Original Article

# Potential Protective Effect of Melatonin on Acetaminophen-Induced Hepatotoxicity in Albino Rats



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## ABSTRACT

**Objective:** To determine the potential protective effect of melatonin on acetaminophen (APAP) induced hepatotoxicity in male Wistar albino rats.

**Study Design:** Experimental study

**Place and Duration of Study:** This study was conducted at the Department of Anatomy, Pharmacology, and Pathology, SRMC, Tando Adam from March 2019 to January 2020 for a period of 10 months.

**Materials and Methods:** 100 adult male rats were divided into five groups; Group A – negative control, Group B (positive control), C, D and E were given APAP (2 gram/Kg daily orally). Groups C, D and E received melatonin therapy 5, 10 and 15 mg/dl daily orally for six weeks. Blood samples were collected and sera separated by centrifugation. Sera were used for the liver functions tests and natural anti – oxidant enzymes. Data was analyzed on statistical SPSS package (ver. 21.0 at $p \leq 0.05$ (Confidence interval 95%).

**Results:** Six weeks melatonin therapy shows significant improvement in serum bilirubin, Prothrombin time (PT), alanine transaminase (ALT), aspartate transaminase (AST) and G-glutamyl transferase (GGT) and significant boosting effect on the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) ($P \leq 0.0005$) was noted.

**Conclusion:** The present study reports potential protective effect of melatonin in acetaminophen (APAP) induced hepatotoxicity in male Wistar albino rats.

**Key Words:** Melatonin, Acetaminophen, Liver toxicity, Rats

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## INTRODUCTION

Melatonin is secreted by the pineal gland during night time. Melatonin is a derivative of tryptophan through serotonin. Serotonin is methylated to yield melatonin. Melatonin is stored in pienalocytes of pineal gland and released into blood capillaries at night time during sleep.[1,2] Pineal gland melatonin regulates the sleep cycle through circadian rhythm. Secretion of melatonin is controlled by the light – dark cycles.

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Melatonin is secreted in nighttime during sleep. It induces biological changes in body systems during sleep. It is sleep inducer that decreases metabolism, body temperature and breathing rats during sleeping. Beside Pineal gland, the melatonin is found in multiple cell and organs in particular the gastrointestinal tract.[1,2] Extra – pineal gland melatonin plays role of anti – oxidant and immunomodulation in local tissues. Melatonin functions in autocoid and paracoid pattern in tissues.[1,3] Melatonin has receptors on target cells present on cell membrane. Melatonin cell membrane receptors are of two types – the MT1 and MT2 that belong to the G-protein coupled receptor family.[1,4] Melatonin protects against oxidative stress as it is part of antioxidant defense system. Melatonin stimulates anti – oxidant enzyme systems and helps scavenge free radicals. Anti – oxidant activity of melatonin is observed in different cell compartments such as the membrane, cytosol, and nucleus. Studies suggest the melatonin exerts direct free radical scavenging activity against the oxygen derived free radicals.[1,3] Its anti – oxidant potential is more effective than glutathione, α – tocopherol, and mannitol[1] hence its protective role against proliferative and degenerative injuries is noticeable. Melatonin protects the macro – and micro – molecules such as the DNA from oxidative injuries.[1,5]

Melatonin shows anti – oxidant potential at very high peak concentrations higher than nighttime peak levels. Thus anti –oxidant activity is noticeable at high pharmacological doses.[6,7] Melatonin stabilizes the inner mitochondria membrane protecting against the electron transport chain (ETC) derived oxygen free radicals.[1,7] Melatonin protects against reactive oxygen species (ROS) and reactive nitrogen species (RNS) especially in the inner cell compartments.[1,7] Melatonin increases the physiological functioning of natural anti – oxidant enzymes such as the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) that maintain normal physiological homeostasis in different organs for example liver, that is the seat of free radical formations.[1,7] As the liver is active in performing different biochemical reactions throughout the clock hence it is vulnerable to oxidative injuries if exposed by chance resulting in hepatocellular necrosis.[1] Liver is major organs of biochemical reactions and injuries and acetaminophen (N-acetyl-para-aminophenol) (APAP) is a common hepatotoxin.[8,9] Accidental acetaminophen[9] injury is common toxicological problem in the patients presenting in the emergency room. Hence the present experimental study was conducted to determine the potential protective effect of melatonin on acetaminophen-induced hepatotoxicity in male Wistar albino rats.

# MATERIALS AND METHODS

The present experimental study was conducted at the Department of Anatomy, Pharmacology, and Pathology, SRMC, Tando Adam in collaboration with Sindh Agriculture University. The study was approved by the Ethics committee of institute and carried out from March 2019 to January 2020. Ethical permission was also taken from the animal house of Sindh Agriculture University for conducting animal research. Protocol of Animal house was maintained strictly and animals handling was in accordance to the ethics regulations. One hundred adult male Wistar rats were purchased from the animal house and selected according to the inclusion criteria of study protocol. A rat of 150 – 200 grams, male gender and Wistar albino strain were inclusion criteria. Rats moving actively around cages, feeding and drinking water well were also inclusion criteria. Any rat found not feeding and drinking well, and moving lazy were excluded. Female rats, sick male with limited mobility in cages were also excluded. Animal house is well equipped with proper ventilation. Animals were housed in 12/12 dark/light cycle. Rats were divided into five groups; 20 rats in each group. Negative control (group A) – no intervention and given Normal saline as placebo therapy. Positive control (group B) was given acetaminophen (APAP) (2g/Kg) – only APAP was given to induce hepatotoxicity not given drug therapy. Group B was taken as positive control for comparison with experimental melatonin treated groups. Experimental groups C – E received APAP+ melatonin therapy as; Group C- was received APAP (2g/Kg)[10] + 5 mg melatonin[11] daily orally, Group D- received APAP (2g/Kg)[10] + 10 mg melatonin [11] daily orally, and experimental Group E- received APAP (2g/Kg)[10] + 15 mg melatonin[11] daily orally. Melatonin was given for six weeks. At the end of experiment, the rats were anesthetized with chloral hydrate and blood samples were taken from the retro – orbital venous plexus using a capillary tube put behind the eyeballs. Blood was taken into disposable syringes (BD, USA) collected in gel tubes. Centrifuged carried at 4000 rpm for 10 minutes get sera that were stored at -20$^0$C. Biochemical analysis was performed on sera for the liver function tests (spectrophotometric). Natural anti – oxidant enzymes (SOD, GPX, CAT) were detected by ELISA assay kit (Fortress Diagnostics). Laboratory findings were entered in a pre – structured proforma and kept in lockers confidential. Statistical analysis was performed on SPSS package (ver. 21.0, IBM, incorporation, USA) using one – way analysis variance (1- ANOVA) post – Hoc Benforinni test. Results significance was analyzed at $p \leq 0.05$ (Confidence interval 95%).

# RESULTS

We observed highly significant effects of melatonin against the acetaminophen induced hepatotoxicity in male rats. The present study observed significant improvement of serum bilirubin, Prothrombin time (PT), alanine transaminase (ALT), aspartate transaminase (AST) and G-glutamyl transferase (GGT) ($P \leq 0.0005$) as shown in table –1. Melatonin therapy in acetaminophen treated rats a significant boosting effect on the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) ($P \leq 0.0005$) as shown in table – 2.

**Table No.1: Liver function tests in control and experimental rats (n=100)**

| | | Mean | SD | P-value |
|---|---|---|---|---|
| Total Bilirubin (mg/dL) | Group. A- Negative Control | 0.57 | 0.11 | ≤0.0002 |
| | Group B. APAP (Positive control) | 2.75 | 0.72 | |
| | Group C. APAP+ Melatonin (5 mg) | 1.50 | 0.74 | |
| | Group D. APAP+ Melatonin (10 mg) | 1.17 | 0.28 | |
| | Group E. APAP+ Melatonin (15 mg) | 1.01 | 0.03 | |
| | Group. A- Negative Control | 7.19 | 1.54 | |
| | Group B. APAP (Positive control) | 13.70 | 1.09 | |

| | | | | |
|---|---|---|---|---|
| PT (sec) | Group C. APAP+ Melatonin (5 mg) | 11.54 | 3.17 | ≤0.0001 |
| | Group D. APAP+ Melatonin (10 mg) | 9.32 | 2.54 | |
| | Group E. APAP+ Melatonin (15 mg) | 9.01 | 1.13 | |
| ALT (U/L) | Group. A- Negative Control | 32.70 | 6.39 | ≤0.0001 |
| | Group B. APAP (Positive control) | 69.82 | 13.19 | |
| | Group C. APAP+ Melatonin (5 mg) | 57.5 | 8.21 | |
| | Group D. APAP+ Melatonin (10 mg) | 53.13 | 7.66 | |
| | Group E. APAP+ Melatonin (15 mg) | 52.2 | 7.2 | |
| AST (U/L) | Group. A- Negative Control | 31.60 | 6.51 | ≤ 0.0005 |
| | Group B. APAP (Positive control) | 42.90 | 19.21 | |
| | Group C. APAP+ Melatonin (5 mg) | 36.53 | 10.71 | |
| | Group D. APAP+ Melatonin (10 mg) | 35.51 | 8.30 | |
| | Group E. APAP+ Melatonin (15 mg) | 33.1 | 3.12 | |
| ALP (U/L) | Group. A- Negative Control | 79.53 | 17.34 | ≤0.0001 |
| | Group B. APAP (Positive control) | 139.50 | 32.34 | |
| | Group C. APAP+ Melatonin (5 mg) | 115.70 | 41.57 | |
| | Group D. APAP+ Melatonin (10 mg) | 97.35 | 31.43 | |
| | Group E. APAP+ Melatonin (15 mg) | 91.34 | 11.03 | |
| LDH (U/L) | Group. A- Negative Control | 109.11 | 16.16 | ≤0.0005 |
| | Group B. APAP (Positive control) | 161.34 | 31.12 | |
| | Group C. APAP+ Melatonin (5 mg) | 139.51 | 31.11 | |
| | Group D. APAP+ Melatonin (10 mg) | 130.15 | 19.61 | |
| | Group E. APAP+ Melatonin (15 mg) | 119.31 | 10.31 | |
| GGT (U/L) | Group. A- Negative Control | 35.15 | 4.91 | ≤0.0001 |
| | Group B. APAP (Positive control) | 71.15 | 17.53 | |
| | Group C. APAP+ Melatonin (5 mg) | 57.25 | 18.81 | |
| | Group D. APAP+ Melatonin (10 mg) | 47.35 | 19.21 | |
| | Group E. APAP+ Melatonin (15 mg) | 41.18 | 11.00 | |

**Table No.2: Anti – oxidant enzymes in control and experimental rats (n=100)**

| | | Mean | SD | P-value |
|---|---|---|---|---|
| Superoxide Dismutase (U/ml) | Group. A- Negative Control | 136.05 | 33.80 | ≤0.0001 |
| | Group B. APAP (Positive control) | 75.35 | 15.34 | |
| | Group C. APAP+ Melatonin (5 mg) | 112.70 | 19.27 | |
| | Group D. APAP+ Melatonin (10 mg) | 121.75 | 13.25 | |
| | Group E. APAP+ Melatonin (15 mg) | 131.43 | 11.31 | |
| Glutathione peroxidase (nM/mL) | Group. A- Negative Control | 137.10 | 31.23 | ≤ 0.0005 |
| | Group B. APAP (Positive control) | 87.14 | 21.35 | |
| | Group C. APAP+ Melatonin (5 mg) | 117.13 | 15.72 | |
| | Group D. APAP+ Melatonin (10 mg) | 121.35 | 11.08 | |
| | Group E. APAP+ Melatonin (15 mg) | 129.78 | 15.76 | |
| Catalase (nM/mL) | Group. A- Negative Control | 307.35 | 30.32 | ≤0.0001 |
| | Group B. APAP (Positive control) | 133.68 | 52.44 | |
| | Group C. APAP+ Melatonin (5 mg) | 201.35 | 71.19 | |
| | Group D. APAP+ Melatonin (10 mg) | 276.15 | 62.35 | |
| | Group E. APAP+ Melatonin (15 mg) | 187.91 | 78.19 | |

# DISCUSSION

Human beings are constantly exposed to chemical pollutants and toxic drugs in the modern era. Many of chemicals are deleterious to the liver producing acute or chronic liver injury. Effects of melatonin against the chemical induced liver injuries have been studied widely.[1,2] The present study is the first experimental research conducted to clarify the effects of melatonin administration in acetaminophen induced liver toxicity in rat model. Ameliorative effects of melatonin against the APAP induced liver toxicity were observed in terms of liver function tests and natural anti – oxidant enzymes. In present study, the six weeks melatonin therapy improved the liver function test markers (Bilirubin, PT, ALT, AST & GGT) and anti – oxidant enzymes (SOD, GPX & CAT). We observed highly significant effects of melatonin against the

acetaminophen induced hepatotoxicity in male rats. Serum bilirubin and PT were improved; ALT, AST and GGT were decreased compared to positive control group B. The findigns of present study are in line keeping full concordant to previous studies.[1-3] We observed the melatonin therapy boosted natural anti – oxidant enzyme (SOD, GPX, CAT) significantly. We suggest the melatonin exerts effects through direct anti – oxidant activity and through inductions of natural anti – oxidant enzymes. It is explained the acetaminophen is converted to N – acetyl – benzoquinone – imine (NAPQI), an electrophilic metabolite. NAPQI is activated by the cytochrome P- 450 system that depletes the hepatic glutathione concentration resulting in ultimately in liver toxicity.[12] In present experimental rat model study, the acetaminophen treated positive control group B revealed peak rise in the liver parenchyma injury as marked by significant rise in the serum bilirubin ALT, AST and GGT that were ameliorated by melatonin therapy in experimental groups C, D and E. Acetaminophen overdose produces liver injury in both humans and animal equally.[12] Complete mechanism of APAP induced liver injury remains not well known but the NAPQI is involved with free radical mediated injury. [12] Several experimental models have shown drug induced liver toxicity with acetaminophen, albendazole, co-amoxiclav, labetaol, etc.[13,14] Many foods are reported to induce hepatotoxicity. Administration of melatonin in animal have shown increase GPX, GSSH, SOD, CAT NADP-dependent - isocitrate dehydrogenase, and glucose-6-phosphate dehydrogenase. A previous study[15] observed hepatic GSH concentration was decreased after melatonin therapy and concluded this was consumed in eradicating the free radical oxidation. In present study, we observed the melatonin therapy induced the natural anti – oxidant enzyme (SOD, GPX, CAT) significantly. We suggest the melatonin exerts effects through direct anti – oxidant activity and through inductions of natural anti – oxidant enzymes. A previous study[16] has shown anti – inflammatory effects of melatonin in experimental rabbits. It was concluded that the melatonin decreased liver injury by reducing apoptosis through attenuating endoplasmic reticulum stress.[17] Previous studies[18,19] investigated the potential of melatonin for treating the nonalcoholic steatohepatitis (NASH). Melatonin therapy was continued for three months and a significant improvement was observed in the liver function tests without any side effects.[18,19] The findings of above study are in agreement with our present study indicating the hepatoprotective efficacy of melatonin. A previous experimental study induced liver fibrosis by carbon tetrachloride ($CCl_4$) and investigated the melatonin effects against liver injury and fibrosis. Attenuation of $CCl_4$-induced liver fibrosis was observed with melatonin therapy. [20,21] This shows the hepatoprotective potential of melatonin similar to our present study. Findings of present study of melatonin against acetaminophen induced liver injury, in light of available literature, shows the drug may prove clinically effective in acetaminophen toxicity.

# CONCLUSION

The present study reports potential protective effect of melatonin in acetaminophen (APAP) induced hepatotoxicity in male Wistar albino rats. It improves liver function tests through boosting of anti – oxidant enzymes; the superoxide dismutase, glutathione peroxidase and catalase. Further animal studies are warranted and similarly the clinical trials be conducted to make melatonin convenient for clinical use in different groups of patients with drug induced liver injury.

**Author's Contribution:**

| | |
|---|---|
| Concept & Design of Study: | Hina Mawani |
| Drafting: | Abdul Majid, Asim Mehmood |
| Data Analysis: | Rashed Ahmed Soomro, Samreen Pandhiani, Aftab Abbasi |
| Revisiting Critically: | Hina Mawani, Abdul Majid |
| Final Approval of version: | Hina Mawani |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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