causes toxicity in many tissues such as liver, kidney, heart, lung, testes, brain, and blood [3,4] as a result of the production of CCl_4 free radicals, which are also produced chemically [2]. In humans, about 50-80% of CCl_4 is absorbed by the lungs. Approximately 4% of the metabolized CCl_4 is directly converted to CO_2 and exhaled from the lungs [5]. The toxic effect of CCl_4 occurs after its conversion to its free radical trichloromethyl by P-450, which is where the lipid peroxidation process begins [10-12].

Melatonin is a neurohormone derives from N-acetylated serotonin [13] and synthesized by bone marrow cells, intestine, lens, pineal gland, retina, Harderian glands, ovary, testicle, and skin [1,9]. Since melatonin is both fat and water soluble, it easily enters every cell in the body, including cytosol and intracellular structures, therefore it is much more effective than vitamin and mineral antioxidants. [14]. Thus, the nuclear DNA, cytosolic proteins and membrane lipids are protected by melatonin against diseases that cause degenerative and proliferative changes [14,15]. This pleiotropic hormone synthesized mainly in the pineal gland, showed positive affects in organism as if regulation of cell apoptosis, inflammation, circadian rhythms, oxidative stress and metabolic disorders [16-18]. In order to diverse uses of melatonin, it has gained all interest as a functional and strategic therapeutic biochemical in an effort to prevent and treat some illness related to muscle [19-21]. Melatonin is a hormone that belongs to the indolamine family and has significant antioxidant properties as a result of its electron donor ability [22-24].

Skeletal muscle plays an important role in energy metabolism, insulin resistance and movement in adults, and accounts for almost 40% of all body mass [25]. The most remarkable feature of the skeletal muscle is the ability to regenerate quickly after injury, made possible by the resident population of stem cells (also called satellite cells (SC)) located between the sarcolemma and the basal lamina of myofibers [26-28].

The soleus muscle is located in the posterior compartment of the lower leg and above the Achilles tendon and has slow-contracting muscle fibers [29]. Soleus is a powerful muscle group that functions while walking and running and it prevents the body from falling forward while standing [30]. Muscle tissue is susceptible to different types of injuries, and the process of

regeneration often leads to the formation of scar tissue. By applying exogenous substances, such as antioxidants, the regeneration processes can be improved, preventing muscle strength loss and scarring [1,31]. Numerous studies have shown that antioxidants prevent different tissue damage caused by CCl_4 administration [32,33]. In this study, melatonin, which is known to have strong antioxidant properties, was investigated for the curative effects on the soleus muscle damaged by carbon tetrachloride (CCl_4).

2. MATERIALS AND METHODS

Eighteen Wistar albino-type male rats were housed in rooms with a 12:12 hour light-dark cycle and a constant temperature for the study. Three groups are formed as control group (group 1), CCl_4 group (group 2), and CCl_4+MEL group (group 3). In group 1 (Control group), corn oil, which dissolves CCl_4, was injected subcutaneously in a volume of 1.5 ml/kg twice a week for 12 weeks. In order to cause muscle injury, the second group was injected subcutaneously in a volume of 1.5 ml/kg with sterile CCl_4 dissolved in corn oil at a ratio of 1:1, 2 times a week for 12 weeks. Group 3 received the same CCl_4 treatment as Group 2, but in addition, 10 mg/kg per day of melatonin was given subcutaneously to the Group 3 starting after the CCl_4 treatment. At the end of the 12^{th} week, intracardiac blood samples and soleus muscle tissues were taken from the rats under ketamine-rompus anesthesia. Standard light microscopy preparation techniques were taken on the muscle tissue specimens. Cross sections 5 μm thick were taken and stained with hematoxylin-eosin and Masson's Trichrome staining technique.

3. RESULTS

It was observed that the morphological structure of the soleus muscle cells in the control group stained with hematoxylin-eosin was smooth and the muscle integrity was normal (Figure 1). Additionally, collagen fibers were found to be at normal levels in Masson's Trichrome stained muscle fibers (Figure 2). Hematoxylin-eosin-stained soleus muscle fibers of Group 2 (CCl_4) displayed protein loss, fibrosis around the blood vessels, atrophy, and hypertrophy in some places (Figure 3 and 4). Also in this group, Masson's Trichrome staining revealed increased connective tissue, atrophy in muscle fibers, and increased collagen fibers around the blood vessels (Figure 5 and 6). Examining the hematoxylin-eosin-stained soleus muscle specimens of the CCl_4 groups treated with melatonin revealed that, in comparison to the CCl_4 group, the tissue integrity was partially preserved, and the morphological

structure of the cells was close to normal (Figure 7). Again, in the same group, Masson's Trichrome stained samples showed that there was a decrease in collagen fibers and a decrease in fibrosis, especially around the blood vessels (Figure 8).

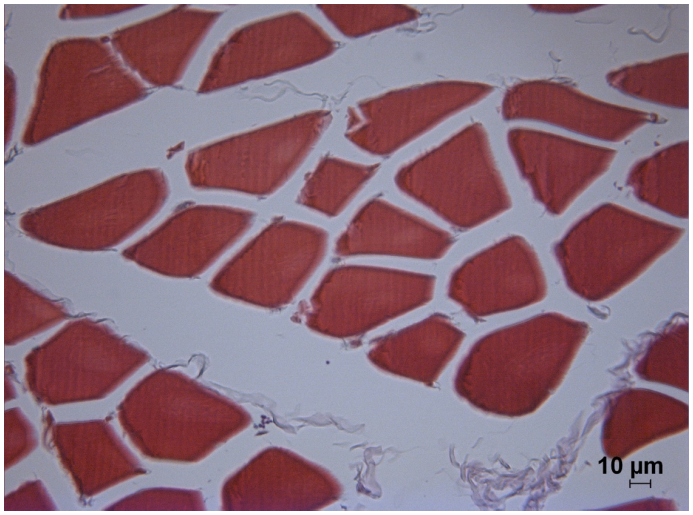

FIGURE 1. View of the soleus muscle tissue of the control group rats, 100µm.

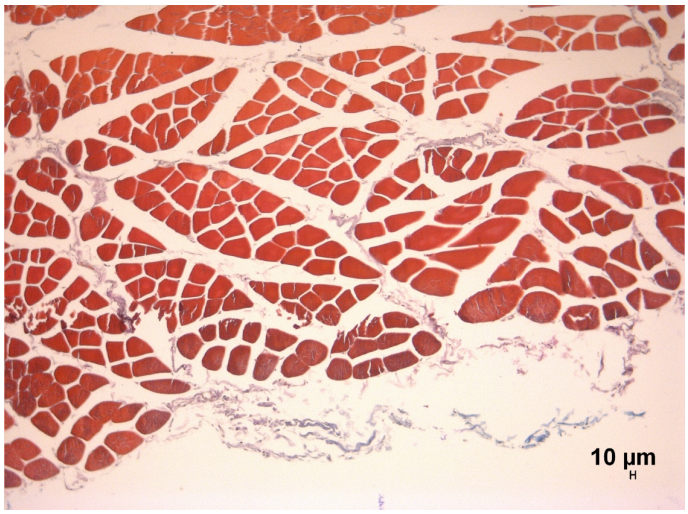

FIGURE 2. Normal levels of collagen fibers in Masson's Trichrome-stained muscle fibers of the control group rats.

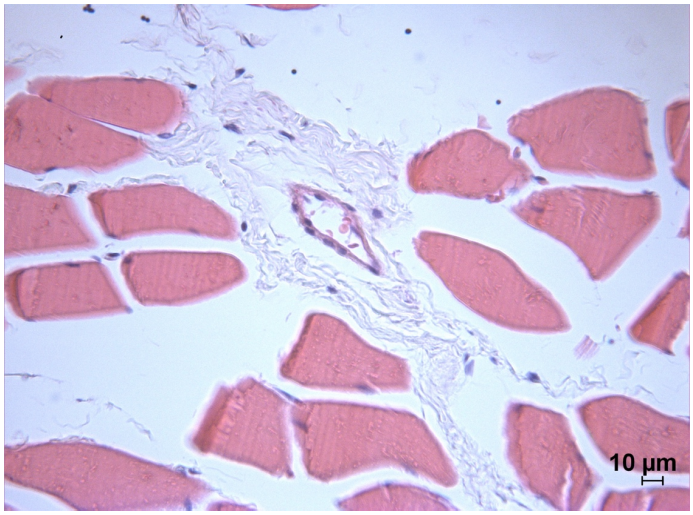

FIGURE 3. Loss of protein, atrophy in some places and fibrosis around the vessels of the CCl_4 group soleus muscle stained with hematoxylin-eosin.

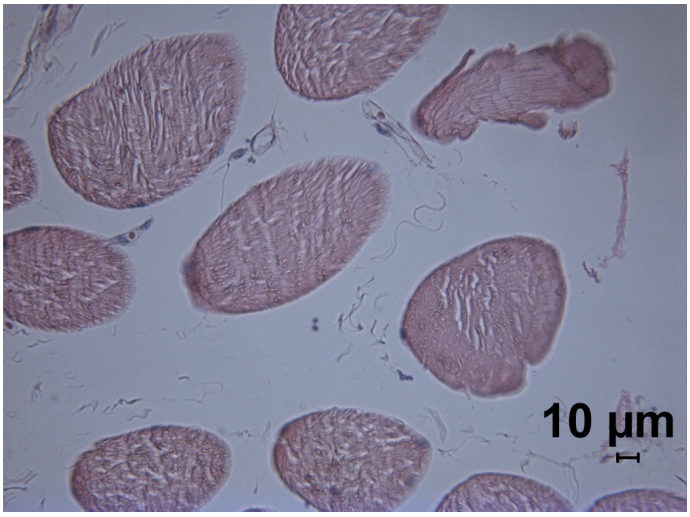

FIGURE 4. Hypertrophy of the muscle fibers in the CCl_4 group.

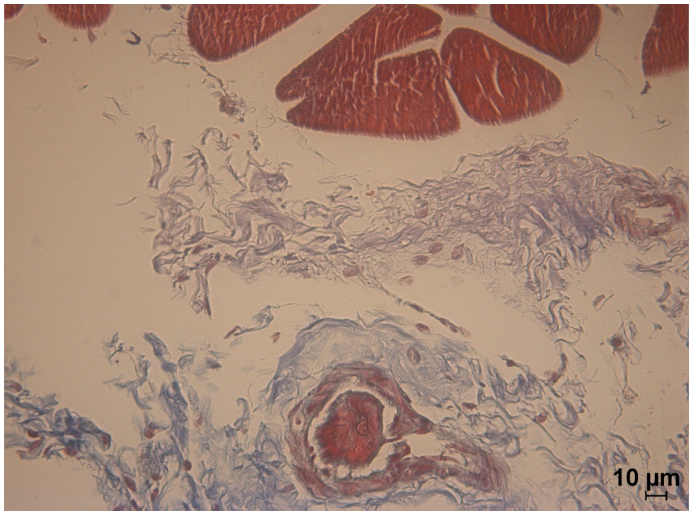

FIGURE 5. Increased collagen fibers around the blood vessel in the CCl_4 group (stained with Masson's Trichrome).

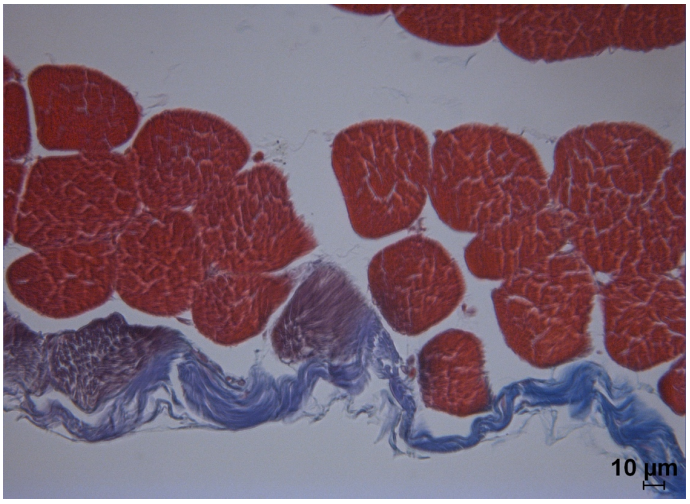

FIGURE 6. Atrophy of muscle fibers and increase in the connective tissue in the CCl_4 group.

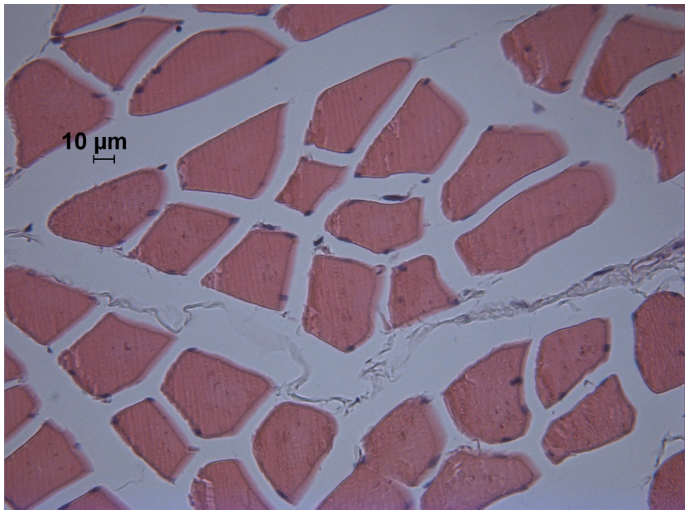

FIGURE 7. Hematoxylin-eosin staining of the CCl_4+MEL group.

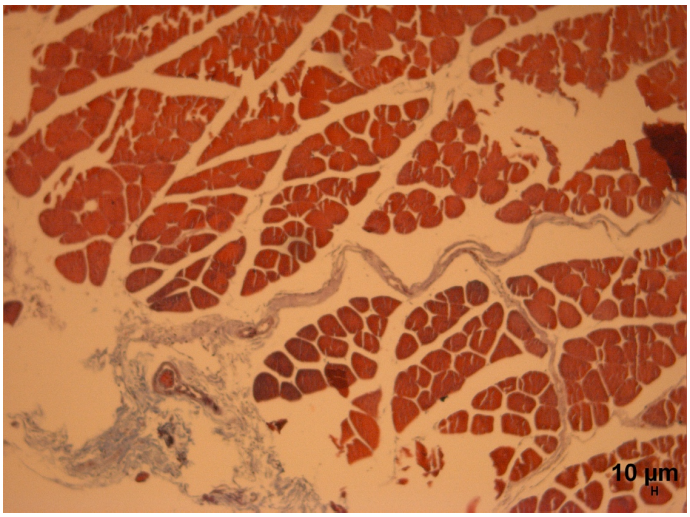

FIGURE 8. Masson's Trichrome staining of the CCl_4+MEL group.

4. DISCUSSION

Carbon tetrachloride (CCl_4) is a molecule produced synthetically in an effort to use in industrial agents such as fire extinguishers, solvents and paints [5].

It has a quite large half-life between 30 to 100 years. It can enter the body through digestion, respiration and dermal absorption and then distributed to tissues such as the brain, kidney, muscle, lung and testes, especially liver. This chlorine-containing substance has been used in animal experiments to cause oxidative damage to several tissues, including the liver, kidneys, brain, muscles, lungs and testicles [33].

Research by Pope and Rall [5] stated that, the general population may be exposed to CCl_4 through ambient air because CCl_4 can evaporate easily and the emissions of CCl_4 as chemical waste into the air, water and soil are not controlled properly. Numerous studies have shown that melatonin has a protective effect against diseases that cause proliferative and degenerative changes to nuclear DNA, membrane lipids and cytosolic proteins. Kuş et al. [36] reported that melatonin reduced kidney and liver damage after CCl_4 intoxication. In another study by Erdem et al. [37] emphasized that melatonin has an important role on striated muscle tissue as a protectant from ischemia-reperfusion injury.

Sokolovic et al. [38] investigated the potency of melatonin to prevent biological and clinical changes in the rat biceps muscle following acute exposure to CCl_4. Microscopic analysis of the biceps muscle from animals exposed to this synthetic chemical come up significant muscle fiber irregularity and intense inflammatory cell infiltration, while significant improvement was observed in the group receiving melatonin. They also investigated serum and tissue biochemistry and revealed that melatonin has a significant effect on preventing CCl_4-induced skeletal muscle damage.

In the study where Chen et al [25] summarized the latest research about the role of melatonin in regulating muscle growth and regeneration, they reported that due to its wide biological functions, application of melatonin is important in regulating muscle and fat metabolism to treat muscle diseases or to improve health in order to its broad-spectrum antioxidant, anti-apoptotic, and anti-tumor properties.

The results of this study were found to be consistent with the afore-mentioned studies. In the CCl_4 group, fibrosis, atrophy in some areas, increase in connective tissue around the nerve plexus, disorientation and hypertrophy in muscle fibers were observed, while in the CCl_4+MEL group, a near-normal appearance was observed with a decrease in fibrosis. In conclusion, when the group given CCl_4+MEL is compared with the group given CCl_4, it is possible to say that melatonin has a stabilizing or even healing effect on the CCl_4-induced muscle injuries.

Author Contribution Statement DFV and DO-experimental design and performance, DFV and HME-manuscript writing. SC-manuscript editing. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest. This study was presented as an oral presentation at the "1st National Zoology Congress", 28-31 August 2013, NEVŞEHİR, TURKEY

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