

The protective role of melatonin against the effect of caffeine on embryonic kidney

Kafeinin embriyonel böbrek gelişimine olan etkisine karşı melatoninin koruyucu rolü

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ABSTRACT

Objective: Oxidative stress is one of the major causes of embryonal developmental disorders. In addition, melatonin reduces oxidative stress in the body and is known as a potential treatment for adverse effects of caffeine on fetal development. In this study, we aimed to investigate the teratogenic effect of caffeine on fetal kidney development and the protection of melatonin against the teratogenicity in terms of biochemical parameters.

Methods: 5-7 months old Wistar-Albino rats (n=24) weighing 180-220 g were divided into seven groups for control, caffeine, melatonin, and caffeine+melatonin (co-injection). Control group: Serum physiological (SF) as 1 mL/kg intraperitoneal (i.p.), Sham group: 0.1 ml hanks as i.p., Low dose caffeine group: 30 mg/kg caffeine as gavage, Low dose caffeine+melatonin group: 30 mg/kg dose gavage with 10 mg/kg melatonin as i.p., High dose caffeine group: 60 mg/kg caffeine as gavage, High dose caffeine+melatonin group: 60 mg/kg dose gavage with 10 mg/kg melatonin as i.p., and Melatonin group: 10 mg/kg melatonin as i.p.

Results: Total antioxidant status (TAS) values were 0.49±0.02 in the control group and 0.14±0.00 in the high

ÖZET

Amaç: Oksidatif stres embriyonel gelişim bozukluklarının en büyük sebeplerinden biridir. Ayrıca, melatonin vücutta oksidatif stresi azaltması nedeniyle kafeinin fetal gelişime olan olumsuz etkilerine karşı potansiyel bir tedavi yöntemi olarak görülmektedir. Bu çalışmada; kafeinin fetal böbrek gelişimine olan teratojenik etkisi ve melatonin maddesinin bu teratojeniteye karşı koruyuculuğunu biyokimyasal parametreler üzerinden incelemeyi hedeflemiştir.

Yöntem: Çalışmada, 180-220 g ağırlığında 24 adet 5-7 aylık Wistar-Albino ırkı ratlar; kontrol, kafein, melatonin ve hem kafein hem de melatonin enjekte edilmek üzere yedi gruba ayrılmıştır. Kontrol grubu: Serum fizyolojik (SF), (1ml/kg) intraperitoneal (i.p.) Sham grubu: 0.1 mL hanks i.p., Düşük doz kafein grubu: 30mg/kg kafein gavaj olarak Düşük doz kafein+Melatonin grubu: 30mg/kg dozda gavaj ile 10 mg/kg melatonin i.p., Yüksek doz kafein grubu: 60mg/kg kafein gavaj, Yüksek doz kafein+Melatonin grubu: 60mg/kg dozda gavaj ile 10 mg/kg melatonin i.p, Melatonin grubu: 10mg/kg melatonin i.p. olarak verilmiştir.

Bulgular: Alınan böbrek dokularında biyokimyasal değerler incelendiğinde, total antioksidan kapasite

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Geliş Tarihi / Received : 19.12.2019

Kabul Tarihi / Accepted : 05.01.2020

DOI ID : 10.5505/TurkHijyen.2020.77675

Yılmaz S, Göçmen AY, Üner AK, Akyüz E, Tokpinar A. The protective role of melatonin against the effect of caffeine on embryonic kidney.

Türk Hij Den Biyol Derg, 2020; 77(1): 51-58

dose caffeine group. In addition, total oxidant status (TOS), oxidative stress index (OSI), glutathione (GSH), glutathione disulfide (GSSG), superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS), calcium (Ca), vitamin D and GSH/GSSG levels were examined ($p<0.001$).

Conclusion: Melatonin has shown that caffeine has an essential role in reducing teratogenic effects and also reducing oxidative stress. Therefore, melatonin is a potential treatment for oxidative stress and caffeine teratogenicity in embryonic development.

Key Words: Caffeine, rat, melatonin, kidney

(TAS) değeri kontrol grubunda $0,49\pm0,02$, yüksek doz kafein grubunda $0,14\pm0,00$ olduğu görülmüştür. Ayrıca total oksidan kapasite (TOS), oksidatif stres indeksi (OSI), glutatyon (GSH), glutatyon disülfid (GSSG), süperoksit dismutaz (SOD), tiyobarbitürik asit reaktif maddeler (TBARS), Kalsiyum (Ca), D vitamini ve GSH/GSSG seviyeleri incelenmiştir ($p<0,001$).

Sonuç: Melatoninin kafeinin teratojenik etkilerini ve oksidatif stresi azaltmada önemli bir role sahip olduğunu göstermiştir. Bu nedenle melatonin, embriyonel gelişimde oksidatif stres ve kafein teratojenitesi için potansiyel tedavi niteliği taşımaktadır.

Anahtar Kelimeler: Kafein, sıçan, melatonin, böbrek

INTRODUCTION

Oxidative stress is defined as the presence of active oxygen species above the available antioxidant buffering capacity. These products may damage proteins, lipids, and DNA that alter reactive oxygen species, the structure, and function of the organism. Tissue damage is caused by a series of enzymes that produce highly reactive intermediate compounds free radicals and non-enzyme-mediated biochemical reactions (1, 2).

Although oxidative stress is a feature of normal pregnancy, it affects the antioxidant capacity of the placenta, leading to the consumption and reduction of antioxidants. The high course of oxidative stress damages the lipids, proteins, and DNA in the placental tissue, causing an accelerated aging form. The premature aging of the placenta is associated with placental insufficiency, which prevents the organ and the fetus from addressing their needs, and as a result, the viability of the fetus is compromised. In addition, high doses of caffeine taken during pregnancy are known to cause oxidative stress (3).

Caffeine (1,3,7-trimethylxanthine) has been a common component of human nutrition for thousands of years, given its behavior and stimulating

effects (4). Caffeine is the most commonly used psychostimulant in Western countries (5, 6). Caffeine is rapidly absorbed from the gastrointestinal tract and the highest caffeine concentration is reached 30-60 minutes after consumption (6, 7). In addition to the organism, factors such as caffeine consumption time, sex, physiological status, and temperature also affect the pharmacokinetics of caffeine. In animal studies, teratogenic and embryotoxic effects of high caffeine doses have been determined (8). Since sufficient enzyme systems do not develop during pregnancy, caffeine metabolism might be extended up to 9-11 hours (9). Because of these effects, caffeine is known to adversely affect fetal development and especially kidney development (10). Therefore, several studies have been conducted on the teratogenic effects of caffeine in recent years. Antioxidants are especially targeted in the studies (11-13).

Melatonin, also known as N-acetyl 5 methoxy tryptamine, is secreted by the pineal gland, especially at night. In addition to adjusting the basic biological rhythm, it is also involved in other basic tasks, such as cell regeneration, strengthening the immune system and regulating body temperature. Melatonin is one

of the most powerful antioxidants known due to its lipophilic structure (14).

High-dose caffeine is known to adversely affect the kidneys and is closely associated with polycystic kidney disease, kidney stones, and hypertension (15). In addition, it has been recently shown that caffeine negatively affects fetal kidney development during the intrauterine period and causes oxidative stress (16). In line with this objective, melatonin may be targeted as a new treatment against the teratogenic effects of caffeine on the kidney.

In recent studies, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), glutathione (GSH), glutathione disulfide (GSSG), superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS) substances such as oxidative stress markers have been shown to be used (17). Therefore, in this study, the parameters were targeted as markers of oxidative stress.

In summary, although the teratogenic effects of caffeine on kidney development were shown in the literature, a study examining the protective effects of melatonin on these effects has not been performed yet. In this study, the teratogenic effect of caffeine on fetal kidney development and the protection of melatonin against the teratogenicity were investigated.

MATERIAL and METHOD

Animals

Wistar-Albino rats (n = 24), 5-7 months old, weighing 180-220 g were used in our study from Erciyes University Experimental Animals and Clinical Research Center (DEKAM). For the research, ethics committee permission of Erciyes University animal experiments local ethics committee dated 11.01.2017 and numbered 17/003 was obtained. The rats were caged at 5.00 pm, with two females and one male, for their match. The following morning, female rats were subjected to a vaginal smear test at 7.00 am.

Females with sperm on the smear test were accepted as 0.5 days pregnant. The rats were kept in specially prepared, automatically air-conditioned rooms with constant temperatures of 19-21 °C and 12 hours of light/dark periods during the study. Rats were fed with normal pellet feed.

1. Control group (C): Serum physiological (SF), (1 mL/kg) was applied i.p.
2. Sham group (S): 0.1 mL of hanks was administered i.p.
3. Low-dose caffeine group (LDC): 30 mg/kg caffeine was administered as gavage.
4. Low dose caffeine + Melatonin group (LDC + M): 30 mg/kg caffeine as gavage and 10 mg/kg melatonin were applied as i.p.
5. High-dose caffeine group (HDC): 60 mg/kg caffeine was administered as gavage.
6. High dose caffeine + Melatonin group (HDC + M): 60 mg/kg caffeine as gavage and 10 mg/kg melatonin were administered as i.p.
7. Melatonin group (M): 10 mg/kg melatonin was given as i.p.

Experimental procedure was shown in (Figure 1).

Preparation of Injections

Caffeine and melatonin powder 98% were obtained from Sigma Aldrich. Drinking water was used as the solvent solution to adjust the amount of caffeine to be given to the rats. In the process of dissolving melatonin in powder form, a hanks solution was used. Both substances were prepared daily and no stock solution was made.

Manipulation of Rats and Obtaining of Fetus

Pregnant rats were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) on the 20th day of gestation. The uterus and the fetuses were dissected individually with their placentas. The kidneys of the animals were collected in sterile plastic bags and stored at -80 °C until biochemical analysis.

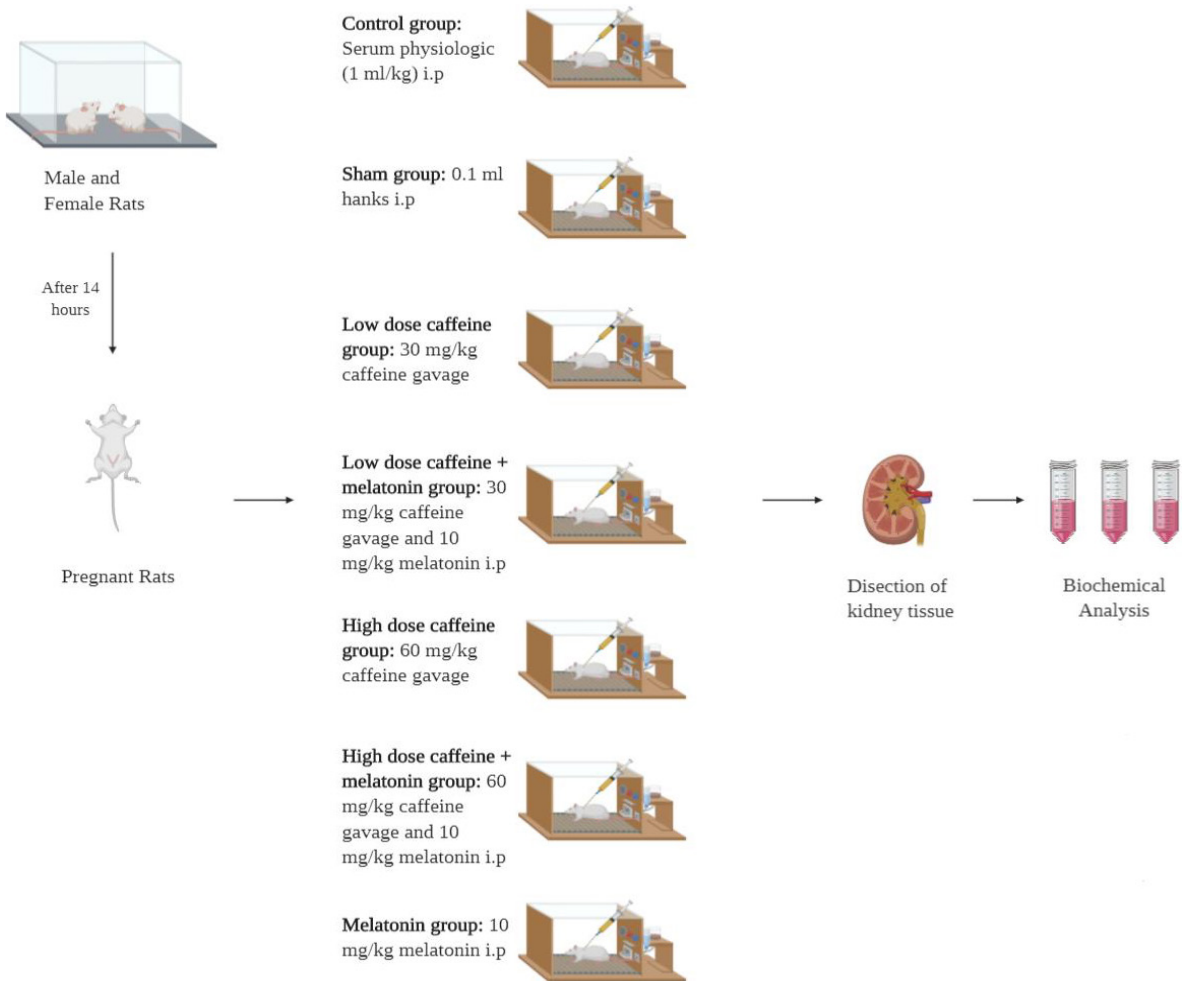


Figure 1. Summary diagram of the whole study. Biochemical analyzes of kidney tissues were performed in seven different groups

Biochemical Analysis

Tissue preparation and protein determination

The kidney tissue used for analysis was placed in microcentrifuge tubes, washed 3 times with 1 mL of 50 mM PBS and aspirated. The whole tissues were homogenized in cold potassium phosphate buffer (50 mM, pH 7.4). Tissue protein levels were measured after appropriate dilutions had been made in the supernatants. The protein content of the samples was determined by the method of Lowry et al. (18) using bovine serum albumin as standard.

Oxidative stress parameters

Oxidative stress parameters (TAS, TOS and SOD levels) were determined by the spectrophotometric method. TAS and TOS tissue levels were calculated according to Erel (19). A known amount of antioxidant (1.65 mmol/l) was used to calculate the antioxidant levels in the samples. The TAS level was presented as mmol Trolox equivalent/l (mmol Trolox equivalent/l). The assay was calibrated with a standard hydrogen peroxide solution (39.16 $\mu\text{mol/l}$) to determine the TOS level. The results are presented as $\mu\text{mol H}_2\text{O}_2$ equivalent/l ($\mu\text{mol H}_2\text{O}_2$ equivalent/l). Other

oxidative stress parameters such as GSH (Cat. No: E-BC-K030-M), GSSG (Cat. No: 703002, Cayman, USA) and SOD (Cat. E-BC-K020, Elabscience, USA) according to the manufacturer's instructions microplate reader (BioTek Instruments, EL × 800 TM, USA). Commercial ELISA kits (EIA 5396 DRG, Germany) were used for vitamin D analysis.

Determination of Ca²⁺

Total Ca²⁺ was assessed using the calcium colorimetric assay kit (ab102505; Abcam) according to the manufacturer's data sheet. 25 standard L of standard solution and 25 sup L of supernatant (diluted 1: 10) extracted from the tissue were mixed with 45 µL of chromogenic reagent and 30 µL of test buffer. The mixture was incubated in the dark for 15 minutes at room temperature. The signal was scanned at 575 nm (Thermo Varioscan). The calcium concentration in the samples was calculated according to the technique described in 2018 by Sen et al.

Statistical analysis

All analyses were performed with SPSS version 23.0 (IBM Co., NY, USA). Data were given as mean (standard deviation). Differences between the groups were analyzed by one-way analysis of variance (ANOVA) and Post hoc Tukey test for continuous variables and parametric data, respectively. Kruskal-Wallis and Post hoc Dunn tests were used for nonparametric data. $p < 0.05$ was considered statistically significant.

RESULTS

The highest TAS values were obtained in the control and melatonin groups, respectively. Melatonin administration significantly increased the antioxidant status in a dose-dependent manner compared to the caffeine group. Dose-dependent melatonin showed a stronger antioxidant effect as used without caffeine. TOS values showed the highest oxidant effect in HDC group. The control group had the lowest value compared to the others. Oxidative stress index was

calculated by TOS/TAS ratio. OSI was significantly higher in the HDC group and lower in the melatonin-treated groups (Table 1). GSH and GSH/GSSG were the highest in melatonin group and GSSG was the highest in HDC group. Melatonin decreased GSSG and increased GSH and GSH/GSSG (Table 1). SOD was the highest in HDC group. Intracellular calcium and vitamin D values of melatonin group were higher than all other groups. According to these results, melatonin significantly reduced oxidative stress by stimulating the antioxidant system.

DISCUSSION

Oxidative stress may disrupt various physiological processes, including cell damage and triggering apoptosis. Melatonin has antioxidant effects and might affect antioxidant enzyme activity and cellular mRNA levels under physiological or high oxidative stress conditions. The beneficial effects of melatonin on cellular toxicity induced by free radicals and peroxynitrite have emerged in numerous experimental and clinical studies (20, 21).

It is emphasized by several researchers that cytotoxic agents negatively affect the development of organs during embryo-fetal development (22).

In 2019, it was aimed to evaluate the possible antioxidant effect of pharmacological doses of melatonin on band-3 protein anion exchange ability. The findings obtained in the study showed the antioxidant power of melatonin for in vitro oxidative stress model (23). In another study, it was purposed to determine the effects of melatonin on antioxidant capacities and arginine metabolism in lung tissue and also on tissue inflammation and oxidative damage caused by carbon tetrachloride (CCl₄) exposure in rats. According to results, melatonin prevented tissue inflammation and the oxidative stress observing after molecules exposure to CCl₄ (24). It was also reported that melatonin, as an antioxidant, provides strong protection to cell organelles exposed to abundant

Table 1. Biochemical parameters of fetus kidney tissue

Parameters	Control	Melatonin Group	LDC Group	HDC group	LDC+Melatonin Group	HDC+Melatonin Group	Sham Group	p
TAS	0.49±0.02a	0.51±0.02a	0.28±0.02b	0.14±0.00c	0.35±0.03d	0.19±0.02e	0.42±0.03f	0.001
TOS	7.48±1.21a	8.12±0.32ab	10.63±0.65c	13.68±0.64d	8.16±0.97ae	8.07±1.22af	9.12±0.79bef	0.001
OSI	1.49±0.19a	1.59±0.10ab	3.73±0.40c	9.53±0.75d	2.34±0.36e	4.19±0.25c	2.20±0.43be	0.001
GSH	2.15±0.06a	2.20±0.15a	1.16±0.12b	0.50±0.14c	1.53±0.15d	0.96±0.07b	1.78±0.18e	0.001
GSSG	0.47±0.07a	0.50±0.02ab	0.65±0.04c	0.83±0.03d	0.50±0.06ae	0.51±0.05af	0.57±0.04bef	0.001
GSH/GSSG	215.34±6.91a	220.87±15.93a	116.60±12.83b	50.72±14.59c	153.64±15.45d	96.45±7.91e	50.52±2.92c	0.001
TBARS	0.84±0.13a	0.91±0.03ab	1.19±0.07c	1.51±0.07d	0.92±0.11ae	0.92±0.10af	1.02±0.08bef	0.001
SOD	9.89±1.60a	10.68±0.43ab	13.83±0.86c	17.50±0.83d	10.68±1.28ae	10.75±1.04af	11.94±0.99bef	0.001
Ca	3.79±0.18a	3.89±0.18a	2.41±0.14b	1.52±0.04c	2.78±0.23d	1.69±0.19c	3.30±0.24e	0.001
VIT D	2.13±0.09a	2.18±0.10a	1.27±0.08b	0.71±0.02c	1.53±0.14d	0.88±0.10c	1.82±0.15e	0.001

There is no significant difference between the groups with the same letters (a, b, c, d, e, f). $p < 0.05$ was considered significant. There was a significant decrease in TAS measurement in HDC group compared to control group. There was a significant increase in HDC+melatonin group compared to HDC group.

free oxygen radicals (25).

In 2019, pregnant mice were injected with energy drinks containing caffeine. As a result of this study, it was observed that perinatal exposure to energy drinks containing caffeine caused oxidative stress, tissue injury and behavioral changes in mouse newborns. Those effects have been reported to occur especially in kidney, liver and brain tissue. Therefore, it has been stated that the consumption of caffeine and energy drink during pregnancy and lactation has a negative effect in newborns and it should be seen as an essential health problem (10).

In another study conducted in 2019, the effects of melatonin on placental oxidative stress and intrauterine inflammation were aimed. It was observed that cardiovascular damage, mal-

perfusion and inflammation caused by oxidative stress decreased to reasonable levels as a result of melatonin exposure (26). It was stated that targeting of the use of melatonin in pregnancy and lactation would be valuable in the prevention of various adult chronic diseases and especially cardiovascular and neurological diseases in the next period (27). However, in the literature, there is no study examining the protective effect of melatonin in spite of the intrauterine oxidative stress caused by caffeine and its teratogenicity on the kidney.

In the light of these information, in this study, we examined the teratogenic effect of caffeine on fetal kidney development and the protection of melatonin against this teratogenicity through biochemical parameters. According to our study, HDC group had

the lowest GSH activity. GSH increased in HDC+M group after caffeine treatment following melatonin administration.

In addition, TAS, TOS, SOD and Vitamin-D are crucial biomarkers in the assessment of oxidative damage. It was determined that melatonin increased TAS levels and decreased TOS levels in HDC group. The highest SOD value was observed in the HDC group and the lowest value was recorded in the control group.

CONCLUSION

The results showed that melatonin has a key role in reducing the teratogenic effects of caffeine and oxidative stress. Therefore, melatonin is a potential treatment for oxidative stress and caffeine teratogenicity in embryonic development.

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