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 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 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. Chlorpromazine (CPZ) is the most extensively of producing hepatocanalicular cholestasis. The mechanism of CPZ-induced liver injury has been many factors found to be implicated in its adverse effect on the liver. Chlorpromazine produces a dose-related impairment in bile secretion and altering consequently affect the functional integrity of these sites. 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. 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 
Accordingly, this 
study was designed to evaluate the possibility that 
MT exerts a protective effect on cholestatic liver 
j injury induced by treatment with CPZ in rats.

**Methods.** Thirty-six adult rats of both gender 
200 were used and housed in the animal house of the 
College of Pharmacy, University of Baghdad, Iraq. 
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 

1 of CPZ-HCL (Medisca, Milan-Italy) alone for 2 
9 Group II = 6 animals 

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 
as negative control. Group IV = 6 animals treated 

1) of MT orally for 3 
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 

in plain tubes to clot, and serum was prepared by 

℃ 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, and 
glutathione (GSH) level according to the method of 

Serum activities of alanine aminotransferase 
(ALT) and aspartate aminotransferase (AST), 
as indices of hepatic cell damage, were assayed 

UK). Serum bilirubin levels (total and conjugated) 

evaluate the histological changes, 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 
p 

**Results.** measured parameters compared to those received only 
saline. The level of serum ALT and AST activities 

ALT and AST activities to levels being comparable 
1), given after treatment with CPZ, attenuates the 
increase in serum ALT and AST, while post-treatment 

AST activity only, while ALT activity remained 
unchanged compared to CPZ- treated group (Table 
1). Serum bilirubin levels (total and conjugated) 

compared to control group, and both pre-treatment 

reduced serum bilirubin levels (total and conjugated); 
on the other hand, treatment with saline after 

serum levels of both total and conjugated bilirubin 
(Table 1). Malondialdehyde levels in liver tissue 
homogenates of CPZ-treated group were found to be 

+ CPZ (controls), and those treated with MT alone. 
Treatment with MT after appearance of hepatotoxicity, 

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 

and negative controls. No one of the other treatments 

affect hepatic GSH levels (Table 2). Concerning 

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 
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
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Table 1 -

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Serum ALT Ul⁻¹</th>
<th>Serum AST Ul⁻¹</th>
<th>Total bilirubin mg dl⁻¹</th>
<th>Conjugated bilirubin mg dl⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>± 1.2a</td>
<td>± 0.2a</td>
<td>± 0.12a</td>
<td>0.20 ± a</td>
</tr>
<tr>
<td>Melatonin + chlorpromazine</td>
<td>± 10.2a</td>
<td>± 0.26b</td>
<td>± 0.19b</td>
<td>± 0.17b</td>
</tr>
<tr>
<td>Chlorpromazine + saline</td>
<td>± 9.7b</td>
<td>± 0.07b</td>
<td>± 0.07b</td>
<td>± 0.07b</td>
</tr>
<tr>
<td>Chlorpromazine + melatonin</td>
<td>± 13.3b</td>
<td>± 0.26b</td>
<td>± 0.26b</td>
<td>± 0.26b</td>
</tr>
<tr>
<td>F = 12.2</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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

Figure 4 - Section showing effects of chlorpromazine plus post-central arteriole and sinusoidal congestion. Bile duct dilatation and proliferation of bile duct (Hematoxylin - eosin stain, magnification x40).
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. Cholestatic liver diseases are characterized by the accumulation of hepatotoxic substances, mitochondrial dysfunction and the impairment of liver antioxidant defense systems. 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, and can be explained as a consequence of generation of CPZ scavenging various types of free radicals, and enhancement of antioxidant enzymes activities that causes changes in carrier mediated transport, activities of membrane bound enzymes, receptor binding, endocytosis and depolarization exocytosis, 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. The protective effect of MT can be explained according lipid bi-layers of membrane phospholipids rather than the polar heads. 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 can resist oxidative damage. Furthermore, several studies have shown that MT stabilizes cell membrane fluidity thereby preserving their functional integrity.

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. Recently, other researchers reported that MT increases bile production and improves the distribution of different histopathological changes in the liver of rats challenged with chlorpromazine (CPZ).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>MDA, μmol g⁻¹ tissue</th>
<th>GSH, μmol g⁻¹ tissue</th>
<th>GSH, μmol g⁻¹ tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MT + CPZ</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CPZ + saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CPZ + melatonin</td>
<td>-</td>
<td>-</td>
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Values presented as Mean ± SD, Values with non-identical superscripts (a, b, c, d) within the same parameter are significantly different (p < 0.05). CPZ - Chlorpromazine, MT - Melatonin.
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 of the drug. The data presented in this study also showed that the studied parameters are restored naturally during post-treatment with saline and non-post-treatment with MT. However, MT provided better effect manifested by reducing serum ALT attributed to post-treatment with saline; this could be related to the stabilizing effect of MT on hepatocyte changes induced by CPZ and reduces the severity of feathery changes and bile duct proliferation (Figure 1). These results are compatible with those reported previously by others.

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.

Acknowledgment.
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