For cited: *Dıraman, E., Kesici, B. & Solmaz, G. F. (2018). The Effect of Nicotine and Melatonin on Kidney Antioxidant Enzyme Activities in Mice. Journal of Current Researches on Health Sector, 8 (2), 103-108.*

Year: 2018
Volume: 8
Issue: 2

Journal of Current Researches on Health Sector
(J o C R e H e S)
www.stracademy.com/jocheres
ISSN: 2547-9636

Research Article/Araştırma Makalesi

Crossref doi: 10.26579/jocrehes_8.2.10

The Effect of Nicotine and Melatonin on Kidney Antioxidant Enzyme Activities in Mice

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Keywords
Nicotine, Melatonin, Superoxide Dismutase, Catalase, Kidney, Mice.

Abstract
In this study, the effects of nicotine and melatonin were researched comparatively on the activities of superoxide dismutase (SOD) and catalase (CAT) as kidney antioxidants enzymes in mice.
For this purpose, nicotine and melatonin were given to mice by intraperitonal injection and after the injection at the 2th, 4th, 8th, 12th and 24th hours mice were killed by servical dislocation; and their kidneys were removed. After total enzyme fractions were obtained by applying the kidneys homogenisation, sonification and centrifugation. These fractions were used for the determination of changes in SOD and CAT activities.
As a result, it was determined that the CAT activity generally was inhibited but there was a few activation 8th hour by the effect of nicotine. Under the effect of melatonin the CAT activity generally was activated but there was a few inhibition in 2th hour. And it was observed that under the effect of nicotine+melatonin the CAT activity activated 4th and 8th hours but inhibited 2th , 12th and 24th hours. It was determined that the SOD activity was inhibited 2th and 4th hours but activated 8th, 12th and 24th hours by the effect of nicotine. Under the effect of melatonin the SOD activity generally was activated all the hours. And it was observed that under the effect of nicotine+melatonin the SOD activity inhibited 2th, 4th and 24th hours but activited 8th and 12th hours.

Article History
Received
9 Oct, 2018
Accepted
1 Dec, 2018

1. Introduction

Free radicals, which have unpaired electrons in their outer orbit and are identified as short-lived reactive atoms and molecules, affect the entire biomolecules in the organic medium. As a result, they may cause death by destroying the balance of biological systems. Radicals can also cause events in cells, such as molecular changes (Florence, 1995) and gene mutations (Fridowich, 1975).

The defense that controls radical reactions and prevents radical formation is made with antioxidants. Enzymatic (SOD, CAT, GSH-PX etc.) and non-enzymatic antioxidants (tocopherol, ascorbate, glutathione, uric acid, glucose etc.) in the cells and tissues react quickly with radicals. As a result of this reaction, the cells are protected from harmful effects of radicals (Aslan et. al., 1995). If the antioxidant

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substances in the cells can not prevent the reactions of free radicals, cell damage will occur (Karlsson et. al., 1997)

Smoking and different forms of tobacco have been consumed in almost every society since centuries. The reason for the abandonment of tobacco, which is mostly used as cigarette, is due to the addictive nature of nicotine, the main substance. Nicotine use is known to have negative effects on nervous system (Wesnes and Warburton, 1983; Seyler et.al, 1986), hormonal system (Seyler et.al.,1986), cardiovascular system (Hartz, 1984), gastrointestinal system (Aşut, 1993; Kayaalp, 1993) and pregnancy (Ammenheuser et.al.., 1994). It has also been reported that the use of nicotine is associated with various cancers (Hect et al., 1993; Kayaalp, 1993).

Melatonin produced by the pineal gland is a good radical scavenger and antioxidant. It is also widely used in medicine and pharmacy. Due to its lipophilic nature, the morphophysiological barriers can pass through easily. For this reason, the cells may protect against oxidative damage. The fact that there is no foreign substance in the body also increases the importance of melatonin as an antioxidant (Reiter, 1997).

In the light of this information, in our study, the effects of nicotine, which causes free radical formation, and the melatonin hormone, which is released from the pineal gland and known as antioxidant, on the activities of SOD and CAT, which are of the renal antioxidant enzymes in mice were researched as to time.

2. Materials and Method

Swiss albino laboratory mice (Mus musculus) weighing 25-30 g were used in the study. No distinction was made between male and female. Melatonin was dissolved in ethanol and nicotine in bidistile water. Melatonin was injected intraperitoneally into rats (10mg/kg) and nicotine (3mg/kg) (Reiter, 1997). In our study, four groups were formed as Nicotine, Melatonin, Nicotine + Melatonin and Control group.

Injection and Obtaining Kidneys

Nicotine + melatonin group was first injected with nicotine and half an hour later with melatonin. Following injection, mice were sacrificed by cervical dislocation at 2^{nd}, 4^{th}, 8^{th}, 12^{th} and 24^{th} hours, and then the

3. Results

Effects of Nicotine : In SOD activity, inhibition was observed at 2^{nd} and 4^{th} hours while activation was observed from 8^{th} to the end of 24^{th} hours according to the control group. In CAT activity, inhibition was observed at all times according to the control group.

Effects of Melatonin: With the effect of melatonin, SOD activity was observed a general activation at all hours compared to the control group. In CAT activity was observed 4% inhibition at 2^{nd} hour. This inhibition turned into 12% activation at 4^{th} hour.

Effects of Nicotine + Melatonin: While nicotine and melatonin together inhibited SOD activity at the 2^{nd} and 4^{th} hours, they activated at 8^{th} and 12^{th} hours. However,

inhibition was observed again at 24th hour. Nicotine and melatonin together inhibited CAT activity at 2nd hours and caused 34% activation at 4th hours. This activation gradually decreased at 8th and 12th hours, and turned into inhibition at 24th hours.

Abdomen and chest were opened. The kidneys of the mice were removed and placed in a beaker containing cold buffer. The kidneys taken with excess water drying paper were weighed on a sensitive scale. They were stored in a freezer at -80ºC in a sucrose solution of 0.25 M until the experimental procedures started. After homogenization and sonication were applied to the kidneys removed from the freezer, the resulting homogenate was centrifuged at 15,000 rpm for 30 minutes at 4 ° C.

4. Analysis

All analyzes were carried out at 0-4 ° C. Protein identification was determined by Lowry et al., (1951), SOD activity by McCord and Fridovich (1969) and Flohe and Otting (1984), CAT activity by Luck (1963).

As a result of the obtained data, Two-way ANOVA test was performed for SOD and CAT activity. The difference in activity between groups and hours for SOD activity was significant ($p < 0.05$). The change in CAT activity between the hours was not statistically significant ($p > 0.05$) but it was statistically significant ($p < 0.05$) between the groups.

Figure 1. Time-Dependent Change of Total SOD Activity of Kidney in Nicotine, Melatonin, Nicotine + Melatonin-Treated Mice

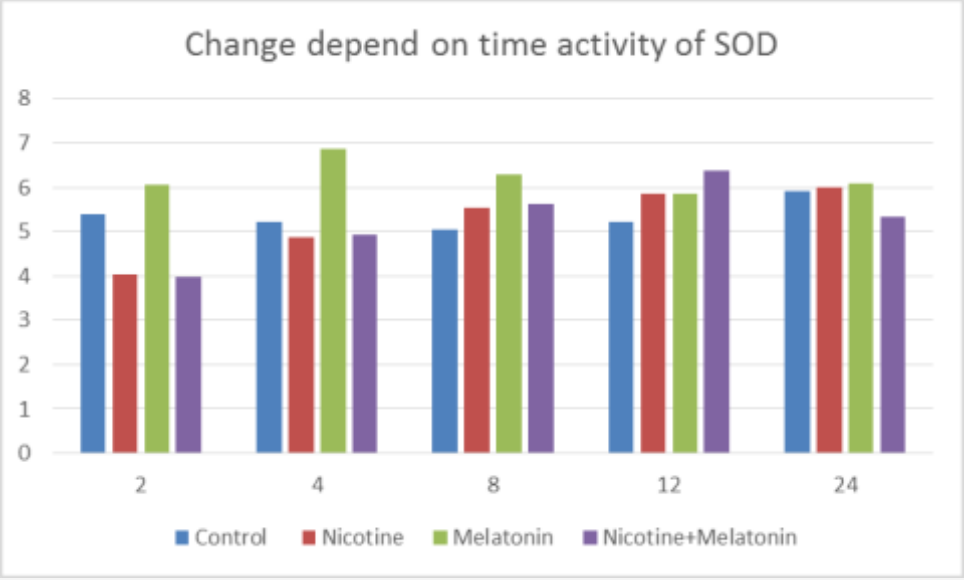
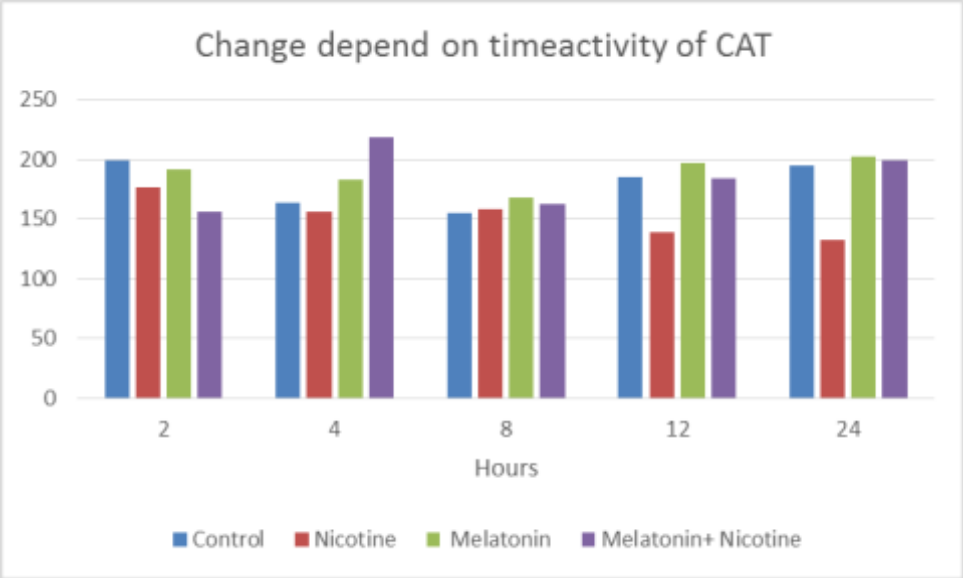


Figure 2. Time-Dependent Change in Total Kinetics of Renal Total CAT in Nicotine, Melatonin, Nicotine + Melatonin-Treated Mice



5. Discussion

In our study, the effects of melatonin, a good radical scavenger and antioxidant, on the activities of SOD and CAT, which are of the renal antioxidant enzymes in mice were investigated in order to reduce the effects of nicotine, which causes free radical production.

Nicotine is one of the tobacco alkaloids and is responsible for the high smoking prevalence due to its addictive properties in the world (Delijewski et al. 2014). Cigarette smoking, tobacco chewing and nicotine replacement therapies are the main sources of human exposure to nicotine. Nicotine has been recognized to result in oxidative stress by inducing the generation of reactive oxygen species (ROS) (Muthukumaran et al. 2008). Husein et.al., (2001) reported increased CAT activity and reduced SOD activity by administration of nicotine in the kidney. In this study, in the SOD activity, inhibition was observed at 2nd and 4th hours while activation was observed from 8th to the end of 24th hours according to the control group, with effect of nicotine. In CAT activity, inhibition was observed at all times according to the control group with effect of nicotine. Therefore, the increase in SOD activity is thought to be the result of induction of enzyme synthesis due to increased free radicals in the medium.

Several studies demonstrated that melatonin treatment prevents tissue damage in various models of oxidative stress (Ramis et al., 2015). Goc et al., ın their study in 2017, they observed that SOD, CAT, and GSH-Px activity increased in all organs after 3 hours in the MEL-treated group, whereas that in liver and kidney increases after 6 hours. In our study, after administration of melatonin, while CAT enzyme activity was increased at 4th hour SOD enzyme activity increased at 8th hours.

In this study, While nicotine and melatonin together inhibited SOD activity at the 2nd and 4th hours, they activated at 8th and 12th hours. However, inhibition was observed again at 24th hour. Nicotine and melatonin together inhibited CAT activity at 2nd hours and caused 34% activation at 4th hours. This activation gradually decreased at 8th and 12th hours, and turned into inhibition at 24th hours.

Our data demonstrate that Melatonin plays a protective role against the imbalance elicited by nicotine between the production of free radicals and antioxidant defense systems. We can say that when melatonin is given with nicotine, it may decrease the effect of nicotine.

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