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Research Article

PROTECTIVE EFFECTS OF MELATONIN AGAINST IDIOSYNCRATIC LIVER INJURY IN RATS CHALLENGED WITH CHLORPROMAZINE AND LIPOPOLYSACCHARIDE

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ABSTRACT
The episode of inflammation during drug treatment predispose animals to tissue injury, raising the possibility that presence or absence of inflammation is an important susceptibility factor for drug toxicity in human , and this phenomenon is hypothesized to be related to idiosyncratic drug reactions. The present study was designed to investigate the possible protective effect of orally administered melatonin against hepatic injury in rats concurrently treated with chlorpromazine (CPZ) and lipopolysaccharides (LPS). The protective effect of melatonin was studied through treatment of rats with single oral dose (10mg/kg), given seven days before and during the day of exposure to both CPZ and LPS. The animals were sacrificed 24 hrs post-challenge with LPS. Hepatic necrosis and cholestasis were assessed by measuring the activities of serum liver enzymes ALT, AST and ALP; in addition, total and direct bilirubin along with evaluation of histological alteration in hepatic tissue. The oxidative stress markers were assessed by measuring the levels of MDA and GSH in hepatic tissue homogenate. Analysis of data showed that melatonin supplementation attenuated markers of oxidative stress by reducing the levels of MDA and restoring GSH levels; melatonin also improved the observed biochemical and histological changes associated with exposure to CPZ and LPS. In conclusion, orally administered melatonin at pharmacological doses have promising protective effects against drug-induced idiosyncratic liver injury that may be of value in clinical practice.
KEY WORDS: melatonin, liver injury, chlorpromazine, drug idiosyncrasy

INTRODUCTION

Idiosyncratic drug hepatotoxicity represents a major problem in drug development[1], because of infrequency of their occurrence and the lack of animal models for preclinical evaluation[2], so that most of adverse drug reactions on the liver are not evident until after approval for human use, which frequently leads to drug withdrawal[3,4]. Many studies proposed that concomitant liver inflammation associated with viral or bacterial infections contribute to the increase in individual susceptibility to drug toxicity[5,6], they found that co-treatment of animals with hepatotoxic agents and bacterial lipopolysaccharides (LPS) results in liver toxicity at doses of the agent that would not cause liver toxicity in the absence of LPS[7]; LPS represent the principle component of Gram negative bacteria that recognized by the immune system of higher vertebrate, they initiate a cascade of events that up regulate the expression of inflammatory cytokines, enhance the production of reactive oxygen species (ROS) and induce migration of inflammatory cells to the liver which implicated in hepatocellular oxidative damage[8-10]. Chlorpromazine (CPZ) was the most extensively studied phenothiazine for its severe incidence of liver injury[11,12] that often described as idiosyncratic hepatotoxicity[13]; there is evidence that several pharmaceutical agents are rendered hepatotoxic upon co-exposure to LPS including halothane, cocaine and CPZ[14-16], and we previously reported that CPZ-induced cholestatic liver injury with marked infiltration of inflammatory cells, typical of idiosyncrasy and these events were significantly reduced by pretreatment with melatonin[17]. Melatonin, the major product of pineal gland, has a fundamental role in neuroimmuno-endocrine system and participate in many physiological functions, including anti-inflammation and immune regulation as well as functioning as abroad spectrum antioxidant[18,19]. Melatonin has been shown to protect liver in several models of liver injury via inhibiting the activity of inducible nitric oxide synthase and hence prevents LPS-induced hepatotoxicity in endotoxemic rats[20-22]. Therefore the present study was designed to investigate the possible protective effect of melatonin against augmented intrinsic hepatotoxicity of CPZ in rats co-treated with small doses of LPS (idiosyncratic -like liver injury).

MATERIALS AND METHODS

Thirty six rats of both sexes (*Rattus norvigus*) weighing 150-200gm were obtained from the animal house of the

College of Pharmacy, University of Baghdad; the animals were maintained on 12 hrs light/dark cycle under conditions of controlled temperature. Both food (rodent chow) and tap water were provided *ad libitum*. The animals were allocated into six groups each of six rats and treated as follows: Control group, the animals received intraperitoneal dose of saline; melatonin treated group, received daily oral dose of melatonin (10mg/kg) for seven days and thirty minutes before the injection of saline at the 8th day. The CPZ-treated group received single i.p dose of CPZ (70mg/kg) 2 hrs after treatment with saline. LPS-treated group, received i.p dose of LPS ($5.4*10^3$ EU/kg) from *E. coli*, serotype 0128:B12, $1.8*10^6$ EU/mg, obtained from sigma chemicals, experimentally this dose alone was not produce overt hepatic injury; then, 2 hrs later they were injected with saline i.p. CPZ-LPS treated group, the animals of this group were challenged by concomitant i.p injection of LPS ($5.4*10^3$ EU/kg) and CPZ (50mg/kg) 2 hrs after LPS treatment[16]. Melatonin pre-treated group, treated with oral daily dose of melatonin (10mg/kg) for seven days prior to and thirty minutes before LPS treatment at the 8th day; then single dose of CPZ was administered i.p 2 hrs after the animals were challenged with the endotoxin dose. The animals in all groups were sacrificed 24 hrs after the last injection. Blood was collected and serum was prepared by centrifugation for 15 min at 2000 rpm. Livers were removed quickly, rinsed in cold phosphate buffer saline (pH 7.4). Both blood and liver tissue samples were stored at -20°C until use for evaluation. Liver sections from all groups were fixed in 10% buffered formalin, processed and thin sections were stained and with hematoxylin-eosin, then examined under light microscope to evaluate histological changes. Samples of the liver were weighed and homogenized in chilled saline phosphate buffer solution to get 10% tissue homogenate, and then centrifuged at 300 rpm for 10 minutes. Aliquots of the supernatants were used for quantitative measurement of lipid peroxidation in the liver according to the method of Buge and Aust[23], while GSH levels were estimated according to the method of Ellman[24]. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) as indices of hepatic cell damage were assayed using commercial diagnostic kits[25,26]. In addition, total and conjugated bilirubin was assayed using commercial test kit for this purpose[27]. Results were expressed as mean±SD. The inter group variation was measured by one way analysis of variance (ANOVA). Statistical significance of the results was calculated at $P<0.05$.

RESULTS

Animals treated with oral melatonin (10mg/kg) alone did not affect any of the measured parameters significantly compared to control group. The animals in groups that received either single dose of CPZ or small dose of LPS alone showed non-significant increase in all measured parameters. As shown in table 1, the activities of serum hepatic enzymes (ALT, AST and ALP) were significantly increased in animals exposed to both CPZ and LPS compared to those of the control group, while seven days pretreatment with 10mg/kg melatonin significantly reduced the increase in ALP and ALT levels and non-significantly reduced the level of AST in comparison to the control group. Total bilirubin in animals simultaneously treated with CPZ+LPS was significantly increased compared to that in the control group; while pretreatment with melatonin significantly reduced the increase in the level of serum total bilirubin. Meanwhile, table 2 showed that MDA levels in the liver tissue homogenates were significantly elevated in CPZ and LPS treated animals, and in co-treated animals higher than those treated with either CPZ or LPS alone, also higher than those of the controls. Pretreatment with melatonin (10mg/kg orally) significantly reduced the levels of MDA compared to that in control group. Histologically, co-exposure of the animals to CPZ and LPS produces several morphological changes in all animals, including feathery changes, intracellular vacuoles, inflammatory cells infiltration, hydropic degeneration and necrosis. All these changes were attenuated in liver sections of all animals pretreated with melatonin as determined by the score system utilized for this purpose (Figure 1).

DISCUSSION

Idiosyncratic drug hepatotoxicity occurs in a small fraction of patients being on certain drug regimens, and it is poorly predicted by standard preclinical models or clinical trials because the susceptibility to drug toxicity is not well defined and thought to be influenced by variety of factors including genetic and environmental factors[5]. Recently, evidences have been reviewed that inflammation is both a result of and susceptibility factor for drug toxicity, which make liver tissue as a target to the deleterious effect of drugs and other agents that are normally not hepatotoxic[7,28]. In the light of these observation they found that low-grade of inflammatory reaction reduces the threshold of toxicity and/or increases the magnitude of response to different chemicals[29,30,14]; thus LPS provide initial activation of proinflammatory cytokines, like TNF-α, which in turn stimulate the production of ROS by activated macrophages, which are consequently implicated in

oxidative hepatocellular injury mediated through several mechanisms, including peroxidation of membrane lipids and oxidative damage of proteins[31]. So, exogenous administration of antioxidants was investigated to prevent oxidative tissue damage. In the present study, we examined the protective effect of melatonin against idiosyncratic like hepatoxicity induced by synergistic effect of LPS and CPZ, characterized by significant elevation of serum markers of hepatocellular and biliary injury[16]. In the present study, animals challenged with both LPS/CPZ showed significant elevation in ALP, ALT and AST serum activities as well as total serum bilirubin; while no significant changes in the serum level of each of the parameters listed above in groups treated with either CPZ or LPS alone; these results agreed with that reported by others[29,32,7]. Melatonin, the chief secretary product of pineal gland was known to have a potent antioxidant capacity[22,33]. Many studies have shown that melatonin attenuates the plasma level of liver enzymes in animal models of sepsis[19,17]; moreover, melatonin protected against liver injury induced by different chemicals and drugs with known hepatotoxic effect including CCl_4, alpha-Naphthylisothiocyanate and CPZ-induced cholestasis in rats; this protective effect was likely due to its antioxidative properties that maintain hepatocyte membrane integrity, thus reducing the leakage of liver enzymes, in addition to its ability to inhibit neutrophil infiltration and accumulation in the damaged hepatic tissue[34-36]. In the present study, pretreatment with melatonin significantly attenuate the elevation of serum levels of ALP, ALT, AST and total bilirubin relative to the control group (Table 1). These results are in accordance with previous findings[17,19,20]. The protective effect of melatonin could be attributed to its immunomodulatory and anti-inflammatory activity on TNF-α release and suppression of nitric oxide synthase expression, in addition to its antioxidant capacity[21,37]. Also, we reported that such protective effects of melatonin in reducing the level of hepatic markers are well correlated with the histological finding (Figure 1), where liver sections revealed that supplementation with melatonin reduced necrotic lesions and inflammatory cell infiltration with regenerative changes, and this result is compatible with previously reported data study[38]. Lipid peroxidation alters membrane fluidity and permeability and increases the rate of protein degradation, which eventually leads to cell lysis. It has been reported that melatonin detoxify a variety of free radicals and prevent oxidative damage through its radical scavenging property. The present study confirm the data reported by others and showed that melatonin pretreatment has antiperoxidative effect by decreasing the rise in MDA levels in liver tissue homogenates of rats challenged with LPS/CPZ treatments (Table 2). This may be due to the capacity of melatonin in scavenging $OH^{\bullet}$ and $ONOO^{\bullet}$ to preserve cellular integrity from oxidative damage[39,17]. Glutathione, the most important cellular antioxidant, functions either by protecting cells from lipid peroxidation or by protecting protein SH group from oxidation by these radicals[40]. In the present study (Table 2), there is significant decrease in GSH level in liver tissue homogenates of animals treated with LPS/CPZ compared to those of control group, while melatonin pretreatment significantly restored GSH levels, these results are in agreement with previously reported data[20]. In conclusion, hepatic degenerative changes caused by LPS/CPZ, which can be considered as idiosyncratic reaction, can be prevented by pre-treatment with melatonin.

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REFERENCES

1. Cosvegrove BD, King BM, Hasan MA, Alexoponlas LG. Synergistic drug-cytokine induction of hepatocellular death as an in vitro approach for the study of inflammation -associated idiosyncratic drug hepatotoxicity. Toxicol Applied Pharmacol 2009; 237:317-330.
2. Roth RA, Luyendyk JP, Maddox JF, Ganey PE. Inflammation and drug idiosyncrasy- is there a connection. J Pharmcol Exp Ther 2003; 307:1-8.
3. Uetrecht J. Screening for potential of drug candidate to cause idiosyncratic drug reactions. Drug Discovery Today 2003; 8:832-837.
4. Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discovery 2005; 4:489-499.
5. Ganey PE, Luyendyk JP, Maddox JF, Roth RA. Adverse hepatic drug reactions: inflammatory episodes as consequence and contributor. Chem Biol Interac 2004; 150:35-51.
6. Peter TS. Do preclinical testing strategies help predict human hepatotoxic potentials? Toxicol Pathol 2005; 33:146-154.
7. Roth RA, Harkema JR, Pestka JP, Ganey PE. Is exposure to bacterial endotoxin determinant of susceptibility to intoxication from xenobiotic agents? Toxicol Appl Pharmacol 1997; 147:300-311.
8. Victor VM, Minano M, Guayerbas N, Del Rio M, Medina S, De la Fuente M. Effects of endotoxic shock in several functions of murine peritoneal macrophages. Mol Cell Biochem 1998; 189:25-31.
9. Victor VM, Fuenten MDL. Changes in the superoxide production and other induced oxidative stress. Physiol Res 2003; 52:100-110.
10. Lexny E, Ruebner BH. Hepatic changes produced by a single dose of endotoxin in the mouse, light-microscopy and histochemistry. Am J Pathol 1967; 51:269-285.
11. George CJ. Hepatotoxicity of commonly used drugs: nonsteroidal anti-inflammatory drugs, antihypertensives, antidiadetic agents, anticonvulsants, lipid lowering agents, psychotropic drugs. Semin Liver Dis 2002; 22:169-183.

12. Guo JJ, Wigle PR, Lammers K, Vu O. Comparison of potentially hepatotoxic drugs among major US drugs compendia. Res Soc Adm Phrm 2005; 1(3):460-479.
13. Wen B, Zhou M. Chlorpromazine and thioridazine to electrophilic imminoquinone species in human liver microsomes. Chem Biol Interact 2009; 181:220-226.
14. Lind RC, Gandolfi AJ, Sipes IG, Brown BRJ. The involvement of endotoxin in halothane associated liver injury. Anesthesiology 1984; 61:544-550.
15. Labib R, Turkall R, Abedel-Rahman MS. Endotoxin potentiates the hepatotoxicity in male mice. Bu J Toxicol Environ Health 2002; 65:977-993.
16. Buchuleitz JP, Gaey PE, Bursian SJ, Roth RA. Underlying endotoximeia augments toxic responses to chlorpromazine, is there a relationship to drug idiosyncrasy? J Pharmacol Exp Ther 2002; 300:460-467.
17. Sener G, Toklu H, Kapucu C, Ercan F, et al. Melatonin protects against oxidative organ injury in a rat model of sepsis. Surg Today 2005; 35(1):35-52.
18. Teixeira A, Morfim MP, Decordova CA, Charao CC, et al. Melatonin protects against pro-oxidant enzyme and reduces lipid peroxidation in distinct membranes induced by the hydroxyl and ascrbyl radicals and by peroxynitrite. J Pineal Res 2003; 35:262.
19. Wangtl WE, Shen YX, Dong C. Protective effect of melatonin against liver injury in mice induced by Bacillus calmatte-Gverin plus lipopolysaccharide. World Gasterenterol 2004; 10:2690-2696.
20. Sewerynek E, Reiter RJ, Lewuski A. lipopoly sacchande induced hepatotoxicity is inhibited by the antioxidant melatonin. Eu J Pharmacol 1995; 293:293-327.
21. Crespo E, Pozo D, Martin M. Melatonin inhibits expression of the inducible NO synthase in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction syndrome in rats. FASEB J 1999; 13:1537.
22. Wu CC, Chiao CW, Hsiao G, Chen A. Melatonin prevents endotoxin-induced circulatory failure in rats. J Pineal Res 2001; 30:147-156.
23. Buge JA, Aust SD. Microsomal lipid peroxidation. Method Enzymol 1978; 52:302-310.
24. Ellman GL. Tissue sulfhydryl group. Arch Biochem Biophys 1959; 82:70-77.
25. Reitman S, Frankle S. A colorimetric method for the determination of serum glutamic oxaloacetic and pyruvic transaminases. Am J Gen Pathol 1957; 28(1):56-63.
26. Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydtolysed phenol with amino-antipyrine. J Clin Pathol 1954; 7(4):322-326.
27. Walter MI, Gerarde RW. Determination and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Microchem J 1970; 15:231.
28. Ganey PE, Roth RA. Concurrent inflammation as adeterminant of susceptibility to toxicity from xenobiotic agents. Toxicology 2001; 169:195-208.
29. Yee SB, Kinser S, Hill DA, Barton CC. Synergistic hepatotoxicity from co-exposure to bacterial endotoxin and pyrrolzidine alkaloid monocrotaline. Toxicol Appl Pharmacol 2000; 166:173-158.
30. Luyendyk JP, Shores KC, Ganey PE, Roth RA. Bacterial lipopolysaccharide exposure alters aflatoxin-B1 hepatotoxicity, benchmark dose analysis for marker of liver injury. Toxicol Sci 2000; 68:220-225.
31. Bharrhan S, Kanwaljit Chopra K, Rishi P. Vitamin E supplementation modulates endotoxin-induced liver damage in a rat model. Am J Biomed Sci 2010; 2(1):51-62.
32. Barton CC, Ganey PE, Roth RA. Development of liver injury in rats treated with aflatoxin B_1 and endotoximn, and protection by neutrophil depletion. Toxicol Sci 1999; 48:55.
33. Reiter RJ, Maestroni GJM. Melatonin in relation to the antioxidative defense and immune systems: Possible implications for cell and organ transplantation. J Mol Med 1999; 77(1):36-39.
34. Ohta Y, Kongo M, Sasaki E, Nishida K, Ishiguro I. Therapeutic effect of melatonin on carbon tetrachloride-induced acute liver injury in rats. J Pineal Res 2000; 28(2):119-126.
35. Calvo JR, Rietter RJ, Garcia JJ, Tan DX, Karbownik M. Charterization of the protective effect of melatonin and related indoles against alpha-naphthylisothiocyanate –induced liver injury in rats. J Cell Biochem 2001; 80:461-470.
36. Sulaiman AA, Al-Shawi NN, Jwaied AH, Mahmood DM, Hussain SA. The protective effect of melatonin against chlorpromazine-induced liver disease in rats. Saudi Med J 2006; 27(10):1477-1482.
37. Bellezzo JM, Leingang KA, Bulla GA, Britton RS, Bacon BR, Fox ES. Modulation of lipopolysaccharide-mediated activation in rat kupffer cells by antioxidants. J Lab Clin Med 1998; 131:36-44.
38. Rishi P, Bharrhan S, Pal M, Bhalla S, Koul A, Chopra K. Inhibition of Endotoxin-induced hepatotoxicity by melatonin in rats. Int J Biomed Sci 2008; 4(2):103-112.
39. Poeggeler B, Reiter RJ, Tan DX, Chen LD. Melatonin, hydroxyl radical-mediated oxidative damage, and aging: a hypothesis. J Pineal Res 1993; 14:151.
40. Haest C, Kramp WMD, Duliker B. Intra and intermolecular cross-linking membrane proteins in intact erythrocyte and ghosts by -SH oxidizing agents. Biochem Biophys Acta 1997; 557:36

Table 1. The protective effect of pre-treatment with melatonin against the liver injury in rats challenged by co-administration of LPS and CPZ.

Group / Parameter	Control	melatonin alone	LPS alone	CPZ alone	LPS+CPZ	Melatonin pre-treatment
MDA nmol/g protein	105.1±8.0	101.3±5.3[a]	112.6±14.4[a]	108.5±13.9[a]	131.3±11.5[*b]	93.0±9.4[c]
GSH µg/g protein	30.0±5.3	29.8±5.0[a]	21.2±5.2[b]	26.8±3.7[a]	16.7±3.3[*c]	27.4±5.1[a]

Each value represent mean± SD; n= 6 animals in each group; * Significant difference compared to the control $P< 0.05$; values with non-identical superscripts are significantly different $P<0.05$.

Table 2. The protective effect of pre-treatment with melatonin against the oxidative stress in liver tissue of rats challenged by co-administration of LPS and CPZ.

Group / Parameters	Control	Melatonin alone	LPS alone	CPZ alone	LPS+CPZ	Melatonin pretreatment
ALP IU/L	41.2± 5.3	39.8±7.5[a]	42.5±5.2[a]	41.3±4.5[a]	67±11.2[*b]	49.8±8.3[c]
ALT IU/L	25.2±5.2	26±7.9[a]	37.2±13.4[a]	31.5±7.1[a]	66±12.8[*b]	38.2±13.4[a]
AST IU/L	11.3±2.4	12.3±2.9[a]	13.7±3.6[a]	13.0±2.2[a]	19.8±5.3[*b]	15.3±3.6[a]
Total Bilirubin mg/dl	0.8±0.13	0.8±0.09[a]	0.98±0.2[b]	0.85±0.1[b]	1.4±0.3[*c]	0.78±0.14[a]

Each value represent mean± SD; n= 6 animals in each group; * Significant difference compared to the control $P< 0.05$; values with non-identical superscripts are significantly different $P<0.05$.

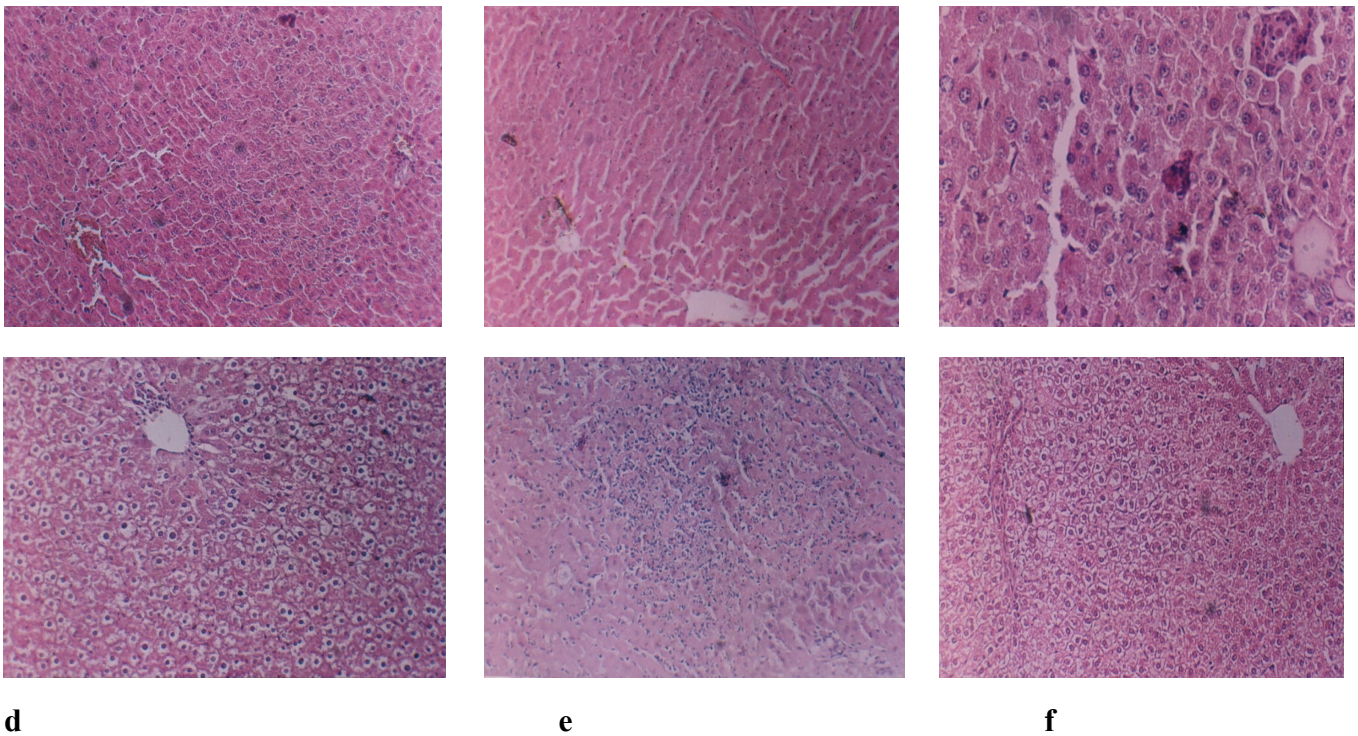

Figure 1. Sections of liver tissues stained with Hemotoxyllin-eiosin showed: **a,** normal hepatic tissue (control) with normal liver morphology; **b,** normal liver histology after treatment with melatonin only; **c,** liver from rats treated with CPZ alone showing no significant histological changes; **d,** liver of rats treated with small dose of LPS showing mild inflammation with normal hepatocellular morphology; **e,** liver from rats treated with both LPS /CPZ showing inflammatory cell infiltration and degenerative ghanges i,e. necrosis in hepatocytes and heavily infiltrated portal tract; **f,** liver from rats pretreated with melatonin before LPS/CPZ challenge showing regenerative changes with mild inflammation and normal liver cells.

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