Protective effect of melatonin against chlorpromazine–induced liver disease in rats

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ABSTRACT

Objective: To evaluate the possible protective effect of orally administered melatonin against Chlorpromazine (CPZ)-induced liver disease in rats.

Methods: We performed this study in the College of Pharmacy, University of Baghdad during the period from May to October 2004. The hepatoprotective effect of melatonin was studied through treatment of rats with single dose (10 mg Kg^{-1}) orally, 7 days before and during the period of CPZ treatment, and 7 days after the induction of suspected hepatotoxicity. The parameters of oxidative stress, malondialdehyde (MDA) and glutathione (GSH) in liver tissue homogenate, activities of the liver aminotransferases, alanine transaminase (ALT) and aspartate transaminase (AST) in serum, in addition to serum level of bilirubin (total and conjugated) were evaluated. Liver tissue sections were examined to follow histological changes.

Results: Analysis of data showed that treatment with melatonin significantly attenuated the oxidative stress parameters as evidenced by lowering MDA levels in tissue homogenate while not affecting GSH levels. Serum activities of ALT, AST and serum bilirubin were normalized with both pre-treatment and post-treatment with melatonin. Data revealed that post-treatments with both saline and melatonin restore hepatic activity; however, melatonin showed significant reduction in ALT activity and bilirubin level than saline post-treatment. Additionally, histological evaluation revealed improvement of liver damage in this respect.

Conclusion: The presented data indicated that orally administered melatonin in pharmacological doses protects against CPZ-induced liver disease in rats.

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Acute, drug-induced hepatocellular cholestasis (either pure or cholestatic hepatitis) is a common manifestation of drug-induced hepatic injury.[1] Chlorpromazine (CPZ) is the most extensively studied neuroleptic agent that has a clear profile of producing hepatocanalicular cholestasis.[2] The mechanism of CPZ-induced liver injury has been proposed but has not been fully clarified, since many factors found to be implicated in its adverse effect on the liver. Chlorpromazine produces a dose-related impairment in bile secretion and altering hepatocyte and canalicular membrane fluidity, which consequently affect the functional integrity of these sites.[3,4] Melatonin (N-acetyl-5-methoxy tryptamine), a secretory product of the pineal gland, functions not only as a direct antioxidant, namely scavenger of various oxygen free radicals and peroxyl radicals, but also as an indirect antioxidant through the enhancement of antioxidant enzymes activities in tissues such as liver and brain.[5,6] Many reports have shown that melatonin (MT) protects against liver injury with intrahepatic cholestasis in rats treated with alpha-

naphthylisothiocyanate (ANIT), possibly through its antioxidant activity and its inhibitory action against hepatic neutrophile infiltration.[7,8] Accordingly, this study was designed to evaluate the possibility that MT exerts a protective effect on cholestatic liver injury induced by treatment with CPZ in rats.

Methods. Thirty-six adult rats of both gender (Rattus norvegicus), 12-14 week old weighing 150-200 were used and housed in the animal house of the Collage of Pharmacy, University of Baghdad, Iraq. All animals were kept at a controlled temperature, fed standard rat chow ad libitum, and had free access to tap water. They were allocated into 6 groups (6 animals in each group) and treated as follows: Group I = 6 animals treated orally with daily doses (40 mg kg^{-1}) of CPZ-HCL (Medisca, Milan-Italy) alone for 2 weeks after saline treatment.[9] Group II = 6 animals treated with 10mg kg^{-1} day-1 MT (ARTI DRUGS Ltd, Tarapur, India) orally for 7 days before and during CPZ treatment as in group I. Group III = 6 animals received normal saline for 3 consecutive weeks, served as negative control. Group IV = 6 animals treated with single daily dose (10mg kg^{-1}) of MT orally for 3 consecutive weeks, served as positive control. Group V = 6 animals treated with 40mg kg^{-1} CPZ-HCl orally for 2 weeks, followed by treatment with 10mg kg^{-1} oral daily doses of MT for 7 days to evaluate the effect of MT during the recovery stage. Group VI - 6 animals treated with saline for 7 days after treatment with CPZ-HCL 40 mg kg^{-1} for 2 weeks. On 22nd day, the animals were killed by cervical dislocation, blood samples were taken by intracardiac puncture and kept in plain tubes to clot, and serum was prepared by centrifugation for 15 minute at 2000 rpm. Liver tissue samples were quickly obtained. Serum and liver tissue samples were stored at -40^{0}C unless immediately analyzed. Liver samples weighed and homogenized in chilled saline phosphate buffer solution to get 10% tissue homogenate, then centrifuged at 3000 rpm for 10 minutes. Aliquots of the supernatants were used for measurement of lipid peroxidation parameters including malondialdehyde (MDA) content by thiobarbituric acid method of Buge and Aust,[10] and glutathione (GSH) level according to the method of Ellman.[11] Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as indices of hepatic cell damage, were assayed colorimetrically utilizing commercial kits (Randox, UK).[12] Serum bilirubin levels (total and conjugated) were assayed using commercial kit (Randox, UK) for this purpose.[13] Liver sections were fixed with 10% formaldehyde, slides made from paraffin embedded blocks and stained by hematoxylin and eosin to

evaluate the histological changes,[14] the samples were introduced randomly and the pathologist was completely blind to the experimental allocation of rats. All results were expressed as mean ± SD, comparisons between groups were performed using unpaired Student's t-test. Values with $p < 0.05$ were considered significantly different.

Results. Treatment of rats with MT (10mg kg^{-1} orally) alone did not significantly affecting any of the measured parameters compared to those received only saline. The level of serum ALT and AST activities were significantly increased in CPZ-treated animals compared to controls. Oral administration of MT (10 mg kg^{-1}), 7 days prior to and during the period of CPZ treatment, significantly reduce the increase in serum ALT and AST activities to levels being comparable to those belong to controls. Melatonin (10mg kg^{-1}), given after treatment with CPZ, attenuates the increase in serum ALT and AST, while post-treatment with saline showed significant reduction in serum AST activity only, while ALT activity remained unchanged compared to CPZ- treated group (**Table 1**). Serum bilirubin levels (total and conjugated) in CPZ-treated group were significantly increased compared to control group, and both pre-treatment and post-treatment with MT (10mg kg^{-1}) significantly reduced serum bilirubin levels (total and conjugated); on the other hand, treatment with saline after CPZ treatment showed insignificant decrease in serum levels of both total and conjugated bilirubin (**Table 1**). Malondialdehyde levels in liver tissue homogenates of CPZ-treated group were found to be significantly higher than those pretreated with MT + CPZ (controls), and those treated with MT alone. Treatment with MT after appearance of hepatotoxicity, induced by CPZ, decreases significantly MDA levels in liver tissue homogenate compared to CPZ-treated group, and those of saline treated post-CPZ challenge. Liver GSH levels of CPZ-treated rats were significantly increased compared to both positive and negative controls. No one of the other treatments affect hepatic GSH levels (**Table 2**). Concerning the histological findings, administration of CPZ produces several morphological changes in 4/6 of the treated rats; the mainly observed pathological changes included: cholestasis manifested by feathery changes, proliferation of bile duct, appearance of pigmented granules and intracellular vacuoles within hepatocytes, inflammatory cell infiltration, ground glass appearance and hydropic degeneration (**Figure 1**). These changes were suppressed in liver sections of all rats pre-treated and post-treated with MT (**Figures 2 & 3**), while livers of the saline- post-CPZ treated

Table 1 - Effects of pre- and post- treatment with 10 mg kg^{-1} melatonin on the liver function indices in rats challenged with chlorpromazine (n=6).

Treatment group	Serum ALT Ul^{-1}	Serum AST Ul^{-1}	Total bilirubin mg dl^{-1}	Conjugated bilirubin mg dl^{-1}
Control	14.0 ± 3.0[a]	50.5 ± 7.4[a]	0.53 ± 0.12[a]	0.20 ± 0.08[a]
Melatonin 10mg kg^{-1}	11.5 ± 1.2[a]	55.8 ± 6.2[a]	0.48 ± 0.07[a]	0.17 ± 0.05[a]
Chlorpromazine 40mg kg^{-1}	24.5 ± 4.6[b]	71.5 ± 13.3[b]	0.95 ± 0.26[b]	0.53 ± 0.15[b]
Melatonin + chlorpromazine	12.3 ± 3.4[a]	53.0 ± 10.2[a]	0.58 ± 0.25[a]	0.25 ± 0.10[b]
Chlorpromazine + saline	23.8 ± 4.0[b]	63.5 ± 9.7[b]	0.83 ± 0.19[b]	0.40 ± 0.17[b]
Chlorpromazine + melatonin	17.7 ± 4.5[a]	52.5 ± 4.5[a]	0.52 ± 0.17[a]	0.22 ± 0.08[a]
	F = 12.2	F = 2.8	F = 8.8	F = 9.4

Values presented as mean ± SD. Values with non-identical superscripts (a, b) within the same parameter are considered significantly different ($p<0.05$). ALT - alanine aminotransferase, AST - aspartate aminotransferase
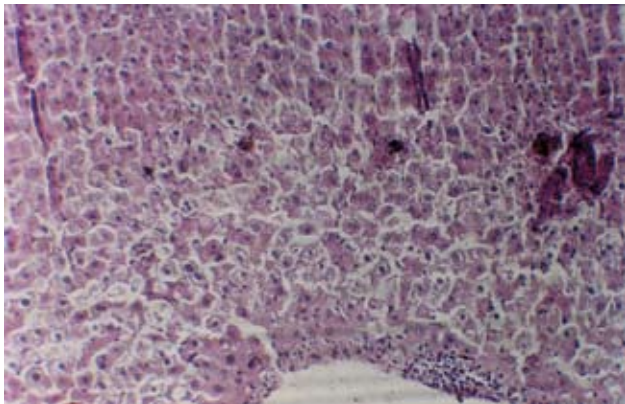

Figure 1 - Section showing morphological alteration of rat's liver after 2 weeks treatment with chlorpromazine 40 mg Kg^{-1} orally. Infiltration of inflammatory cells around the portal tract and hepatocytes. Intracellular vacuoles within hepatocytes, severe feathery changes and focal necrosis. Appearance of pigment granules, hydropic degeneration. Ground glass appearance and loss of nuclei. (Hematoxylin - eosin stain, magnification x40).
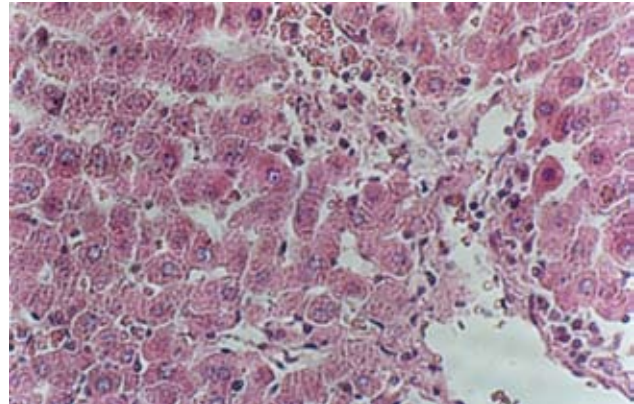

Figure 3 - Section showing that melatonin administration after chlorpromazine treatment restores the normal morphology of hepatocytes and reduces the inflammatory cell infiltration (Hematoxylin - eosin stain, magnification x40).
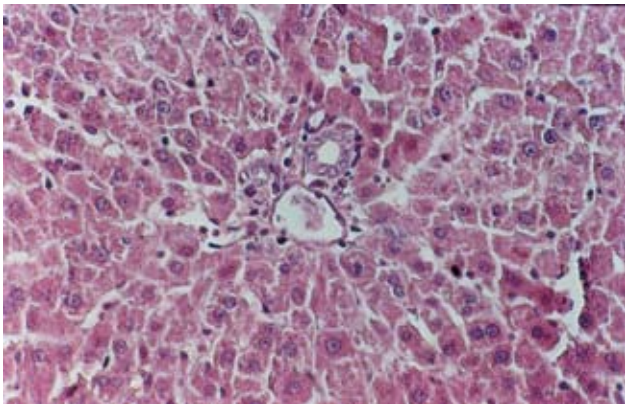

Figure 2 - Section showing the improvement of liver histology by treatment with 10mg Kg-1 melatonin starting 7-days prior to and during chlorpromazine treatment. Normal portal tract. Normal hepatocytes (Hematoxylin - eosin stain, magnification x40).
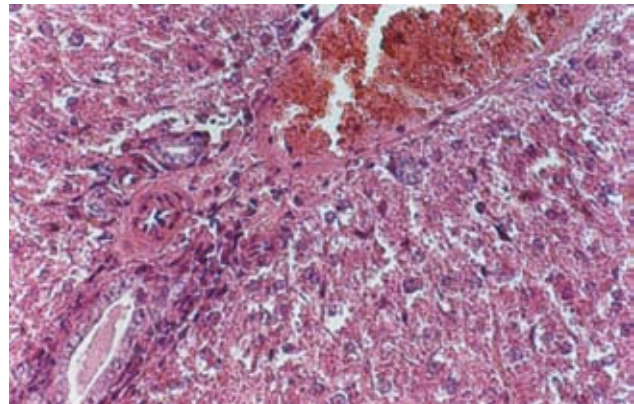

Figure 4 - Section showing effects of chlorpromazine plus post-treatment with saline. Moderate inflammation around central arteriole and sinusoidal congestion. Bile duct dilatation and proliferation of bile duct (Hematoxylin - eosin stain, magnification x40).

Table 2 - Effects of treatment with 10mg kg^{-1} melatonin on Malondialdehyde (MDA) and glutathione (GSH) levels in liver tissue homogenate of rats challenged with chlorpromazine (n=6).

Treatment group	MDA nmol g^{-1} tissue	GSH μmol g^{-1} tissue	GSH μmol g^{-1} tissue
Control	61.9 ± 12.4[a]	10.9 ± 2.1[a]	10.9 + 2.1[a]
MT 10mg kg^{-1}	56.7 ± 8.4[a]	10.2 ± 1.6[a]	10.2 + 1.6[a]
CPZ 40mg kg^{-1}	134.2 ± 16.0[b]	18.1 ± 4.2[b]	18.1 + 4.2[b]
MT + CPZ	45.5 ± 6.5[c]	15.5 ± 3.3[b]	14.8 + 3.3[b]
CPZ + saline	73.2 ± 10.8[a]	18.6 ± 4.3[b]	15.5 + 3.3[b]
CPZ + melatonin	55.6 ± 9.2[a]		18.6 + 4.3[b]
	F = 51.7	F = 7.0	F = 7.0

Values presented as Mean + SD, Values wit non-identical superscripts (a, b, c, d) within the same parameter are significantly different ($p<0.05$). CPZ - Chlorpromazine, MT -Melatonin

Table 3 - Effects of pre- and post-treatment with melatonin (MT) on the distribution of different histopathological changes in the liver of rats challenged with chlorpromazine (CPZ).

Treatment group	Types of histopathological changes			
	Cholestasis	Necrosis	Degeneration	Inflammation
Control	-	-	-	-
MT 10mg kg^{-1} alone	-	-	-	-
CPZ 40mg kg^{-1} alone	++	+	+	++
MT+CPZ	-	+/-	-	+/-
CPZ+ Saline	+/-	+	-	++
CPZ+ MT	-	+/-	-	+

- = sections were devoid of detectable changes, + = slightly positive changes, ++ = moderately positive changes.

rats showed less improvement in the affected hepatic tissue (**Figure 4**) as evidenced by the score system utilized for this purpose (**Table 3**).

Discussion. Cholestatic hepatitis is one of the most common forms of drug related injury, and has been associated with numerous agents from variety of the pharmacologic categories such as estrogen, erythromycin and various phenothiazines.[15,16] Cholestatic liver diseases are characterized by the accumulation of hepatotoxic substances, mitochondrial dysfunction and the impairment of liver antioxidant defense systems.[17] The data reported in this study demonstrated the implication of oxidative stress in hepatic tissue damage induced by CPZ-treatment, manifested by elevation of MDA contents in liver tissue (**Table 2**), this result is consistent with other studies that show the contribution of oxidative stress in the pathogenesis of cholestasis,[18,19] and can be explained as a consequence of generation of CPZ cation radicals and/or metabolic activation of CPZ to quinoneimine derivatives.[20] Meanwhile, retention of hydrophobic bile acids and toxic substances and or infiltration of inflammatory cells may participate in the generation of reactive oxygen species (ROS) with consequent production of oxidative damage.[21] It has been shown that in rats intoxicated with alpha-Naphthylisothiocyanate (ANIT), liver GSH level increases in accordance with the appearance of liver injury.[22,23] Based on these findings, the results of this study (**Table 2**) are compatible with those reported previously,[23,24] where an increase in hepatic GSH level was observed, thought to result from inhibition of GSH efflux across the canalicular membrane of hepatocyte, since GSH represents the major osmotic solute for the generation of bile salt-independent

bile flow. The protective effect of MT observed in this respect can be attributed to its high efficacy in scavenging various types of free radicals,[25,26] and enhancement of antioxidant enzymes activities.[27] Alteration of membrane fluidity of liver cells that causes changes in carrier mediated transport, activities of membrane bound enzymes, receptor binding, endocytosis and depolarization exocytosis,[28] results in leakage of certain intracellular enzymes, such as ALT and AST, to the plasma increasing their activities there. In the present study, **Table 1** showed an elevation in serum ALT and AST activities in animals treated with CPZ alone compared to controls, which are consistent with those reported by others.[29-31] The protective effect of MT can be explained according to its localization in a superficial position within the lipid bi-layers of membrane phospholipids rather than the polar heads.[32] Thus, in this position it is capable of functioning as a free radical scavenger, and it may also provide an indirect means by which the membrane can resist oxidative damage. Furthermore, several studies have shown that MT stabilizes cell membrane fluidity thereby preserving their functional efficiency.[33,34] It has been reported that MT receptors subtype MT1 were expressed in human gall bladder epithelium, suggesting that in addition to its profound receptor independent effects as antioxidant, MT could also act through receptor mediated process thereby influencing gall bladder functions.[35] Recently, other researchers reported that MT increases bile production and improves bile/bilirubin excretion through the improvement of ATP levels within hepatocyte, which consequently increases the efficiency of ATP-dependent transporters involved in bile flow and secretion,[36] and what obtained in the present study is compatible with previous results, where there is

a significant decrease in the level of serum bilirubin in animals pre-treated with MT compared to CPZ-treated group (**Table 1**). Cholestatic injury induced by CPZ is a self-limiting effect, where complete recovery achieved within 2-4 weeks after discontinuation of the drug.[37] The data presented in this study also showed that the studied parameters are restored naturally during post-treatment with saline and non-significantly different with those observed during post-treatment with MT. However, MT provided better effect manifested by reducing serum ALT activity and bilirubin level more efficiently than those attributed to post-treatment with saline; this could be related to the stabilizing effect of MT on hepatocyte membrane and enhancement of bile flow. The present work also showed that MT improves the histological changes induced by CPZ and reduces the severity of inflammatory cells infiltration, sinusoidal congestion, feathery changes and bile duct proliferation (**Figure 1**). These results are compatible with those reported previously by others.[38,39]

In conclusion, MT when administered orally in pharmacological doses protects against CPZ-induced liver injury in rats, as both a preventive and treatment measures, and further investigations are required to clarify the detailed mechanism.

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