Research Article

Functional and Histological Study of Liver in Adult Rats Treated with Different Doses of Melatonin

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Abstract | This study aimed to evaluate the effects of different doses of melatonin on liver function in adult rats. Eighteen Wistar adult albino rats (*Rattus norvegicus*), approximately 13–16 weeks old and weighing 230 ± 10 g, were randomly divided into three groups (n=6 per group) and treated orally for 30 days as follows: Group A1 received 10 mg/kg body weight (B.W) of melatonin; Group A2 received 20 mg/kg B.W of melatonin; and the control group (Group A) received distilled water. At the end of the treatment period, blood samples were collected via cardiac puncture, and serum was separated for biochemical analysis. Parameters assessed included oxidative stress markers (malondialdehyde (MDA), reduced glutathione (GSH)) and liver enzymes (aspartate aminotransferase (AST), alanine transaminase (ALT)). The results showed that melatonin administration led to a significant (P<0.05) reduction in final body weight and a marked (P<0.01) increase in MDA and AST levels in Group A2 compared to Groups A1 and control. Conversely, in Group A1, melatonin significantly (P<0.05) reduced MDA and AST levels while significantly increasing GSH levels compared to A2 and control. No significant differences were observed in ALT levels among the three groups. Histopathological examination of liver tissue revealed vascular and sinusoidal congestion in Group A2, which was absent in Groups A1 and control. In conclusion, melatonin at a dose of 10 mg/kg B.W demonstrated beneficial effects on liver function, antioxidant status, and body weight. However, a higher dose of 20 mg/kg B.W had a detrimental impact on liver function.

Keywords | ALT, Antioxidant status, AST, Body weight, Wistar albino rats

Received | March 17, 2025; **Accepted** | April 27, 2025; **Published** | May 15, 2025
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Citation | Youssef MH, Arrak JK, Ramadhan SJ (2025). Functional and histological study of liver in adult rats treated with different doses of melatonin. J. Anim. Health Prod. 13(2): 362-367.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.2.362.367
ISSN (Online) | 2308-2801

SINTRODUCTION

The liver, one of the primary metabolic organs found exclusively in vertebrates, performs numerous essential biological functions, including detoxification and the synthesis of proteins and other biochemicals necessary for growth and digestion (Kamoldinova, 2023). Among its key metabolic roles are the breakdown of red blood cells, conversion and storage of nutrients such as glucose and glycogen, hormone synthesis, and carbohydrate metabolism (Nogueira-Ferreira *et al.*, 2024). Additionally, the liver acts as an accessory digestive organ by producing bile an alkaline fluid containing bile acids and cholesterol which aids in the emulsification and digestion of dietary fats. The liver is composed primarily of hepatocytes, highly specialized cells responsible for regulating a wide range of high-volume biochemical activities, including the synthesis and degradation of both simple and complex organic compounds, many of which are essential for maintaining vital physiological functions (Hyder *et al.*, 2023).

The pineal gland produces melatonin (N-acetyl-5-methoxytryptamine or MEL), a hormone primarily secreted at night (Ahmad *et al*., 2023). Melatonin plays a significant role in regulating the biological clock, promoting proper sleep, and modulating immune function (Abdullah *et al*., 2022). It facilitates deep sleep by suppressing wake-promoting signals (Pandi-Perumal *et al*., 2008). Additionally, melatonin acts as a potent free radical scavenger, eliminating reactive species such as hydrogen peroxide, thereby enhancing antioxidant activity in tissues (Boutin *et al*., 2024). Its anti-inflammatory properties are attributed to its ability to neutralize free radicals and regulate inflammatory responses by increasing pro-inflammatory markers during the early stages of inflammation (Hernández-Velázquez *et al*., 2016). Melatonin is also believed to protect the liver from damage induced by certain drugs by reducing oxidative stress, mitochondrial and microsomal lipid peroxidation, and inflammatory cell infiltration (Colares *et al*., 2022). Furthermore, its antioxidant capabilities extend to protecting renal tissues from drug-induced damage (Dun *et al*., 2022). The current study aimed to investigate the effects of different doses of melatonin on liver function in adult rats.

MATERIALS AND METHODS

Animals management and study design

The animals were housed in well-ventilated plastic cages, given the unrestricted availability of water and food throughout the study period. The ambient temperature was kept at 22±2°C with a 12-hour light/dark cycle for acclimatization and research purposes. Eighteen Wistar adult Albino Rats (*Rattus norvegicus*) (between the ages 13-16 weeks and weighing 230±10g) were separated equally among the following groups. Group A (Control): orally gavage with 0.5 ml D.W Once daily, Group (A1): orally gavage with melatonin once daily (10 mg/kg/b.w), Group (A2): orally gavage with melatonin (20 mg/kg/b.w) once daily for 30 days (Oleshchuk *et al*., 2019). At the final stage of the experiment blood and tissue samples were taken from euthanized animals and given intramuscular injections of ketamine 90 mg/kg B.W and xylazine 40 mg/kg B.W. The examination of the following parameters was conducted: oxidant and antioxidant status, including MDA and reduced GSH concentrations, measured according to the methods described by Burtis and Ashwood (1999) and Guidet and Shah (1989), respectively. Liver enzyme activities, specifically aspartate aminotransferase (AST) and alanine transaminase (ALT), were assessed using the COBAS INTEGRA e411 analyzer (Roche). Liver tissue samples were collected and preserved in 10% neutral buffered formalin for histological analysis. Paraffin blocks were prepared, and tissue sections were stained with hematoxylin and eosin (H&E) following the procedures outlined by Bancroft and Gamble (2008).

Body weight measurements

Animals' body weights were measured at the start of the experiment and then weekly until the completion of experiment. Weight gain was also monitored.

Statistical analysis

The Statistical Analysis System (SAS, 2018) software was used to analyze the effects of various factors on the study parameters. Differences between means were evaluated using analysis of variance (ANOVA), and significant differences were determined using the least significant difference (LSD) test.

RESULTS

Final weight and weight gain

The results showed that the final body weight was significantly decreased ($P \leq 0.05$) in the A2 group compared to the control and A1 groups. In contrast, no significant differences were observed in final weight and weight gain between the control and A1 groups (Table 1).

Table 1: Effect of different melatonin doses on body weight of rats.

Groups	Mean ± SE of body weight (g)		
	Initial weight	Final weight	Weight gain
Control (A)	234.60 ±29.67	249.43 ±29.10 a	14.83 ±2.02
A1	185.06 ±10.61	203.22 ±9.28 ab	18.16 ±3.56
A2	179.31 ±11.28	190.58 ±12.65 b	11.28 ±2.94
LSD value	NS	*	NS
P-value	0.126	0.0492	0.284

Means with different letters in the same column varied significantly * ($P \leq 0.05$). Data are expressed as mean ± SE, N = 6 per group. A: control group rats were given D.W; A1: group got 10 mg/kg/B.W/day of melatonin; A2: group received 20 mg/kg B.W/day of melatonin for 30 days.

Antioxidative enzymes concentration

Table 2 shows that MDA activity significantly decreased ($P \leq 0.01$) in the A1 group, while GSH levels significantly increased ($P \leq 0.01$) in the same group compared to the control and A2 groups. Additionally, the A2 group showed no significant differences in MDA and GSH concentrations when compared with the control group.

Liver enzymes activity

The results illustrated in Table 3 revealed a significant decrease ($P \leq 0.05$) in AST enzyme concentration in the A1 group compared with the control and A2 groups, while ALT enzyme concentrations showed no significant differences between the control and the treated groups.

Table 2: Effect of different doses of melatonin on malondialdehyde (MDA), and reduced glutathione (GSH) concentration in rats.

Groups	Mean ± SE	
	MDA (µmol/L)	GSH (µmol/L)
Control (A)	11.43 ±0.53 a	24.15 ±0.41 b
A1	7.92 ±0.34 b	31.83 ±0.45 a
A2	11.84 ±0.44 a	23.63 ±0.50 b
LSD value	1.367 **	1.415 **
P-value	0.0001	0.0001

Means with different letters in the same column varied significantly. ** ($P \leq 0.01$). Data are expressed as mean ± SE, N = 6 per group. A: control group rats were given D.W; A1: group got 10 mg/kg/B.W/day of melatonin; A2: group received 20 mg/kg B.W/day of melatonin for 30 days.

Table 3: Effect of different doses of melatonin on aspartate aminotransferase (AST), and Alanine transaminase (ALT) activity in rats.

Groups	Mean ± SE	
	AST (U/L)	ALT (U/L)
Control (A)	125.40 ±8.67 ab	41.40 ±4.37
A1	117.00 ±3.84 b	41.00 ±2.96
A2	145.00 ±6.99 a	39.00 ±1.52
LSD value	20.971 *	9.786 NS
P-value	0.0357	0.851

Means with different letters in the same column varied substantially. * ($P \leq 0.05$). Data are expressed as mean ± SE, N = 6 per group. A: control group rats were given D.W; A1: group got 10 mg/kg/B.W/day of melatonin; A2: group received 20 mg/kg B.W/day of melatonin for 30 days.

Histopathological study

The histopathological images (Figures 1 and 2) showed the normal histological structure of the liver in the control group. Meanwhile, the A1 group (Figures 3 and 4) displayed normal architecture, including intact sinusoids, hepatocytes, and central veins. In contrast, the liver sections from the A2 group (Figures 5 and 6) showed central vein congestion and sinusoidal dilation.

DISCUSSIONS

Melatonin, a neurohormone primarily secreted by the pineal gland, is widely recognized for regulating circadian rhythms. However, recent studies have expanded its role to include the modulation of metabolic processes, body weight, and oxidative stress. In our study, we observed compelling evidence supporting the beneficial effects of melatonin, particularly at physiological or moderate doses. These findings align with earlier research, which demonstrated that exogenous melatonin can reduce body weight and fat accumulation, especially in animals exposed to high-fat, high-sugar diets (Ríos-Lugo *et al.*, 2010; Tan *et al.*, 2011). Long-term melatonin use was also associated with significant reductions in total cholesterol levels and weight gain, likely due to its ability to inhibit cholesterol synthesis, block absorption, and enhance catabolic pathways (Tung *et al.*, 2020).

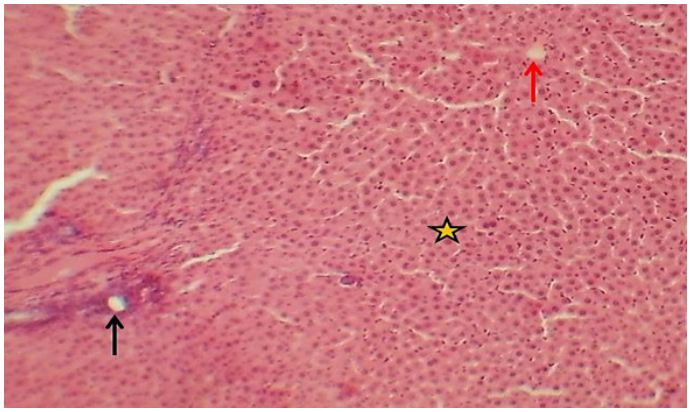

Figure 1: A slice of the liver of control group shows a normal appearance of the central vein (red arrows), portal triad (black arrow), and hepatic cords (asterisk). H & E staining 100x.

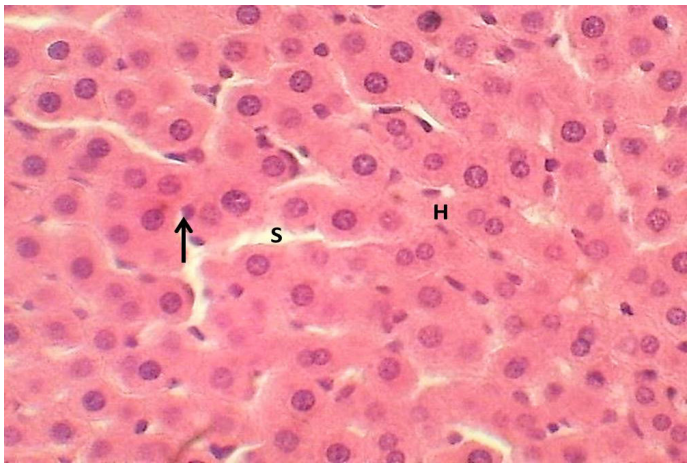

Figure 2: A slice of the liver of control group reveals the usual appearance of Kupffer cells (arrows), sinusoids (S), and hepatocytes (H). H & E stains.400x.

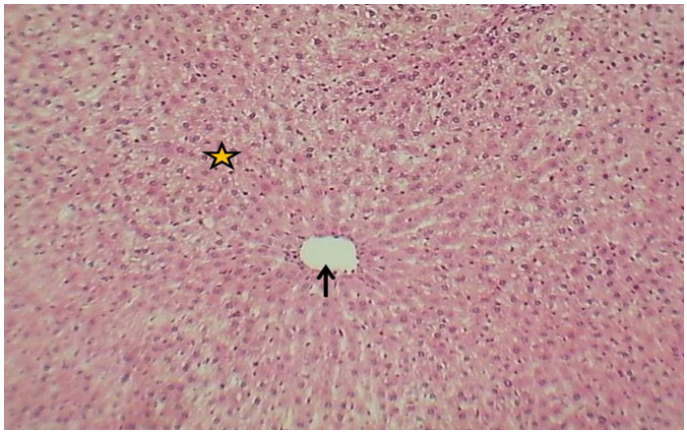

Figure 3: A slice of the liver (A1 group) showing the typical central vein (arrow) and hepatic cords (asterisks). H & E stain.100x.

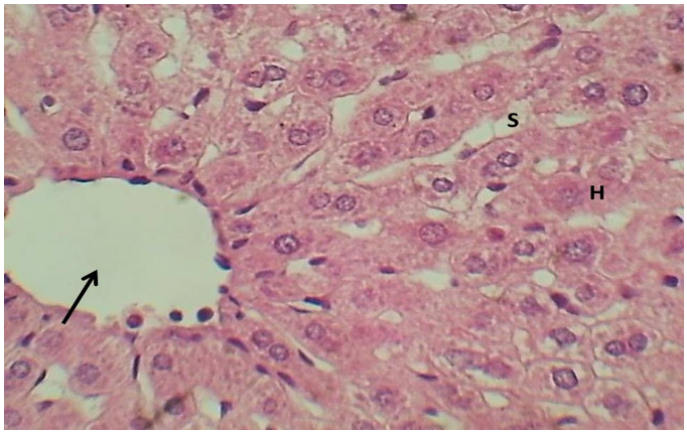

Figure 4: A slice of the liver (A1 group) reveals typical sinusoids (S), hepatocytes (H), and the central vein (Arrow). H & E stain 400x.

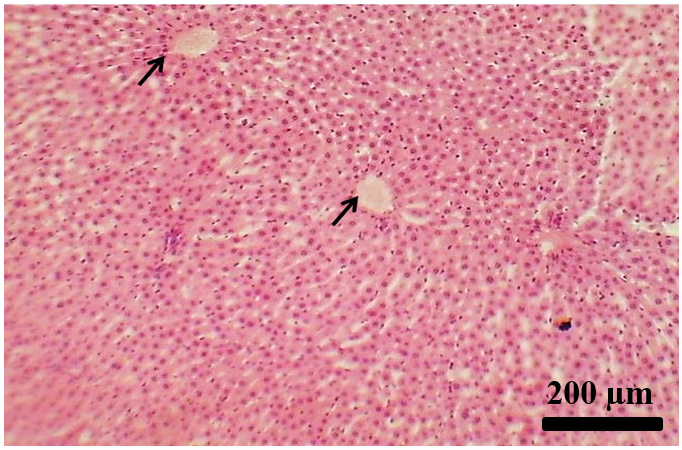

Figure 5: A slice of the liver (A2 group) with vascular congestion (arrows). H&E stain. 100x.

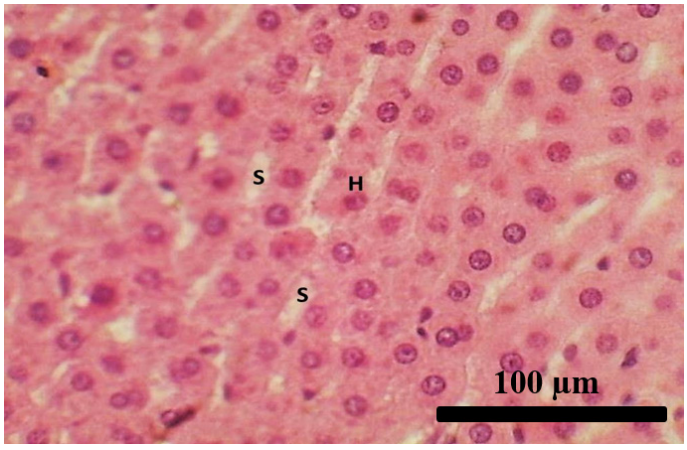

Figure 6: A slice of the liver (A2 group) showing sinusoidal congestion (S) and normal hepatocytes (H). H&E staining 400x.

In addition to its role in metabolic regulation, melatonin exhibits potent antioxidant properties. It can directly scavenge free radicals and stimulate the activity of endogenous antioxidants, such as GSH, SOD, and glutathione peroxidase (GPx). Our findings confirm this protective effect, as animals treated with moderate doses of melatonin showed increased antioxidant enzyme activity, reduced levels of lipid peroxidation (LPO), and lower nitric oxide (NO) concentrations in liver and kidney tissues. These effects are consistent with previous reports suggesting that melatonin, even in small quantities, can neutralize up to two hydroxyl radicals and exert long-lasting antioxidant effects (Han *et al*., 2017; Colares *et al*., 2022). This makes melatonin a highly effective molecule for counteracting oxidative stress and maintaining cellular homeostasis. However, a key observation in our study is the dose-dependent nature of melatonin's effects. While moderate doses promoted antioxidant defense and preserved tissue function, high doses were associated with potential toxicity, particularly in liver and kidney tissues. This was evident through biochemical and histological alterations, including elevated liver enzymes (AST, ALT), which serve as reliable indicators of hepatocellular injury (Kalas *et al*., 2021). These adverse outcomes suggest that melatonin, at supraphysiological levels, may act as a pro-oxidant rather than an antioxidant. Excessive ROS production, mitochondrial dysfunction, and disrupted redox balance are likely mechanisms underlying this toxicity, as proposed by Othman *et al*. (2020) and Al-Olayan *et al*. (2020).

The potential shift from a protective to a harmful role highlights the dual nature of melatonin, depending on its concentration. While low to moderate doses enhance mitochondrial efficiency and reduce oxidative load, high doses may overstimulate mitochondrial respiration, leading to electron leakage, increased ROS, and damage to cellular membranes through lipid peroxidation. Such changes impair membrane fluidity, permeability, and integrity eventually triggering apoptosis (Ighodaro *et al*., 2012). Furthermore, the liver's central role in metabolism and detoxification makes it particularly vulnerable to oxidative damage, especially under prolonged exposure to high-dose melatonin.

CONCLUSION

The results of our study indicate that a daily dose of 10 mg of melatonin provides significant protection against both acute and chronic liver damage caused by various diseases. Additionally, it may serve as a useful therapeutic agent to minimize oxidative stress, which in turn helps reduce liver damage. However, the use of a high, long-term dose of 20 mg melatonin may pose risks to the animal, as it negatively affects body weight and disrupts the oxidant status.

ACKNOWLEDGEMENTS

For their collaboration on this study, the authors would like to thank the Department of Physiology, Biochemistry, and Pharmacology at the College of Veterinary Medicine, University of Baghdad, Iraq.

NOVELTY STATEMENT

This article provides novel insights into the different doses of melatonin on liver function and oxidative stress in adult rats. Unlike previous studies that often focused on either low-dose or generalized antioxidant roles of melatonin, this research offers a direct comparison between two distinct doses (10mg/kg and 20mg/kg) and reveals that while the lower dose exerts protective antioxidant and hepatoprotective effects, the higher dose may lead to hepatic toxicity, offering new evidence on its potential risks at supraphysiological levels.

AUTHOR'S CONTRIBUTION

Muna Hassan Yousef wrote the original draft of the study and carried out the experiments. Jawad Kadhim Arrak contributed to designing the study, the statistical evaluation, and the biological analysis. Sadiq Jaffer Ramadhan contributed to the manuscript's histological examination and critical revision. The article's final draft was reviewed and approved by all authors.

Ethical approval

The processes used in this study were reviewed and approved in compliance with animal welfare ethical standards by the Scientific Committee of the Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq, as well as the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Conflict of interest

The authors have declared no conflict of interest.

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