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Original Research Article

Effect of melatonin in amelioration of piroxicam induced liver and renal oxidative stress in rats

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ABSTRACT

Background: Piroxicam, a nonsteroidal anti-inflammatory drug, induces oxidative stress affecting liver and kidney function. Melatonin, a potent antioxidant, may mitigate this damage through its free radical-scavenging and tissue-protective properties.
Methods: The piroxicam-induced oxidative stress model was used to evaluate the amelioration effect of melatonin at two doses (10 mg/kg and 25 mg/kg BW). The changes in the physical, physiological and biochemical characteristics in the serum, liver and renal tissue oxidative stress markers, the histopathological changes and insilico docking of melatonin with various oxidative stress marker were also studied.
Results: The blood serum and tissue stress markers in the rats receiving piroxicam was considerably higher than those in the normal control ($p<0.05$). Pre-treatment with melatonin at doses of 10 mg/kg and 25 mg/kg resulted in a substantial amelioration in tissue oxidative stress as compared to the control group. Other than lipid peroxidation, which exhibited a significant ($p<0.05$) plunge when compared to the piroxicam treated group, the serum liver and renal comprehensive metabolic panel profile was significantly reduced. In the pre-treatment group, the level of total protein contents likewise increased. Further it is confirmed from the histological studies that the pre-treatment of melatonin group's liver and kidney show less tissue damage than the piroxicam-induced group, and the histo-architectural of hepato-renal cells gradually recovered and in silico docking study of melatonin with oxidative stress maker shown potential interaction with high binding energy.
Conclusions: It can be concluded that pretreatment of melatonin is effective against piroxicam- induced liver and renal oxidative stress in the Wister rats.

Keywords: Oxidative stress, Piroxicam-induced, Tissue markers, Melatonin, Metabolic panel levels, Docking

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for their analgesic and anti-inflammatory effects; however, they often pose significant risks to vital organs, particularly the liver and kidneys. Piroxicam, a commonly prescribed NSAID, is known to induce oxidative stress, leading to hepatic and renal toxicity.[1] Oxidative stress occurs due to an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defence mechanisms, ultimately causing cellular and tissue damage.[2] The liver and kidneys, being highly metabolically active organs, are particularly susceptible to oxidative stress, which can result in hepatotoxicity and nephrotoxicity.[3] Melatonin, a naturally occurring hormone secreted by the pineal gland, is a potent

antioxidant with the ability to scavenge free radicals, enhance the expression of antioxidant enzymes, and reduce oxidative stress-induced damage.[4] Numerous studies have reported melatonin's protective effects against drug-induced toxicity, including those caused by NSAIDs.[5] Its antioxidative properties help mitigate lipid peroxidation, protein oxidation, and DNA damage, which are common indicators of oxidative stress.[6]

The aim of the study was to investigate the protective role of melatonin in mitigating piroxicam-induced oxidative stress in Wistar rats. By analyzing oxidative stress markers in serum, liver, and renal tissues, as well as conducting histopathological evaluations, we aim to elucidate melatonin's potential in reversing tissue damage. Additionally, in silico docking studies were performed to determine melatonin's binding affinity with oxidative stress-related markers. We hypothesize that melatonin administration at doses of 10 mg/kg and 25 mg/kg body weight (BW) will significantly reduce oxidative stress markers and improve tissue integrity, thereby demonstrating its hepatoprotective and nephroprotective effects.

METHODS

All experiments were carried out with male Wistar albino rats weighing 230-250 g. Obtained from the Central Animal House, Krupanidhi College of Pharmacy, Animals were housed in polypropylene cages (3 rats per cage) lined with husk, renewed every 24-hr. under a 12-hr light/dark cycle at around 24°C with 50% humidity. The rats had free access to tap water and a standard pellet diet (Purina Chow). The protocol of the study was approved by Institutional Animal Ethic Committee. The animals were maintained under standard condition in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experimental on Animals (CPCSEA). The acute oral toxicity studies have been already conduced on the melatonin (OCED 425) in the previous studies, and as per the study, the low dose of melatonin is 10 mg/kg and high dose of melatonin is 25 mg/kg per body weight of the animal.

Statistical analysis

The results were expressed as mean±SEM. The results are analyzed by one-way ANOVA followed by multiple comparison test i.e., Dunnett's test using graph prism pad version 9.4.2 software. P<0.05 will be considered as significant.

RESULTS

Physical and physiological parameters

All the values are expressed as mean±standard error of the mean (S.E.M) n=6. The data was carried out by GraphPad Prism 9.4 and analysed by one-way ANOVA, followed by Dunnett's Multiple Comparison Test significance value '***' indicates p<0.001 and '**' indicates p<0.01 negative control verses positive control and *p<0.05, **p<0.01, respectively when all treated group verses normal group.

In vivo study

Piroxicam treatment (7 mg/kg administered intra-peritoneally for 42 days consecutively) caused significant increase in the serum activities of liver and renal marker enzymes (alkaline phosphatase, creatinine, alanine aminotransferase, serum bilirubin, BUN, aspartate aminotransferase, triglycerides, cholesterol and serum uric acid levels) and significant decrease in some enzymes (total protein and albumin) when compared to control rats. Prior treatment with melatonin and silymarin showed a significant decrease in serum and increase in protein levels respectively and restored the serum diagnostic marker enzymes to near- normal levels following piroxicam treatment.

In vivo antioxidant activity

Lipid peroxidation

Malondialdehyde (MDA), a thiobarbiturate reactive substance, was measured as a marker for oxidative stress in liver and renal tissue homogenates. To determine the lipid peroxidation, MDA levels were measured in liver and renal tissue homogenates. The liver and renal MDA levels were considerably increased in Piroxicam induced group rats alone in comparison with normal control group. However, Pre-treatment with Melatonin significantly suppressed the rise of MDA levels in the homogenate. The value of MDA in tissue was calculated from standard curve of MDA.

In the present investigation we observed a significant elevation in lipid peroxidation to an extend of, (38.27±1.70 nmoles/ml) and (231.47±4.90 nmoles/ml) in piroxicam induced group as compared to normal control rats which exhibited (74.52±2.70 nmoles/ml) and (69.28±5.30 nmoles/ml) in liver and renal tissue respectively. The results are clearly depicting the injured state of liver and renal tissue. Pre-treatment with melatonin, and silymarin showed significant inhibition of piroxicam induced changes in lipid peroxidation.

It is evident from the results that Pre-treatment of Melatonin for 42 days, with high dose 25 mg/kg (62.16±1.10 nmoles/ml) and (116.97±2.9 nmoles/ml) showed a clinical significant in the reduction of lipid peroxidation compared to low dose group whereas on comparing with the standard, silymarin the same was found to be statistically non-significant. The groups pretreated with silymarin showed much action by exerting antioxidant effect by blocking the induction of lipid peroxidation (68.32±09 nmoles/ml) and (86.95±2.70 nmoles/ml) in liver and renal tissue respectively. Same way carried out in superoxide dismutase, reduced glutathione, catalase.

Histopathological studies

Histopathological evaluation of liver

Histopathological observations of normal group exhibited clear integrity of show uniform polyhedral hepatocytes, normal sinusoids, and portal veinsas. Conversely piroxicam induced group (7 mg/kg) treatment resulted in biliary hyperplasia with ballooning of hepatocytes- severe, fatty degeneration of the liver cells with pyknotic nuclei, prominent Kupffer cells, and dilation and congestion of the portal veins, pointing to suggesting of severe tissue damage. Pre-treatment with melatonin for 42 days at 10 mg/kg showed the few inflamed portal area, reduced ballooning of hepatocytes- moderate, central vein (CV) portal vein (PV), improvement in the hepatic histological appearance that varied from moderate lymphocytic infiltration, fatty degeneration, and normal sinusoids whereas at 25 mg/kg showed notably restored normal hepatic architecture when compared to lower dose (10 mg/kg). Pre-treatment with silymarin also showed restored normal hepatic cellar structure.

Normal saline group

The cells exhibited clear integrity of show uniform polyhedral hepatocytes, normal sinusoids, and portal veins.

Piroxicam induced group: the rat shows biliary hyperplasia with ballooning of hepatocytes- severe, fatty degeneration of the liver cells with pyknotic nuclei, prominent Kupffer cells, and dilation and congestion of the portal veins, pointing to suggesting of severe tissue damage.

L MEL and Px group (10 mg/kg p. o.)

Few inflamed portal area, reduced ballooning of hepatocytes- moderate, central vein (CV) portal vein (PV), improvement in the hepatic histological appearance that varied from moderate lymphocytic infiltration, fatty degeneration, and improved sinusoids.

H MEL and Px group (25 mg/kg p. o.)

The cells showed notably restored normal hepatic architecture when compared to lower dose.

SLY + Px group (100 mg/kg p. o.)

The liver cells with Pre-treatment with Silymarin also showed absence of inflammatory hepatic cellar structure.

Histopathological evaluation of kidney

Histopathological observations of normal group exhibited clear integrity of the glomeruli, vessels and renal tubular epithelia appeared normal . In contrast, in the piroxicam induced group (7 mg/kg) treatment we noticed severe degradation of the proximal convoluted tubules (PCT), as indicated by the presence of tubular dilatation, epithelial degeneration, and severe loss of the brush border due to retraction or destruction of microvilli, inter tubular inflammatory cellular leakage, and shrinkage and atrophy of the glomeruli. The various sections of the loop of Henle were minimally affected. Nevertheless, pre-treatment with melatonin for 42 days at 10 mg/kg demonstrated improvement in the hepatic histological appearance that, moderate loss in renal tubular epithelial cells, hydropic degeneration, cytoplasmic vacuolation, tubular dilatation, and interstitial lymphocytic infiltration with the low dose and nearly complete recovery at melatonin high dose (25 mg/kg). Pre-treatment with silymarin also showed normal glomeruli, tubules and vessels were evident and restored normal renal cellar structure was observed.

Shrinkage and atrophy of the glomeruli

The various sections of the loop of Henle were minimally affected. Nevertheless, pre-treatment with melatonin for 42 days at 10 mg/kg demonstrated improvement in the hepatic histological appearance that, moderate loss in renal tubular epithelial cells, hydropic degeneration, cytoplasmic vacuolation, tubular dilatation, and interstitial lymphocytic infiltration with the low dose and nearly complete recovery at melatonin high dose (25 mg/kg).

Pre-treatment with silymarin also showed normal glomeruli, tubules and vessels were evident and restored normal renal cellar structure was observed.

Normal saline group

The cells retained integrity of glomerulus, vessels and renal tubules.

Piroxicam induced group

In piroxicam exposed rat exhibits proximal tubular dilatation with severe loss of the brush border, intertubular inflammatory cellular leakage with glomerulus, atrophytubular fibrosis and inflammatory cells with glomerular hyperplasia.

L MEL and Px group (10 mg/kg p. o.)

In this, animals reveal improvement in the renal histological appearance the moderate loss in renal tubular epithelial cells, hydropic degeneration, cytoplasmic vacuolation, tubular dilatation, and interstitial lymphocytic infiltration was observed.

H MEL and Px group (25 mg/kg p. o.)

The cells show prominent improvement in the histology of the kidney compared to the low dose treatment group (G, glomerulus; H and E).

Table 1: Water consumption in normal and piroxicam induced animals.

Days	Normal	Piroxicam induced
Days 0	30.08±0.58	33.27±0.66
Days 21	32.38±0.91	48.87±0.68*
Days 42	31.54±0.56	70.12±0.55**

Note: '***' indicates p<0.001 and '**' indicates p<0.01 negative control verses positive control and *p<0.05, **p<0.01, respectively when all treated group verses normal group.

Table 2: Body weight in normal and piroxicam induced animals.

Days	Normal	Piroxicam induced
Days 0	251.44±2.69	253.42±4.23
Days 21	256.12±2.33	280.56±3.98*
Days 42	259.65±2.67	301.34±3.64**

Note: '***' indicates p<0.001 and '**' indicates p<0.01 negative control verses positive control and *p<0.05, **p<0.01, respectively when all treated group verses normal group.

Table 3: Weight of liver and kidneys in normal and piroxicam induced animals.

Days	Normal		Piroxicam induced	
	Liver	**Kidney**	**Liver**	**Kidney**
Days 0	6.64±1.1	0.762±0.012	6.85±0.92	0.723±0.038
Days 21	8.82±0.51	0.81±0.025	11.18±0.22*	1.11±0.036**
Days 42	9.67±0.95	0.86±0.064	14.36±1.01**	1.38 ±0.055**

Note: '***' indicates p<0.001 and '**' indicates p<0.01 negative control verses positive control and *p<0.05, **p<0.01, respectively when all treated group verses normal group.

Table 4: Effect of melatonin and silymarin on the activities of serum AST, ALT, ALP and albumin in piroxicam induced liver and renal oxidative stress in rats.

Groups	AST (U/l)	ALT (U/l)	ALP (U/l)	Albumin (g/dl)
Normal group	114±1.8	22.66±2.1	701.8±12.30	4.76±0.2
Piroxicam induced (7 mg/kg, I. P.)	363.58±2.3**	117.8±2.7**	1076.02±11.2**	2.28±0.18**
Low dose of melatonin (10 mg/kg, p. o. L MEL + Px)	201.62±2.02*	75.04±2.3**	863.8±10.3*	2.82±0.68*
High dose of melatonin (25 mg/kg, p.o H MEL + Px)	201.62±2.48*	65.04±2.5**	803.2±10.4*	3.36±0.3*
Silymarin (100 mg/kg, p.o. SLY+ Px)	147.52±2.9*	58.27±1.9*	771.1±12.31**	3.41±0.16*

Note: '***' indicates p<0.001 and '**' indicates p<0.01 negative control verses positive control and *p<0.05, **p<0.01, respectively when all treated group verses normal group.

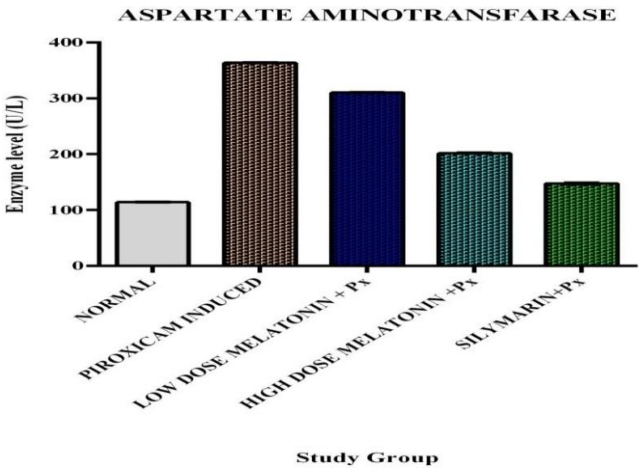

Figure 1: Effect of melatonin and silymarin on the activities of serum AST in piroxicam induced liver oxidative stress in rats.

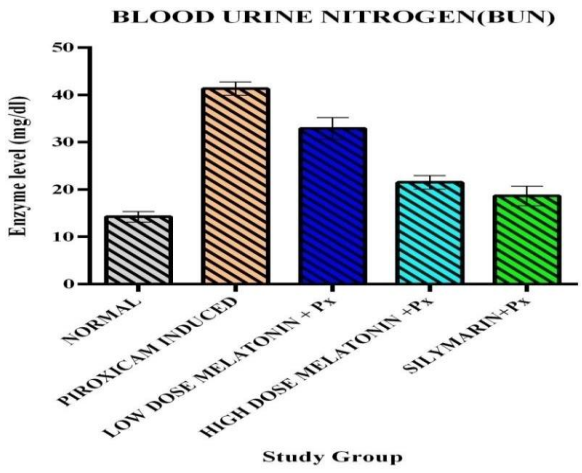

Figure 2: Effect of melatonin and silymarin on the activities of serum BUN in piroxicam induced renal oxidative stress in rats.

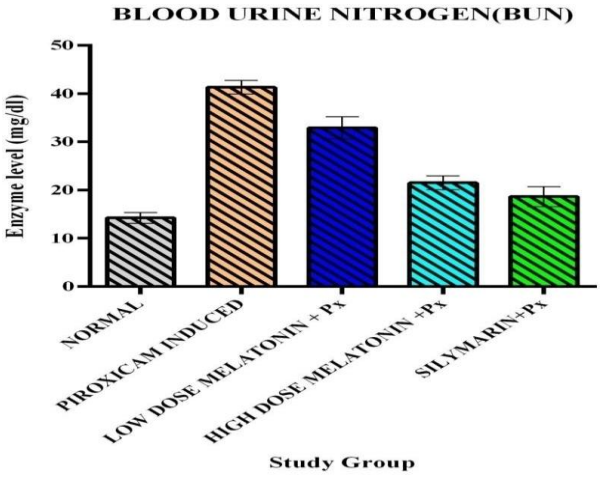

Figure 3: Effect of melatonin and silymarin on the activities of serumt in piroxicam induced liver oxidative stress in rats.

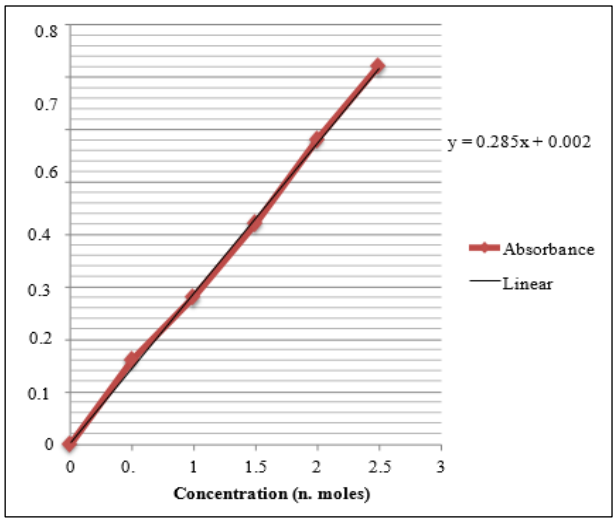

Figure 4: Calibration curve for the estimation of MDA levels in liver tissue.

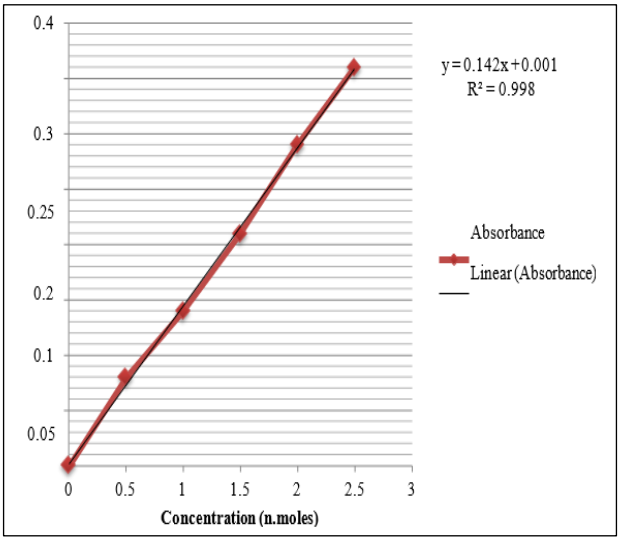

Figure 5: Calibration curve for the estimation of MDA levels in renal tissue.

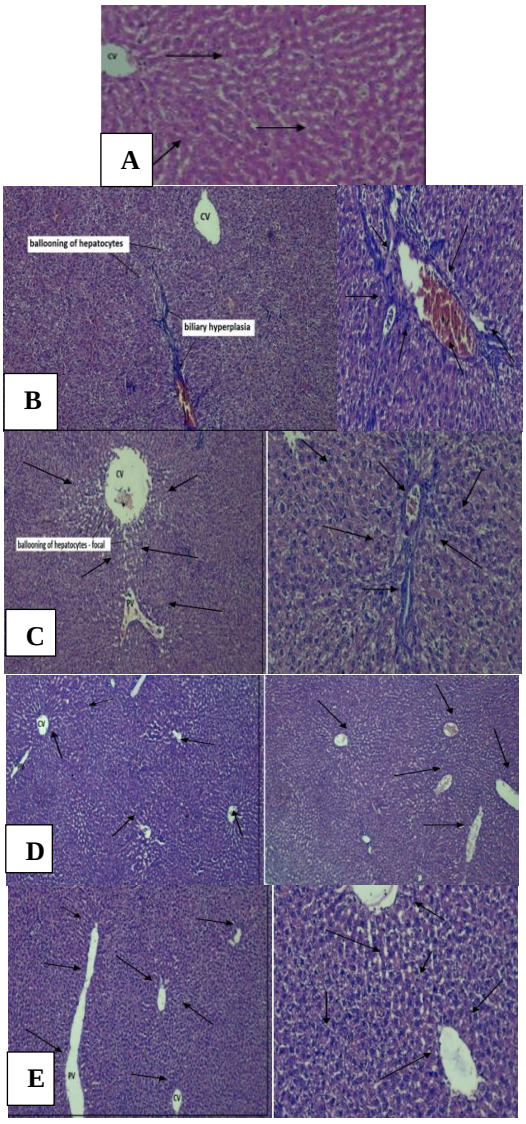

Figure 6 (A-E): Histopathological findings of liver in normal, experimental and standard group animals.

SLY and Px group (100 mg/kg p. o.)

The renal cells with pre-treatment with silymarin also showed absence of inflammatory renal cellar structure.

DISCUSSION

The results of this study indicate that piroxicam administration significantly increases oxidative stress in hepatic and renal tissues, as evidenced by elevated levels of oxidative stress markers in the serum and tissue samples. This aligns with previous studies reporting that prolonged NSAID use disrupts cellular redox homeostasis, leading to hepatotoxicity and nephrotoxicity.[7] The increase in lipid peroxidation, a hallmark of oxidative damage, further confirms the deleterious effects of piroxicam.[8]

Pre-treatment with melatonin at both 10 mg/kg and 25 mg/kg BW resulted in a marked reduction in oxidative stress markers, indicating its potential to mitigate piroxicam-induced toxicity. The significant decrease in lipid peroxidation suggests melatonin's role in neutralizing ROS and preventing peroxidative damage to cell membranes.[9] Additionally, the improvement in the Comprehensive Metabolic Panel profile further supports melatonin's hepatoprotective and nephroprotective properties, as previous studies have highlighted its ability to enhance antioxidant enzyme activities and modulate inflammatory responses.[10]

Histopathological analysis corroborated these findings, revealing reduced tissue damage in melatonin-pretreated groups compared to the piroxicam-only group. The preservation of hepato-renal architecture suggests that melatonin aids in cellular recovery and tissue integrity maintenance.[11] Moreover, in silico docking studies demonstrated high binding energy interactions between melatonin and oxidative stress markers, reinforcing the molecular basis of its protective effects.[12]

Limitations

However, limitations exist. The study was conducted on Wistar rats, limiting direct human applicability. Optimal dosing needs further exploration. Additionally, while oxidative stress markers were analyzed, molecular mechanisms require deeper investigation. Future studies should explore melatonin's long-term effects and interactions with other drugs.

CONCLUSION

In conclusion, the present study demonstrated that the oral pre-treatment with melatonin significantly potentiated antioxidant effect against piroxicam-induced liver and renal oxidative stress. The overall antioxidative effect of melatonin is probably related to its direct quenching of oxidative free radicals or its ability to maintain the activities of free radical scavenging enzymes, which protect hepato-renal tissue membranes against oxidative damage by decreasing lipid peroxidation. These observations highlight that Melatonin is a promising supplement for improving defense mechanisms in the liver and kidney against oxidative stress caused by piroxicam. It would also be worthwhile to study the effects of melatonin with different drugs used in piroxicam treated patients, in the hope of reducing morbidity and mortality. This study demonstrates that melatonin effectively mitigates piroxicam-induced oxidative stress in hepatic and renal tissues. The reduction in oxidative stress markers, improved metabolic profiles, and preserved tissue architecture highlight melatonin's antioxidative and protective potential. Furthermore, in silico docking analysis confirms its strong interaction with oxidative stress markers, reinforcing its mechanistic role in reducing oxidative damage.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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