ORIGINAL PAPER

doi: 10.5455/medarh.2025.79.86-91
MED ARCH. 2025; 79(2): 86-91
RECEIVED: MAR 05, 2025
ACCEPTED: APR 12, 2025

[1]Department of Dermatovenerology, Clinical Center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
[2]Department of Histology and Embryology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Corresponding author: Mersiha Krupalija - Fazlic MD, Clinical Center, Department of Dermatovenerology, University of Sarajevo, Bolnicka 25, 71000 Sarajevo, Bosnia and Herzegovina; Phone: +387 61 205 640., E-mail: dr.mersihakrupalija@gmail.com. ORCID ID: http//www.orcid.org/0000-0000-0000-0000.

The Beneficial Effect of Melatonin on Gentamicin-induced Liver Injury in Rats

Mersiha Krupalija-Fazlic[1], Dina Kapic[2], Maida Sahinovic[2], Samra Custovic[2], Visnja Muzika[2]

ABSTRACT

Background: Gentamicin is a potent, broad-spectrum aminoglycoside antibiotic used in the treatment of many infections. Gentamicin can induce hepatotoxicity through oxidative stress and apoptosis. Since melatonin has antioxidant properties, its protective effects on liver tissue damage were evaluated in this study. **Objective:** The aim of this study was to conduct our investigation to assess the hepatoprotective effects of melatonin in rats treated with gentamicin. **Methods:** Forty eight adult male Wistar rats were used. The animals were randomly distributed into six groups of equal size. During the period of 11 days, three control groups of rats were daily injected i.p. with the vehicle or with melatonin at a dose of 5 or 10 mg/kg. The gentamicin group was injected with gentamicin at a dose of 80 mg/kg during 8 days and vehicle for 11 days. The other two experimental groups were administered gentamicin (80 mg/kg during) 8 days and melatonin (doses of 5 and 10 mg/kg) 3 days before and 8 days concomitantly with melatonin. Obtained liver sections were analyzed using qualitative, semi-quantitative, and stereological analysis. **Results:** Gentamicin expressed hepatotoxic effects inducing congestion of lobular blood vessels, hydropic degeneration of periportal hepatocytes and mononuclear infiltration in the portal tract. Treatment with gentamicin resulted in an increase in the Vv of blood vessels, a decrease in the Vv of hepatocytes, and a decrease in the glycogen content in all three lobular zones. Melatonin administration reduced the liver alterations induced by gentamicin; the higher dose had a more potent protective effect. **Conclusion:** Melatonin has a beneficial effect on gentamicin-induced liver damage and the effect is dose-dependent.

Keywords: gentamicin, liver, melatonin, rats.

1. BACKGROUND

Gentamicin is a broad-spectrum aminoglycoside antibiotic with well-known nephrotoxic and ototoxic effects (1, 2). Although, hepatic alterations induced by this agent are not often reported in the clinical practice (3), previous research on animal models has shown that gentamicin causes hepatocyte apoptosis, mononuclear infiltration, bile duct hyperplasia, depletion of glycogen and vascular lesions (4-6). The proposed mechanisms of gentamicin-induced hepatotoxicity are mitochondrial alteration and oxidative stress (6).

Melatonin is a neuroendocrine hormone produced by the pineal gland and some extrapineal tissues (retina, skin, gut, endocrine glands, and some immune and hematopoietic cells) which regulates circadian rhythm and has immunomodulatory and antioxidant properties (7-9). These effects are mediated through the activation of cell-surface melatonin receptors expressed on numerous cells (10). Hepatoprotective effects of exogenous melatonin have been demonstrated in numerous studies dealing with oxidative-stress mediated liver damage induced by a different agents (11, 12).

2. OBJECTIVE

We conducted this investigation to assess the hepatoprotective effects of melatonin in rats treated with gentamicin. The obtained results will contribute to the overall scientific knowledge, especially regarding the effect of different doses of exogenous melatonin.

3. MATERIAL AND METHODS

Animals

Adult male Wistar rats, weighing cca 200g, were acclimatized seven days prior to the experiment. The animals were maintained under standard laboratory conditions (temperature 22 ± 2°C, 12h dark/light cycle, summer) and were supplied with standard commercially available pellet food for rodents and water ad libitum. Maintenance and care of animals were in accordance with internationally accepted guidelines for biomedical research (The Council for International Organisations of Medical Sciences – CIOMS and The International Council for Laboratory Animal Science – ICLAS).

Experimental design

All animals (n=48) were randomly allocated into 3 control and 3 experimental groups (n=8/group). The substances were applied intraperitoneally (i.p.), daily, as follows:

- Vehicle group (V): vehicle 1% ethanol in Ringer solution, 11 days.
- Melatonin 1 group (M1): melatonin (5 mg/kg), 11 days.
- Melatonin 2 group (M2): melatonin (10 mg/kg), 11 days.

Gentamicin group (G): gentamicin (80 mg/kg) during consecutive 8 days + vehicle three days before and 8 days concomitantly with gentamicin.

Gentamicin + melatonin 1 group (GM1): gentamicin (80 mg/kg) during 8 days + melatonin (5 mg/kg) three days before and 8 days concomitantly with gentamicin.

Gentamicin + melatonin 2 group (GM2): gentamicin (80 mg/kg) during 8 days + melatonin (10 mg/kg) three days before and 8 days concomitantly with gentamicin

Substances

Freshly prepared 1% ethanol in Ringer solution was used as vehicle. Melatonin (Sigma-Aldrich, St. Louis, MO, USA) dissolved in absolute ethanol and diluted in commercially available Ringer solution was prepared in two different solutions (0.675 mg/ml; 1.25 mg/ml) with final concentration of 1% ethanol. All animals were administered with the same volume of used substances (8 ml/kg). Application of the substances was done daily (from 6 p.m. to 7 p.m.), and rats were injected with gentamicin 1h after melatonin administration.

Histotechnology

All animals were sacrificed by inhalation of diethyl ether 24h after last application of the substances. Right medial liver lobe was dissected and fixed in 10% buffered formalin. The tissue was then routinely processed, embedded in paraffin, and thin sections (5 μm) were made and stained with the haematoxylin-eosin (HE) and periodic-acid Schiff (PAS) staining method to detect deposits of glycogen.

Qualitative and quantitative histological analysis

Qualitative histological analysis was performed using light microscopy with the 40x, 100x and 400x magnifications. Representative photomicrographs were made using a light microscope (Eclipse 400, Nikon) with an installed digital camera (DN 100, Nikon).

Quantitative histological analysis included stereological determination of volume density (Vv) of hepatocytes, their nuclei and cytoplasm, and of the vascular compartment of the liver lobule (central vein and sinusoids). Semi-quantitative scoring of the deposited glycogen was done as proposed by Kaya et al (13) and Stojiljkovic and Stoiljkovic (14). For the semi-quantification, 30 hepatocytes were analyzed per animal and graded as follows: normal amount of glycogen (+++), I grade decrease (++), II grade decrease (+), and total absence (-).

Statistical analysis

The Shapiro-Wilk test was used to determine the normality of data distribution. All data were expressed as mean ± SD or median with interquartile range.

Differences between the corresponding groups were tested using the Kruskal-Wallis and Man Whitney U test. Spearman's rank correlation was calculated to assess the relationship between the variables. Results of the semi-quantitative scoring were analyzed using the chi-square test of independence to determine the association between two variables.

4. RESULTS

Qualitative histological analysis

Liver architecture appeared preserved in the control group of animals (Figure 1).

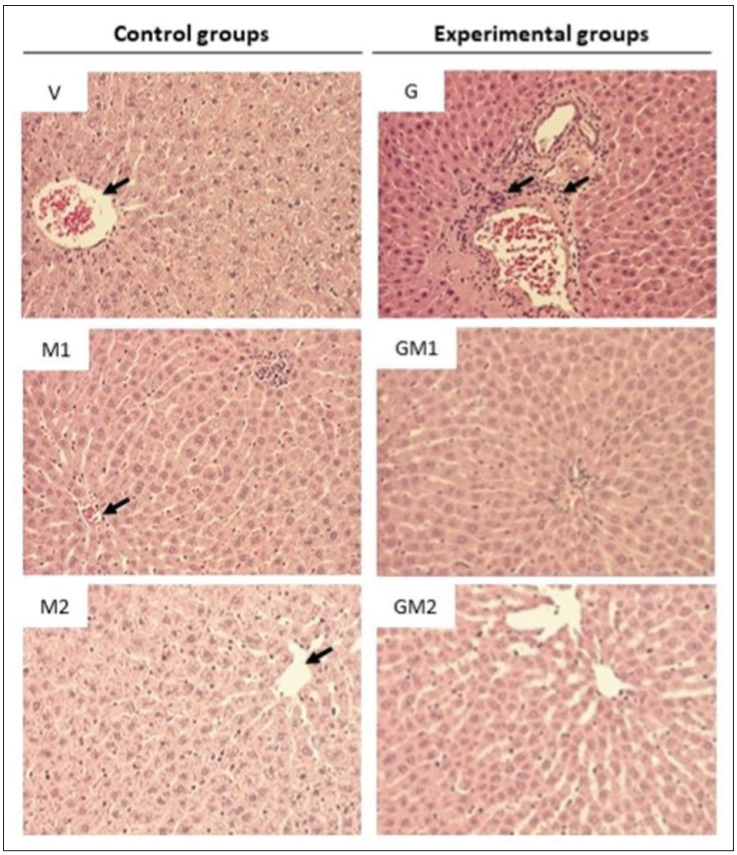

Figure 1. Representative photomicrographs of the liver of control and experimental groups. Preserved architecture of liver in control groups (V, M1, M2); arrows pointing to the central vein. Dilation and congestion of blood vessels and mononuclear infiltration of portal areas (arrow) were found in G group, while other experimental groups (GM1 and GM2) showed almost regular appearance. HE staining, 100x magnification.

Group	Vv blood vessels	Vv hepatocytes	Vv cytoplasm	Vv nucleus
V (N=8)	0,12(0,10-0,13)	0,88(0,87-0,89)	0,80(0,78-0,80)	0,083(0,074-0,093)
M1 (N=8)	0,15(0,10-0,18)	0,85(0,82-0,90)	0,77(0,72-0,82)	0,082(0,072-0,105)
M2 (N=8)	0,14(0,13-0,19)	0,86(0,84-0,87)	0,78(0,75-0,79)	0,083(0,072-0,093)
G (N=8)	0,19(0,18-0,23)*	0,81(0,77-0,82)*	0,68(0,67-0,70)*	0,106(0,091-0,130)*
GM1 (N=8)	0,18(0,15-0,19)*	0,82(0,81-0,85)*	0,70(0,67-0,73)*	0,122(0,099-0,141)
GM2 (N=8)	0,16(0,14-0,20)**	0,84(0,80-0,86)**	0,72(0,67-0,757)*	0,119(0,103-0,146)

Table 1. Results of the stereological analysis *statistically significant result in comparison to V group of animals (p<0.05); ** statistically significant result in comparison to G group of animals (p<0.05); results were presented as median [IQR, 25th-75th percentile]

The liver tissue of animals treated with the lower dose of melatonin (M1 group) was well preserved. The sinusoids appeared wider in comparison to the control group of animals but without congestion. Mononuclear infiltration was observed around the central venule and the hepatocytes in the periportal area were markedly hypertophic (Figure 1).

Findings in the group of animals treated with the higher dose of melatonin (M2 group) were similar to the M1 group, but without the mononuclear infiltration detected in the previous group (Figure 1).

The most prominent changes in the experimental groups of rats were detected in the gentamicin treated animals (G group). In this group dilation and congestion of blood vessels and mononuclear infiltration of portal areas was noted. In the liver lobule signs of hydropic degeneration of hepatocytes with hyperchromatic or picnotic nuclei in the periportal and intermediate zone were found (Figure 1).

Analysis of the liver structure in the GM1 group revealed only mild reactive changes in the marginal plate of hepatocytes containing prominent hyperchromatic nuclei. Other structural components appeared relatively preserved (Figure 1).

In the GM2 group both, liver lobules and portal areas showed regular architecture with occasional signs of hydropic degeneration of the hepatocytes in the intermediate region of the liver lobule (Figure 1).

Stereological analysis

Treatment with gentamicin resulted in an increase in the Vv of blood vessels and a decrease in the Vv of hepatocytes in the G group of rats in comparison to the V group. Administration of melatonin at a dose of 10 mg/kg in the GM2 group was accompanied with the reduction of Vv of the lobular blood vessels and an increase of the Vv of hepatocytes in comparison to the G group. A statistically significant, strong and negative correlation was found between the Vv of lobular blood vessels and the Vv of the hepatocyte cytoplasm (r=-0,893, p<0,0005) as well as the Vv of the liver lobule blood vessels and the Vv of the hepatocytes (r=-1, p<0,0005) in the experimental group of rats (Table 1).

Semi-quantitative analysis of the glycogen deposits in hepatocytes

The PAS staining method revealed that in the vehicle treated rats the hepatocyte glycogen content was well preserved. The glycogen deposits in hepatocytes of rats treated with either dose of melatonin were slightly reduced in comparison to the animals treated with the vehicle (Figure 2), but no relationship was found between the type of treatment and the glycogen content in the periportal ($\chi2$=0,8 df = 1 p=0,377), intermediate ($\chi2$ =0,0 df = 1 p=1,0) or perivenular ($\chi2$ =0,0 df = 1 p=1,0) hepatocytes of the liver lobule in the groups of control animals (Figure 3).

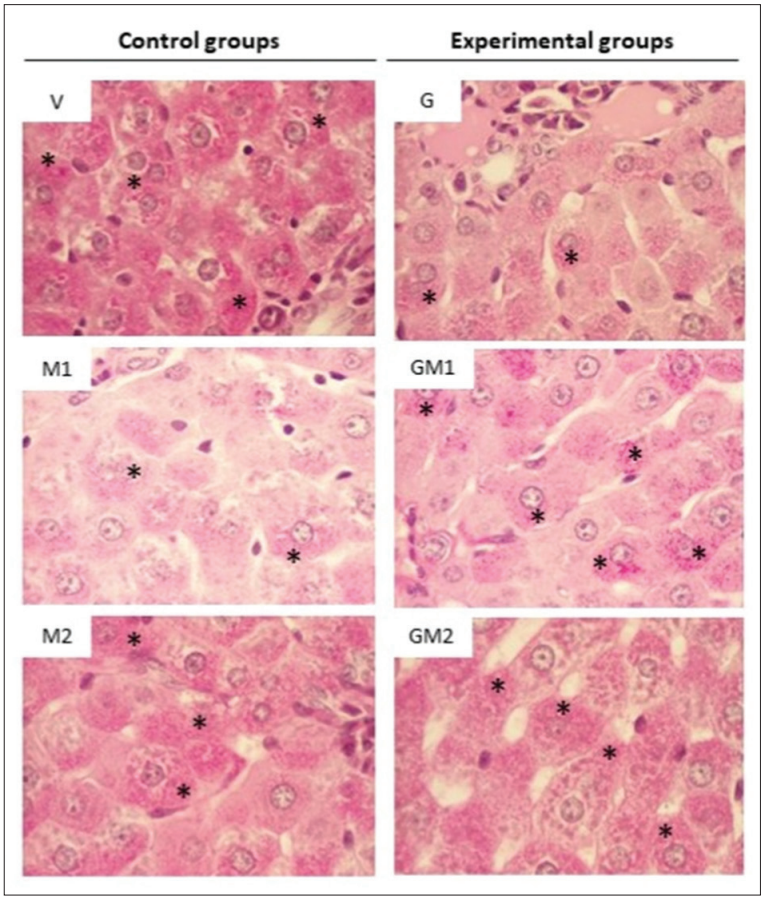

Figure 2. Glycogen deposits in periportal zone of liver lobule of control and experimental groups. Control V group showed regular glycogen deposits, while deposits were slightly decreased in melatonin treated control groups (M1 and M2). Glycogen content was markedly reduced in G group, while it was relatively preserved in melatonin treated experimental groups (GM1 and GM2). Asterisk pointing to the PAS positive deposits of glycogen. PAS staining, 400x magnification.

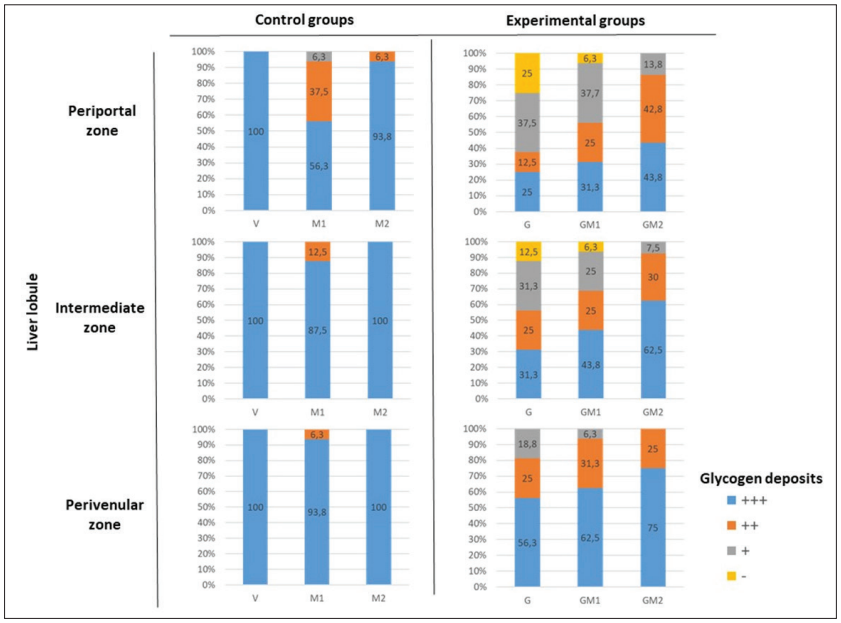

Figure 3. Distribution of the glycogen deposits in all three observed liver lobule zones in the experimental and control groups.

The glycogen content was reduced in hepatocytes in the G group compared to the control group of animals; the changes were especially pronounced in the periportal zone of the liver lobule. Administration of melatonin in the GM1 and GM2 groups had a beneficial effect; the glycogen content was increased in comparison to the G group of animals (Figure 2 and Figure 3).

There was a significant relationship between the glycogen content and combined treatment of gentamicin and melatonin in the perivenular zone ($\chi 2$= 20,57 df=4 p<0,0005), in the intermediate zone ($\chi 2$= 30,54 df =6 p<0,0005), and in the periportal zone of the liver lobule ($\chi 2$= 54,169 df=6 p<0,0005).

5. DISCUSSION

Gentamicin is a widely used antibiotic in the treatment of Gram negative infections (1, 2). Besides its nephro- and ototoxicity, it has been shown that it exhibits hepatotoxic effects (4-6), primarily through oxidative stress (6). Since melatonin has potent direct and indirect antioxidative actions, the present study was conducted in order to investigate the potential hepatoprotective effects of two different doses of exogenous melatonin in gentamicin treated rats.

Application of melatonin to healthy animals in our study did not alter liver microstructure. Although melatonin treatment in healthy animals reduced glycogen deposits in comparison to the vehicle treated group, the altered glycogen content was not dose-dependent. These effects of melatonin on the rat liver were also described by other authors (17, 18).

Our qualitative histological analysis of the liver showed that gentamicin induced changes in both the portal area and the liver lobule. The most prominent findings were mononuclear infiltration of the portal canal, congestion of central vein and perivenular sinusoids, and hydropic degeneration of periportal hepatocytes. The PAS staining revealed depletion of glycogen in all three observed zones, but it was most pronounced in the periportal region of the liver lobule. Gentamicin administration caused a reduction in the volume density of hepatocytes and an augmentation in the volume density of liver lobule blood vessels. Similar findings were reported by other authors (4, 6, 15, 16).

Both, the lower and the higher dose of melatonin resulted in a hepatoprotective effect. In the lower melatonin dose group of animals treated with gentamicin the hepatic lobules showed only mild reactive changes, while in the higher melatonin dose experimental group the hepatic lobule had a relatively preserved structure. No signs of vascular congestion or mononuclear infiltration were found in either of the experimental groups. The results of our qualitative histological analysis were confirmed by our stereological findings, which showed that both doses of melatonin had a protective effect, but that the melatonin dose of 10 mg/kg was more potent in context of the liver parenchyma protection than the 5 mg/kg dose.

Specifically, melatonin administration at a dose of 10 mg/kg resulted in a decrease of the volume density of the lobular blood vessels and an increase of the volume density of hepatocytes in comparison to the G group. The volume density of lobular blood vessels and the volume density of the hepatocyte cytoplasm in the experimental groups of rats showed a strong and negative correlation (r=-0,893, p<0,0005) as well as the Vv of the liver lobule blood vessels and the Vv of the hepatocytes (r=-1, p<0,0005).

Additionally, we have performed a semi-quantitative analysis of the hepatocyte glycogen deposits. It is well know that the liver glycogen maintains the blood glucose levels and that the hepatocyte glycogen content mirrors the state of glucostatic function (17). Different physiological and pathological conditions change the deposits of glycogen in hepatocytes (18). Since the distribution of enzymes involved in the carbohydrate metabolism depends on the zonation of liver lobules (19), we have analyzed the glycogen content in all three liver lobule zones. This analysis has shown that the glycogen content of hepatocytes in the gentamicin-treated rats was reduced; total absence of glycogen was observed in 25% of periportal hepatocytes and a II grade decrease in 37,5% of the cells. Alkahtani et al. (6) have also found a reduction in glycogen in the hepatocytes of gentamicin-treated animals. These changes in glycogen content are probably due to mitochondrial alterations with the consequent increase in oxidative stress, which is a well-known pathway leading to necrosis.

Melatonin administration to animals that were concomitantly treated with gentamicin preserved the deposits of glycogen in the hepatocytes and the effect

was dose dependent in all three observed hepatic lobule zones. In a study done by Mazepa et al. (20) melatonin preserved the liver glycogen deposits in exercised rats. Melatonin was also able to restore the glycogen content in high fat induced diabetic mice (21) and in conditions of smoking-induced hyperglycemia (22).

Thus, the hepatoprotective effects of melatonin pretreatment and its co-administration with gentamicin were evident in this study. Melatonin exerts protective effects in different conditions, such as heavy metal induced testicular damage, gentamicin induced nephrotoxicity, liver cirrhosis, ischemia/reperfusion liver injury, or age-related hepatic alterations, which was shown in various previous studies (1, 23-26).

Konturek et al (27) found that the majority of the endogenous melatonin production in the liver, pancreas and bile ducts is during the day in contrast to the pineal melatonin activity. This means that in our study, application of exogenous melatonin in the early evening (6 – 7 p.m.) augmented its level during the low activity of pineal melatonin and exerted this way its protective effects.

6. CONCLUSIION

Our results indicate a dose-dependent beneficial effect of exogenous melatonin against gentamicin induced liver alterations. Thus, melatonin might be considered as a relatively safe supportive treatment in conditions of gentamicin intake.

- **Author's contribution**: The all authors were involved in all steps of preparation this article. Final proofreading was made by the first author.
- **Conflict of interest**: The authors declare that there is no conflict of interest
- **Financial support and sponsorship**: The authors received no specific funding for this work.

REFERENCES

1. Kapić D, Mornjaković Z, Ćosović E, Šahinović M. A histological study of the effect of exogenous melatonin on gentamicin induced structural alterations of proximal tubules in rats. Bosn J Basic Med Sci. 2014; (1):30-34. doi: 10.17305/bjbms.2014.2293.
2. Turnidge JD, Waterston JA. Gentamicin and ototoxicity: why this drug is still in use. Med J Aust. 2012; 196(11): 665-666. doi: 10.5694/mja12.10733.
3. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012–. Aminoglycosides. 2019. PMID: 31643557. [access:10.9.2024.]
4. Soliman KM, Othman AI, Abdul-Hamid M. Carnosine ameliorates liver and jejunum toxicity induced by gentamicin. The Egyptian Journal of Histology. 2006; 29(2): 269-280.
5. Lietz T, Bryła J. The effect of various aminoglycoside antibiotics on glycogen phosphorylase activity in liver and kidney medulla of rabbit. Acta Biochim Pol. 1990; 37(1): 187-190.
6. Alkahtani S, Alarifi SA, Al-Doaiss AA. Detection of Apoptotsis Induced by Gentamicin in Rat Hepatocytes. International Journal of Zoological Research. 2009; 5(4): 161-170.
7. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine. 2005; 27(2): 101-110. doi: 10.1385/ENDO:27:2:101.
8. Bondy SC, Campbell A. Melatonin and Regulation of Immune Function: Impact on Numerous Diseases. Curr Aging Sci. 2020; 13(2): 92-101. doi: 10.2174/1874609813666200711153223.
9. Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol. 2002; 54(10): 1299-1321. doi: 10.1211/002235702760345374.
10. Stauch B, Johansson LC, Cherezov V. Structural insights into melatonin receptors. FEBS J. 2020; 287(8): 1496-1510. doi: 10.1111/febs.15128.
11. Grigorov I, Bogojević D, Jovanović S, Petrović A, Ivanović-Matić S, Zolotarevski L, Poznanović G, Martinović V. Hepatoprotective effects of melatonin against pronecrotic cellular events in streptozotocin-induced diabetic rats. J Physiol Biochem. 2014; 70(2): 441-450. doi: 10.1007/s13105-014-0322-7.
12. Ullah U, Badshah H, Malik Z, Uddin Z, Alam M, Sarwar S, Shah FA. Hepatoprotective effects of melatonin and celecoxib against ethanol-induced hepatotoxicity in rats. Immunopharmacology and Immunotoxicology. 2020; 42(3), 255–263.
13. aya, O., Kilic, M., Celik, I., Baltaci, AK. And Mogulkoc, R. Effect of melatonin supplementation on plasma glucose and liver glycogen levels in rats subjected to acute swimming exercise. Pak J Pharm Sci. 2010; 23(3): 241-244.
14. Stojiljković N, Stojiljković M. Micromorphological and histochemical characteristics of a rat's liver treated with gentamicin. Acta Medica Medianae. 2006; 45: 24-28.
15. Morin JP, Viotte G, Vandewalle A, Van Hoof F, Tulkens P, Fillastre JP. Gentamicin-induced nephrotoxicity: A cell biology approach. Kidney International. 1980; 18: 583-590.
16. Guliaev AE, Kivman Gia. Quantitative indicators of gentamicin and sisomicin interaction with isolated subcellular fractions of the liver and kidneys and intracellular distribution of these antibiotics. Antibiot Med Biotekhnol. 1985; 30(1): 50-54.
17. Kanungo S, Wells K, Tribett T, El-Gharbawy A. Glycogen metabolism and glycogen storage disorders. Ann Transl Med. 2018 Dec; 6(24): 474. doi: 10.21037/atm.2018.10.59. PMID: 30740405; PMCID: PMC6331362.
18. Soon GST, Torbenson M. The Liver and Glycogen: In Sickness and in Health. Int J Mol Sci. 2023 Mar 24; 24(7): 6133. doi: 10.3390/ijms24076133. PMID: 37047105; PMCID: PMC10094386.
19. Cunningham RP, Porat-Shliom N. Liver Zonation–Revisiting Old Questions With New Technologies. Front Physiol. 2021 Sep 9; 12:732929. doi: 10.3389/fphys.2021.732929. PMID: 34566696; PMCID: PMC8458816.
20. Shieh JM, Wu HT, Cheng KC, Cheng JT. Melatonin ameliorates high fat diet-induced diabetes and stimulates glycogen synthesis via a PKCzeta-Akt-GSK3beta pathway in hepatic cells. J Pineal Res. 2009 Nov; 47(4): 339-344. doi: 10.1111/j.1600-079X.2009.00720.x. Epub 2009 Oct 10. PMID: 19817973.
21. Li T, Ni L, Zhao Z, Liu X, Lai Z, Di X, Xie Z, Song X, Wang X, Zhang R, Liu C. Melatonin attenuates smoking-induced hyperglycemia via preserving insulin secretion and hepatic glycogen synthesis in rats. J Pineal Res. 2018 May; 64(4): e12475.

doi: 10.1111/jpi.12475. Epub 2018 Mar 25. PMID: 29437243; PMCID: PMC5947659.

22. Mazepa RC, Cuevas MJ, Collado PS, González-Gallego J. Melatonin increases muscle and liver glycogen content in nonexercised and exercised rats. Life Sci. 2000; 66(2): 153-160. doi: 10.1016/s0024-3205(99)00573-1. PMID: 10666011.
23. Wu J, Danielsson A, Zern MA. Toxicity of hepatotoxins: new insights into mechanisms and therapy. Expert Opin Investig Drugs. 1999; 8: 585-607.
24. Rosa DP, Bona S, Simonetto D, Zettler C, Marroni CA, Marroni NP. Melatonin protects the liver and erythrocytes against oxidative stress in cirrhotic rats. Arq Gastroenterol. 2010; 47(1): 72-78.
25. Liu Y, Zhang H, Zhang X, Xie Y, Zhao W. Melatonin modulates acute testicular damage induced by carbon ions in mice. Pharmazie. 2009; 64(10): 685-689.
26. Kim SH, Lee SM. Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. J Pineal Res. 2008; 44(2): 165-171.
27. Konturek SJ, Konturek PC, Brzozowska I, Pawlik M, Sliwowski Z, Cze-Guzik M et al. Localization and biological activities of melatonin in intact and diseased gastrointestinal tract (GIT). J Physiol Pharmacol. 2007; 58: 381-405.