

## MELATONIN AMELIORATES DECREASE IN RAT GASTROCNEMIUS MUSCLE CATALASE ACTIVITY INDUCED BY CARBON TETRACHLORIDE

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Skeletal muscle tissue is known to be susceptible to oxidative tissue damage that accompanies different disorders. Carbon tetrachloride (CCl<sub>4</sub>) is dangerous chemical that is used to mimic disorders, in experimental animals, related to reactive oxygen species induced tissue damage. It is well established that antioxidants, both natural and synthetic ones, are able to alleviate tissue damage caused by reactive oxygen species. The aim of the present study was to determine the effects of melatonin (MLT), a strong naturally occurring antioxidant, on changes in Wistar rat skeletal muscle catalase activity acutely induced by CCl<sub>4</sub>. Gastrocnemius muscle tissue, in which catalase activity was determined, was obtained from three groups of animals i.e. control (untreated), CCl<sub>4</sub> treated and MLT and CCl<sub>4</sub> group. The results revealed statistically significant decrease in muscle tissue catalase activity in rats exposed to CCl<sub>4</sub>, while in the group that received MLT and CCl<sub>4</sub> this decrease was insignificant. The protective activity of MLT could be contributed to its different mechanisms since it is known to directly scavenge free radicals, increase tissue antioxidant capacity and to up-regulate antioxidant enzyme gene expression.

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**Key words:** carbon tetrachloride, gastrocnemius muscle, catalase

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### Introduction

Skeletal muscles (SM) are prone to oxidative tissue damage, apart from different organ underlying disorders, after a mild/extensive exercise (1-3). One of the major factors that makes SM tissue prone to this type of damage is the presence of myoglobin, a catalyst for formation of reactive oxygen species (ROS) (1). Affected SM tissue is fighting ROS growth by increasing tissue blood flow (perfusion), as well as cell enzymatic antioxidative defences (1). Enzymes involved in defending SM cells from ROS include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The first two are mainly localized in cytoplasm and mito-

chondria, while the last one (CAT) can be found predominantly in lysosomes (1). Lysosomes are important cell organelles with main function in polymer molecules degradation, which could be found near cell membrane of almost all animal cells (4). In mitochondria, CAT is thought to act as a "safety device" since it might prevent excessive leakage of generated H<sub>2</sub>O<sub>2</sub>, thus preventing lipid and protein oxidative damage caused by myoglobin derived ferryl products (1).

Carbon tetrachloride (CCl<sub>4</sub>) is a dangerous chemical known to induce different experimental animal tissue damage, which is mediated via trichloromethyl free radicals generated in liver. When applied, it causes liver, kidneys, brain, hearth, muscles, lungs, testis, etc. cell (cell structures) oxidative damage (5, 6). When administered, CCl<sub>4</sub> is metabolised by liver Cytochrome P450 into highly reactive trichloromethyl radicals that further cause damage to different cell macromolecules (lipids, proteins, nucleic acids) (5). Muscle tissue is relatively susceptible to different types of injuries and the process of its regeneration often leads to scar tissue formation. These regeneration processes could be enhanced, thus preventing scarring and loss in muscle strength, by applying different exogenous compound e.g. antioxidants (7).

Melatonin is a neurohormone synthesized by bone marrow cells, gut, lens, pineal gland cells, in the retina, Harderian glands, ovary, testes and skin

(8). Besides its role as a "biological clock" regulator, MLT possesses strong antioxidant activity i.e. acts as a scavenger for hydroxyl and peroxy radicals (9). Both synthesized and inject MLT exert its activity via three melatonin type receptors (MT1-3) that are distributed throughout the body, where the MT3 is directly related to MLT potential antioxidative defence since it involves enzyme quinone reductase 2 that prevents electron transfer of quinones (10). In rat SM crash injury model MLT was shown to exhibit significant modulation of apoptotic pathways mediated by MT1 receptors, thus preserving SM tissue function (7).

### Aim of the study

The aim of the present study was to estimate MLT potential in preventing changes in rat gastrocnemius muscle CAT activity occurring after an acute CCl<sub>4</sub> application.

### Methods

#### Animals and housing

Male Wistar rats, weighting 250-300 g, were obtained from the Vivarium of the Institute of Biomedical Research, Faculty of Medicine, University of Niš, Serbia, housed in groups of 6. The animals were maintained under standard laboratory conditions: temperature 22 ± 2 °C and humidity 60%, with food and water available ad libitum. All experimental procedures with the animals were conducted in compliance with the Declaration of Helsinki and European Community guidelines for the ethical handling of laboratory animals (EU Directive of 2010; 2010 /63/EU) and were also approved by the local Ethics Committee.

#### Muscle tissue damage induction

Before the experiment, all animals were divided into three groups of 6 rats each:

- Group I - control group animals were administered with vehicle (olive oil, 10 ml/kg),
- Group II - group treated with CCl<sub>4</sub> (1 ml/kg),

- Group III – group treated with MLT (50 mg/kg) 1 h prior to CCl<sub>4</sub> application.

All animals were treated by an intraperitoneal injection and all were sacrificed by an overdose of ketamine 24 h after the last injection. Skeletal muscle tissue samples collected for tissue biochemical analyses included the gastrocnemius (GCM) muscle.

#### Tissue biochemical analysis

Collected GCM tissue samples were homogenized in distilled water (10%, w/v) and centrifuged afterward (4000 rpm, 10 min, 4 °C) in order to obtain supernatant. Total protein content in GCM homogenates was determined using standard Lowry method (11). The calculations were performed based on albumin standard curve (0-12 mg/ml).

Catalase activity determined following standard method previously described (12). Briefly, equal volumes of tissue homogenates and substrate, H<sub>2</sub>O<sub>2</sub>, dissolved in phosphate buffer saline were incubated for 5 minutes at room temperature. Afterward, the same volume of ammonium molybdate was added to reaction mixture in order to stop the reaction. The intensity of formed yellow dye was measured at 405 nm and the results were expressed as U/g of proteins.

#### Statistical analysis

The obtained results were given as mean values ± standard deviation (SD) and were further compared using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons (GraphPad Prism, ver. 5.03; San Diego, CA). Probability values (p) less than 0.05 were considered to be statistically significant.

### Results

The results obtained for CAT activity in GCM of animals from Group I-III are given in Table 1. Acute administration of CCl<sub>4</sub> caused statistically significant decrease in GCM CAT activity. Application of MLT 1h before CCl<sub>4</sub> prevented a significant decrease in CAT activity (Table 1).

**Table 1.** Gastrocnemius muscle catalase activity in rats acutely administered with CCl<sub>4</sub> and MLT

Biochemical parameter/ Group (treatment)	Group I (Vehicle)	Group II (CCl <sub>4</sub> )	Group II (MLT + CCl <sub>4</sub> )
Gastrocnemius muscle catalase activity (U/g of tissue proteins)	134.4 ± 11.9	115.7 ± 2.2*	146.9 ± 17.2 <sup>#</sup>

The results are presented as mean value ± SD (n=6); \*p<0.001 vs. Group I; <sup>#</sup>p<0.001 vs. Group II.

## Discussion

Different disorders affecting SM, as well as some intense exercise protocols are strongly related to free reactive oxygen species (ROS) damaging effects (13). Generation of ROS can be traced back to either SM cells and/or interstitial inflammatory cells, where over time myofibrils are losing their ability to cope with excessive ROS (13). At physiological levels ROS are needed for proper SM functioning, however their excessive production leads to contractile dysfunction (13). Enzymatic antioxidant capacities of SM exposed to significant increase in ROS is reported to be both increased and decreased by different authors (14-16). All cells, including SM cells, have their own antioxidative enzyme systems that precisely regulate the amounts of ROS in physiological conditions (13). However, these enzymes could be significantly altered and their deficiencies lead to wide range of different disorders such as metabolic and autoimmune disorders (17).

The presence of CAT in skeletal muscle tissue cells has been pointed out during 1950s (18) and enzyme localisation and function have not been completely understood. Namely, the loss in rat GCM wet weight, seen after animals were exposed to starvation, is reported to linearly follow an increase in tissue catalase activity, suggesting that CAT might be used as a biomarker for muscle waist (18). On the other hand, more recent study revealed that progressive sarcopenia (loss of SM mass), a frequent disorder seen in older people, is followed by an increase in H<sub>2</sub>O<sub>2</sub> and decreases in tissue CAT activity (14). Also, they postulated that H<sub>2</sub>O<sub>2</sub> represents the most important ROS involved in the onset of sarcopenia (14).

Since the CAT is reported to be an important enzyme involved in different neuromuscular disorders (13, 14) the present study evaluated its activity in CCl<sub>4</sub> induced SM damage model. The activity of the CAT, an important enzyme involved in ROS metabolism, was found to be significantly altered in animals that received CCl<sub>4</sub>, whereas the activity was found to be increased in liver and heart, while in brain and kidneys its activity was decreased (19). The results of our study suggest that CCl<sub>4</sub> acutely administered to rats leads to CAT activity decrease in the investigated SM (Table 1).

Melatonin is proven to be powerful antioxidant both in *in vivo* and *in vitro* conditions, where it is able to scavenge ROS and to affect tissue enzymatic and non-enzymatic antioxidant defences (20). There are studies reporting that MLT restorative effect on oxidative status is related to its capability to diminish H<sub>2</sub>O<sub>2</sub> and lipid peroxidation (20). One of the suggested mechanisms of MLT antioxidant action is its potential to directly scavenge ROS, where some authors suggest that its activity could be complementary to the function of catalase and glutathione peroxidase (21). Namely, MLT was found to directly scavenge H<sub>2</sub>O<sub>2</sub> forming N(1)-acetyl-N(2)-formyl-5-methoxykynura-mine that can further be transformed by CAT (21).

Also, previous studies revealed that MLT is able to increase SOD and CAT activity in skin cells and lymphocytes by up-regulating their gene expression (17, 22). Such activity is proposed to be related to the melatonin ability to prevent nuclear factor-E2-related factor (Nrf2) degradation, thus enabling Nrf2 to stimulate the transcription of enzymes involved in cell antioxidant defenses (17). Melatonin is known to modulate tissue antioxidant activity via MT1 receptors (17) that are expressed in rat skeletal muscle cells as well (23). Additionally, in an *in vivo* model of doxorubicin induced rat cardiac muscle damage melatonin lead to statistically significant increase in tissue catalase activity (24).

## Conclusion

The results of the present study revealed that acutely administered CCl<sub>4</sub> caused significant decrease in rat gastrocnemius muscle catalase activity. Additionally, when melatonin was administered prior to CCl<sub>4</sub> such drastic decrease in catalase activity was prevented. The activity of melatonin could possibly be attributed both to its ability to directly scavenge H<sub>2</sub>O<sub>2</sub> and to increase catalase production by enhancing mRNA synthesis.

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doi:10.5633/amm.2019.0301**MELATONIN SPREČAVA SMANJENJE AKTIVNOSTI KATALAZE  
INDUKOVANO APLIKACIJOM UGLJEN-TETRAHLORIDA U *M.*  
GASTROCNEMIUS-U PACOVA**

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Poznato je da je skeletno mišićno tkivo podložno oštećenjima koja su indukovana reaktivnim vrstama kiseonika, koje prate različite poremećaje skeletnih mišića. Ugljen-tetrahlid (CCl<sub>4</sub>) je opasna supstanca koja se koristi u eksperimentalne svrhe za indukciju oštećenja tkiva i posredovana je reaktivnim kiseoničnim vrstama. Takođe, utvrđeno je da antioksidansi, prirodni ili sintetički, mogu da spreče/ublaže oštećenja izazvana kiseoničnim vrstama. Cilj ove studije bio je da utvrdi uticaje melatonina (MLT), snažnog antioksidansa, na promene u aktivnosti mišićne katalaze kod Wistar pacova koji su akutno tretirani CCl<sub>4</sub>. Tkivo *m. gastrocnemius*-a, u kome je određivana aktivnost katalaze, uzeto je iz životinja koje su svrstane u tri grupe, i to iz životinja kontrolne grupe (netretirane), grupe tretirane CCl<sub>4</sub> i iz grupe pacova koja je tretirana MLT i CCl<sub>4</sub>. Dobijeni rezultati ukazuju na statistički značajno smanjenje u aktivnosti katalaze kod životinja koje su tretirane CCl<sub>4</sub>, dok ovako značajan pad aktivnosti katalaze nije primećen kod životinja koje su primile MLT i CCl<sub>4</sub>. Protektivna aktivnost MLT može biti posledica različitih mehanizama dejstva s obzirom da je poznato da MLT direktno vezuje reaktivne kiseonične vrste, povećava antioksidativni kapacitet tkiva, ali i povećava gensku ekspresiju antioksidantnih enzima u tkivima.

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**Ključne reči:** ugljen-tetrahlid, *m. gastrocnemius*, katalaza

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