Original Article

Effect of trazodone and nefazodone on hepatic injury induced by carbon tetrachloride

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ABSTRACT: The present study aimed to evaluate the effect of the serotonin antagonists and reuptake inhibitors trazodone and nefazodone on liver injury induced by treatment with carbon tetrachloride (CCl_4) in rats. Liver damage was induced in rats by oral administration of CCl_4 (2.8 mL/kg in olive oil). Nefazodone (5, 10, or 20 mg/kg), trazodone (5, 10, or 20 mg/kg), silymarin (25 mg/kg), or saline (control) was orally administered once daily in association with CCl_4 and for one week thereafter. Liver damage was assessed by determining serum enzyme activities and hepatic histopathology. In CCl_4-treated rats, treatment with trazodone (5, 10, 20 mg/kg), reduced serum alanine aminotransferase (ALT) levels by 24, 38.6, and 49.3%. Serum aspartate aminotransferase (AST) levels were decreased by 18.1, 37.9, and 42.2%, and alkaline phosphatase (ALP) levels decreased by 25.7, 32.6, and 39.7%, respectively. Nefazodone (5, 10, 20 mg/kg) in a dose-dependent manner reduced the elevation of ALT levels by 15.6, 36.5, and 45.9%, AST levels by 16.7, 17.3, and 43%, and ALP by 30.5, 37.5, and 42.9%, respectively. Silymarin treatment reduced the levels of ALT, AST, and ALP by 56.1-62.8, 56.0-64.0, and 50.1-58.2%, respectively. The administration of CCl_4 decreased levels of reduced glutathione in blood compared to the vehicle-treated group. In CCl_4-treated rats, reduced glutathione levels increased after trazodone in a dose-dependent manner. Reduced glutathione was increased by nefazodone at concentrations of 5 and 10 mg/kg, but not after 20 mg/kg nefazodone. Reduced glutathione levels were increased by the administration of silymarin to near normal values. The administration of CCl_4 resulted in a marked increase in nitric oxide levels in serum (the concentrations of nitrite/nitrate) as compared to the control group. Treatment with trazodone or nefazodone caused a dose-dependent decrease in serum nitric oxide levels compared with the CCl_4 control group. Histopathological and histomorphometric examinations also indicated that CCl_4-induced liver injury was less severe in trazodone and nefazodone-treated groups than in the CCl_4 control groups. Metabolic perturbations caused by CCl_4 in the form of decreased intracellular protein and mucopolysaccharide content in hepatocytes were improved by treatment with trazodone and nefazodone. It is concluded that administration of serotonin antagonists and reuptake inhibitors trazodone and nefazodone is associated with a reduction in experimental liver injury induced by CCl_4.

Keywords: Trazodone, nefazodone, serotonin antagonists, liver injury, rats

1. Introduction

The effect of drugs acting on serotonin (5-HT) neurotransmission or serotonin modulators on liver function is of particular interest for several reasons. First, these drugs are widely prescribed for the pharmacotherapy of depression and accordingly their effect on liver in healthy patients and more importantly in patients with liver disease is of clear clinical significance. In patients with hepatitis C viral infection, these agents have been used for the treatment of depression especially that observed after treatment with interferon-alpha/ribavirin combination therapy (*1,2*). Second, serotonin has been linked to liver disease in man, where in patients with liver cirrhosis, a significant fall of serotonin concentration in sera and urine has been noted, which might contribute to development of the hyperdynamic state of circulation observed in this condition (*3*). In addition, alterations of the serotonin system have been implicated in the

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pathogenesis of hepatic encephalopathy, a serious neuropsychiatric complication of chronic liver disease (*4*) and therapeutic approaches aiming at the normalization of serotonin turnover could be beneficial in the prevention and treatment of early neuropsychiatric symptoms of hepatic encephalopathy (*5*). Third, changes in levels of neuropeptides in the brain can have substantial effects on liver function. For example, intracisternal administration of thyrotropin-releasing hormone has been shown to protect the liver (*6*), while intracisternal injection of corticotropin releasing factor exacerbated hepatic injury induced by CCl_4 in rats (*7*). Consequently, increased central serotonergic activity could affect liver function. Fourth, serotonin appears to play an important role in liver regeneration (*8-10*).

Trazodone is a phenylpiperazine with complex pharmacological actions, possessing antidepressant, anxiolytic and hypnotic activities. Trazodone is primarily a serotonergic antidepressant, blocking the reuptake of 5-HT presynaprtically, thereby, increasing its availability in the synapse (*11,12*). In the brain, this latter effect accounts for the alleviation of the symptoms of depression (*13*). It also blocks postsynaptic 5-HT_2 receptors leading to facilitated neurotransmission through 5-HT_{1A} receptors, which reduces anxiety levels (*12*). Nefazodone which is chemically related to trazodone is also a 5-HT_2 receptor antagonist and a reuptake inhibitor. It has a weak affinity for cholinergic and noradrenaline alpha 1-adrenergic receptors (*12,14-16*). In view of their mixed serotonin effects, these agents can be described as serotonin modulators. Both trazodone and nefazodone are also capable of inducing hepatotoxicity (*17-20*), with the result that nefazodone was withdrawn from the US market (*21*).

The aim of the present study was to examine whether trazodone and nefazodone would modulate the development of hepatic injury caused by CCl_4 in the rat. Hepatic injury was determined *via* liver enzymes and histological analysis of necrotic areas as well as histochemical investigation of intracellular protein and mucopolysaccharide content. The effect of trazodone and nefazodone on CCl_4-induced hepatic damage was compared to that of silymarin, a herbal remedy widely used for its hepatoprotective effects (*22*).

2. Materials and Methods

2.1. *Animals*

Sprague-Dawley rats of both sexes, weighing 120-130 g were used throughout the experiments and fed with standard laboratory chow and water *ad libitum*. All animal procedures were performed in accordance to the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985). The drug doses for rats used were based upon the human dose after conversion to that of the rat according to Paget and Barnes conversion tables (*23*).

2.2. *Drugs and chemicals*

Carbon tertrachloride (BDH Chemicals, Poole, UK), silymarin (Sedico Pharmaceutical Co., Giza, Egypt), nefazodone hydrochloride (Bristol-Myers Squibb, Cairo, Egypt) and trazodone hydrochloride (Egyptian International Pharmaceutical Industries Co., Cairo, Egypt) were used. All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use.

2.3. *CCl_4-induced hepatic injury*

Two separate studies evaluated the effect of trazodone and nefazodone on CCl_4-induced hepatic damage. In each study rats were divided into 6 equal groups (6 rats each). Groups 1-5 received CCl_4 in olive oil (1:1, v/v) at a dose of 2.8 mL/kg through an orogastric tube. Starting on the first day of CCl_4 administration, rats were also treated with the test drug (trazodone or nefazodone) at doses of 5, 10, and 20 mg/kg, silymarin (25 mg/kg) or saline only (positive control, treated with CCl_4 and not receiving drugs) once daily orally and for one week thereafter. All treated rats were administered half the initial dose of CCl_4 (0.14 mL/100 g body weight) 3 days after the first administration of CCl_4 so as to maintain hepatic damage. In addition, a 6th group of rats (n = 6) received the vehicle (olive oil) at 2.8 mL/kg followed 3 days later by an additional dose of 1.4 mL/kg olive oil. Rats had free access to food and drinking water during the study. After 7 days of CCl_4 or olive oil administration, rats were killed under ether anaesthesia.

In a third study, the effect of the highest dose (20 mg/kg) of trazodone or nefazodone was examined on serum aminotransferases in normal rats (n = 6/group). Drugs were given once daily orally for one week.

2.4. *Biochemical assessment*

At the end of the experiments, blood samples were obtained from the retro-orbital vein plexuses, under ether anaesthesia. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum were measured according to the Reitman-Frankel colorimetric transaminase procedure (*24*), whereas colorimetric determination of alkaline phosphatase (ALP) activity was done according to the method of Belfield and Goldberg (*25*), using commercially available kits (BioMérieux, Craponne, France). Blood reduced glutathione (GSH) level was estimated spectrophotometrically by the method of Beutler *el al.* (*26*) using a commercial kit (Biodiagnostic, Giza, Egypt). Glutathione levels were expressed as mg/dL. Nitric oxide estimated as nitrite/nitrate, was determined in serum according to the method of Miranda *et al.* (*27*). The level of total nitrite/nitrate in

serum samples was expressed in μM and was calculated using the standard curve constructed with the prepared serial dilutions of sodium nitrite.

2.5. *Histological and histochemical studies*

At the end of the treatment period, rats were killed, livers excised and fixed in 10% formalin saline. Sections were prepared and stained with haematoxylin and eosin stain (H&E) for histological examination. Livers of all animals were dissected immediately after death. The specimens were then fixed in 10% neutral-buffered formal saline for at least 72 h. All specimens were washed in tap water for half an hour and then dehydrated using incresing grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections 6 μm thick were cut and stained with H&E for histopathological investigation (*28*). Protein (*29*) and mucopolysaccharide (*30*) staining were also performed. All sections were investigated using the light microscope. Further histopathological evaluation was done with morphometry. The percentage of liver tissue affected by necrosis and fibrosis (damaged area) were determined using a computer assisted automated image analyzer. The Qwin Leica image processing and analysis system (Cambridge, UK) was used for interactive automatic measurement of the percentage of damaged areas on slides stained with H&E by analyzing 15 random fields per slide.

The image analyzer was also used to evaluate the DNA content. The image analysis system automatically expresses the DNA content of each individual cell measured then gives the percentage of each cell out of the total number of cells examined. Also, it classifies the cells into 4 groups; diploid (2C), proliferating cells (3C), tetraploid (4C) and aneuploid cells (> 5C). The proliferating cells were further classified according to Lee *et al.* (*31*) into: (< 10%) low proliferating index, (10-20%) medium proliferating index, and (> 20%) high proliferating index.

2.6. *Statistical analysis*

All results are expressed as means ± S.E. Multiple group comparisons were performed by one way ANOVA followed by Duncan test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. *Biochemical changes*

In normal rats, trazodone or nefazodone administerted at a dose of 20 mg/kg had no effect on serum ALT, AST, or ALP (Table 1). In rats treated with CCl_4, the levels of ALT, AST, and ALP in plasma were markedly raised indicating the severity of hepatic injury and cholestasis caused by CCl_4. Trazodone (5, 10, or 20 mg/kg) given at the time of CCl_4 administration caused a dose-dependent reduction of raised ALT by 24, 38.6, and 49.3%, AST by 18.1, 37.9, and 42.2%, and ALP levels by 25.79, 32.6, and 39.7%, respectively. Meanwhile, silymarin treatment reduced the levels of ALT, AST, and ALP by 62.8, 64.0, and 50.1%, respectively (Table 2). In CCl_4-treated rats, glutathione level in blood was reduced by 40.9% compared with vehicle-treated control group (21.4 ± 0.7 *vs.* 36.2 ± 0.1 mg/dL). Reduced glutathione was increased by 19.6, 37.8, and 54.2% by trazodone administered at doses of 5, 10, and 20 mg/kg, respectively, compared with the CCl_4-control group. Meanwhile, reduced glutathione increased after silymarin treatment by 62.1% compared with the CCl_4-control group (Table 2). The administration of CCl_4 induced a significant increase in serum concentrations of nitric oxide (nitrite/nitrate). Treatment with trazodone significantly protected against the CCl_4-induced elevation in nitric oxide level which decreased by 27.7, 45.6, and 52.6% after 5, 10, and 20 mg/kg trazodone, respectively, as compared to control CCl_4. On the other hand, silymarin significantly lowered the raised nitric oxide level by 57.4% as compared to CCl_4 control group (Table 2).

Table 1. Effect of trazodone and nefazodone on serum ALT, AST, and ALP in normal rats

Treatments	ALT (U/L)	AST (U/L)	ALP (U/L)
Vehicle	61.0 ± 3.1	66.4 ± 2.5	59.0 ± 1.9
Vehicle + trazodone 20 mg/kg	57.4 ± 1.5	55.8 ± 2.1	53.3 ± 3.1
Vehicle + nefazodone 20 mg/kg	55.4 ± 3.4	57.2 ± 1.7	50.3 ± 2.4

Data are means ± S.E.

Nefazodone administered to CCl_4-treated rats at doses of 5, 10, or 20 mg/kg, reduced the elevation of ALT levels by 15.6, 36.5, and 45.9%, AST levels by 16.7, 17.3, and 43.0%, and ALP by 30.5, 37.5, and 42.9%, respectively. On the other hand, ALT, AST, and ALP decreased by 56.1, 56.0, and 58.2%, respectively, by treatment with silymarin. In CCl_4-treated rats, glutathione level in blood was reduced compared with vehicle-treated control group (21.0 ± 0.6 *vs.* 36.6 ± 0.8 mg/dL). Reduced glutathione was increased to 30.9 ± 0.8 and 29.95 ± 1.3 mg/dL by nefazodone given at doses of 5 or 10 mg/kg, respectively (47.1% and 42.6% increase *vs.* CCl_4 control group). With nefazodone at 20 mg/kg, reduced glutathione levels were reduced again to 24.3 ± 0.8 mg/dL (15.7% increase *vs.* CCl_4 control group). In contrast, reduced glutathione in blood increased by 66.2% after silymarin treatment compared with the CCl_4-control group (34.9 ± 0.6 *vs.* 21.0 ± 0.6 mg/dL) (Table 3). Treatment with nefazodone at 10 or 20 mg/kg also resulted in a significant decrease of the CCl_4-induced elevation in nitric oxide level by 27.5 and 54.0%, respectively, as compared to control CCl_4. Nitric oxide level decreased by 61.2% after silymarin treatment compared to the CCl_4 control group (Table 3).

Table 2. Effect of trazodone on serum ALT, AST, ALP, nitric oxide (NO), blood GSH, and histological area of damage in carbon tetrachloride-treated rats

Treatment groups	ALT (U/L)	AST (U/L)	ALP (U/L)	NO (μM)	GSH (mg/dL)	Area of damage (%)
Vehicle	52.0 ± 6.0	57.2 ± 4.8	60.2 ± 3.6	53 ± 3.2	36.2 ± 0.1	0.21 ± 0.1
CCl_4 (control)	144.3 ± 11.1[a,c]	155.4 ± 12.1[a,c]	116.2 ± 7.0[a,c]	512 ± 17.8[a,c]	21.4 ± 0.8[a,c]	56.96 ± 6.5[a,c]
CCl_4 + trazodone 5 mg/kg	109.7 ± 5.5[a,b,c] (–24.0%)	127.2 ± 8.5[a,b,c] (–18.1%)	86.3 ± 6.2[a,b,c] (–25.7%)	370 ± 16.1[a,b,c] (–27.7%)	25.6 ± 0.5[a,b,c] (19.6%)	47.76 ± 1.4[a,c] (–16.2%)
CCl_4 + trazodone 10 mg/kg	88.6 ± 4.9[a,b,c] (–38.6%)	96.5 ± 3.6[a,b,c] (–37.9%)	78.3 ± 6.1[a,b,c] (–32.6%)	278.3 ± 16.0[a,b,c] (–45.6%)	29.5 ± 0.6[a,b,c] (37.8%)	34.87 ± 3.9[a,b,c] (–38.8%)
CCl_4 + trazodone 20 mg/kg	73.1 ± 4.3[a,b,c] (–49.3%)	89.8 ± 7.5[a,b,c] (–42.2%)	70.1 ± 6.7[b] (–39.7%)	242.6 ± 18.0[a,b] (–52.6%)	33.0 ± 0.7[a,b] (54.2%)	17.10 ± 2.0[a,b,c] (–69.9%)
CCl_4 + silymarin 25 mg/kg	53.6 ± 2.4[b] (–62.8%)	56.0 ± 3.9[b] (–64.0%)	58.0 ± 3.6[b] (–50.1%)	218.2 ± 15.0[a,b] (–57.4%)	34.7 ± 0.1[a,b] (62.1%)	10.90 ± 1.3[a,b] (–80.9%)

Data are means ± S.E. Six rats were used for each group. The percent change from the CCl_4-control group is also shown in parenthesis. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's multiple range test. $p < 0.05$ was considered statistically significant. [a] $p < 0.05$ *vs.* vehicle control group. [b] $p < 0.05$ *vs.* CCl_4 control group. [c] $p < 0.05$ *vs.* silymarin-treated group. Serum ATL, AST, and NO levels were significantly less in CCl_4 + trazodone 10 mg/kg- and CCl_4 + trazodone 20 mg/kg-treated groups than CCl_4 + trazodone 5 mg/kg-treated group.

Table 3. Effect of nefazodone on serum ALT, AST, ALP, NO, blood GSH, and histological area of damage in carbon tetrachloride-treated rats

Treatment groups	ALT (U/L)	AST (U/L)	ALP (U/L)	NO (μM)	GSH (mg/dL)	Area of damage (%)
Vehicle	58.4 ± 3.9	62.2 ± 6.1	51.0 ± 4.3	58.0 ± 2.6	36.6 ± 0.8	0.16 ± 0.2
CCl_4 (control)	134.9 ± 8.7[a]	148.2 ± 12.8[a]	115.9 ± 8.1[a]	531.1 ± 19.0[a,b]	21.0 ± 0.6[a,b]	43.5 ± 4.5[a,b]
CCl_4 + nefazodone 5 mg/kg	113.8 ± 9.9[a,b,c] (–15.6%)	123.4 ± 4.1[a,b,c] (–16.7%)	80.5 ± 7.3[a,b,c] (–30.5%)	503.9 ± 24.5[a,c] (–5.3%)	30.9 ± 0.8[b] (47.1%)	37.53 ± 2.6[a,c] (–13.7%)
CCl_4 + nefazodone 10 mg/kg	85.6 ± 6.3[a,b,c] (–36.5%)	122.5 ± 6.8[a,b,c] (–17.3%)	72.2 ± 6.3[a,b,c] (–37.7%)	385.3 ± 8.0[a,b,c] (–27.5%)	29.95 ± 1.3[a,b] (42.6%)	24.5 ± 3.0[a,b,c] (–43.7%)
CCl_4 + nefazodone 20 mg/kg	72.9 ± 6.1[b] (–45.9%)	84.4 ± 5.1[a,b,c] (–43.0%)	66.2 ± 6.0[a,b,c] (–42.9%)	244.0 ± 15.5[a,b] (–54.0%)	24.3 ± 0.8[a,c] (15.7%)	20.4 ± 1.8[a,b,c] (–53.1%)
CCl_4 + silymarin 25 mg/kg	59.2 ± 3.1[b] (–56.1%)	65.2 ± 6.2[b] (–56.0%)	48.4 ± 4.2[b] (–58.2%)	206.0 ± 8.3[a,b] (–61.2%)	34.9 ± 0.6[b] (66.2%)	9.6 ± 1.3[a,b] (–77.9%)

Results are means ± S.E. Six rats were used for each group. The percent change from the CCl_4-control group is also shown in parenthesis. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's multiple range test. $p < 0.05$ was considered statistically significant. [a] $p < 0.05$ *vs.* vehicle control group. [b] $p < 0.05$ *vs.* CCl_4 control group. [c] $p < 0.05$ *vs.* silymarin-treated group. Serum ATL and NO levels were significantly less in CCl_4 + trazodone 10 mg/kg- and CCl_4 + trazodone 20 mg/kg-treated groups than CCl_4 + trazodone 5 mg/kg-treated group. Serum AST level was significantly less in CCl_4 + trazodone 20 mg/kg-treated group than CCl_4 + trazodone 5 mg/kg-treated group.

3.2. *Histological and histochemical results*

Trazodone (20 mg/kg) or nefazodone (20 mg/kg) only treated rats showed a normal hepatic appearance (Figure 1). Treatment of rats with CCl_4 led to marked liver tissue damage in the form of complete distortion of the tissue architecture, a variable degree of vacuolar degeneration in many of the hepatocytes, cellular infiltration as well as hemorrhage (Figure 2A). Examination of sections from the liver of rats treated with trazodone and CCl_4 revealed that trazodone administered at 20 mg/kg had a protective effect against the damaging effect of CCl_4 (Figure 2D). With trazodone at 5 and 10 mg/kg, a variable degree of vacuolar degeneration was still present in many of the hepatocytes. Also, cellular infiltration was marked (Figures 2B and 2C). The highest dose caused regain of normal tissue architecture although mild cellular infiltration was noticed (Figure 2D). Histochemical investigation for protein and mucopolysaccharide confirmed these results as only at the highest dose of trazodone did their levels return to normal (Figures 3 and 4).

Examination of liver sections from CCl_4 + nafazodone-treated rats revealed a dose-dependent improvement in hepatic architecture compared to the CCl_4 control group (Figure 5). In sections from rats treated with 5 mg/kg nefazodone, vacuolar degeneration and pyknotic nuclei were present in most of the hepatocytes. These findings became less prominent after 10 mg/kg nefazodone, while marked improvement of liver tissue except for slight dilatation and congestion of some blood sinusoids was observed with the highest dose (20 mg/kg) of nefazodone examined (Figure 5). Histochemical investigation for protein and mucopolysaccharide content in hepatocytes were in agreement with these findings (Figures 6 and 7). Sections taken from the liver of silymarin + CCl_4-

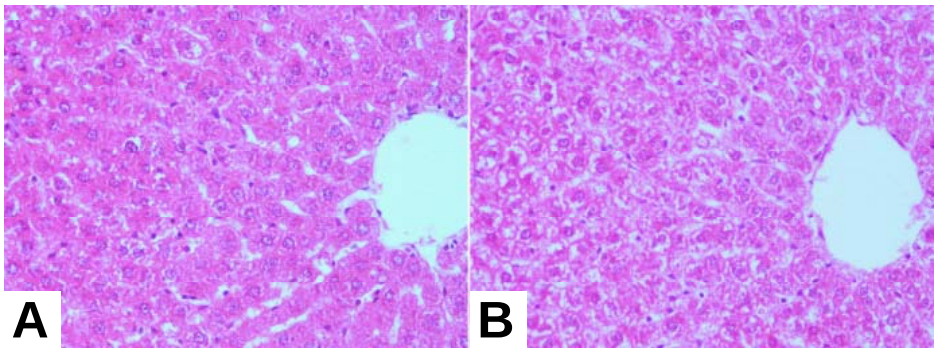

Figure 1. Typical liver tissue sections from rats treated with trazodone (20 mg/kg) (A) and nefazodone (20 mg/kg) (B). Each tissue stained with H&E showed normal hepatic architecture.

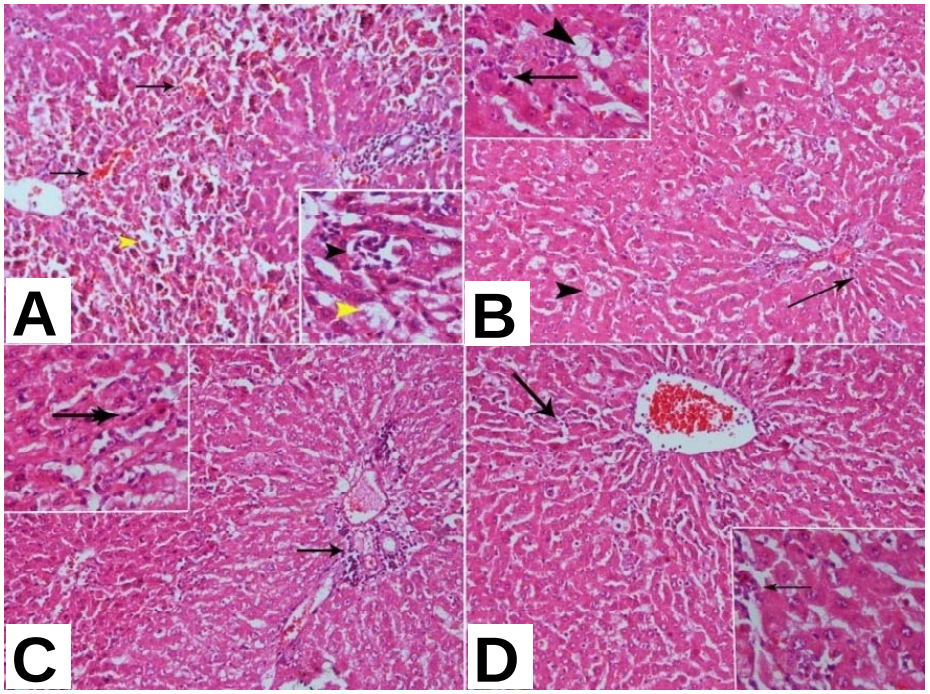

Figure 2. H&E stain of liver tissue sections from rats treated with CCl_4 and various doses of trazodone. (A) section of liver tissue from a rat treated with CCl_4 alone showing complete distortion of the liver architecture, variable degrees of vacuolar degeneration in many of the hepatocytes (yellow arrow head), cellular infiltration (black arrow head), and areas of hemorrhage (arrow) over the hepatic lobule. **(B)** section of liver tissue from a rat given CCl_4 and trazodone (5 mg/kg) showing that the liver tissue architecture was still distorted with mild degree of fibrosis and cellular infiltrate around the main vessels (arrow). The cellular infiltrate was also seen in the blood sinusoids. Many of the hepatocytes showed vacuolar degeneration (arrow head). **(C)** section of liver tissue from a rat treated with CCl_4 and trazodone (10 mg/kg) showing no vacuolar degeneration observed in hepatocytes, while marked cellular infiltrate was still present in the blood sinusoids and around the main blood vessels. **(D)** section of liver tissue from a rat that received CCl_4 and trazodone (20 mg/kg) showing regain of normal architecture of liver tissue, although diffuse cellular infiltrate was still observed in blood sinusoids (arrow). No fibrosis was noticed.

treated rats showed almost normal hepatic architecture (Figures 5-7).

3.3. *Quantitative analysis of the area of damage*

A significant increase in the percentage of damaged areas was observed in CCl_4-treated rats when compared to normal animals. Morphometric analysis of liver sections showed that trazodone or nefazodone administration to CCl_4-treated rats resulted in a dose-dependent decrease in damaged areas compared with the CCl_4 control group (Tables 2 and 3).

3.4. *Quantitative analysis of the DNA content*

Normal distribution of DNA content in the liver of the control group showed that 22.5% of the examined cells contained DNA at a value < 1.5C, 60.0% of the examined cells contained a diploid DNA value of 2C, 16.66% of the examined cells contained a 3C DNA value (medium proliferating index) and 0.83% of the examined cells were at a 4C level. The group subjected to CCl_4 showed that 0.0% of the examined cells contained DNA (< 1.5C), which means a decrease in DNA content (hypoploidy) compared to the control group (Table 4).

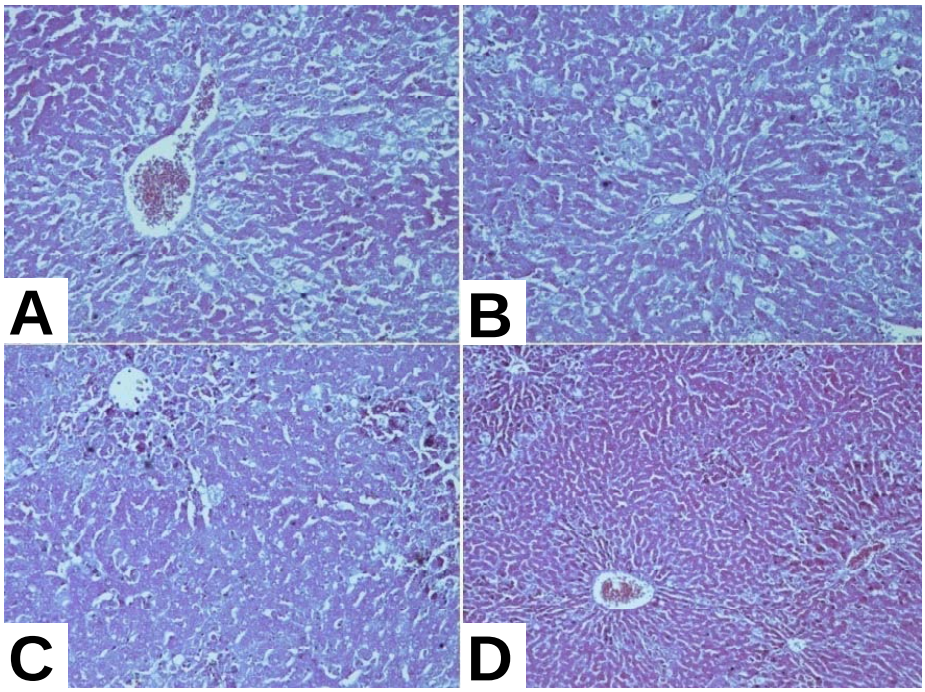

Figure 3. Protein stain of liver tissue sections from rats treated with CCl_4 and various doses of trazodone. (A) section of liver tissue from a rat treated with CCl_4 alone showing marked decrease in protein content of hepatocytes in a patchy manner over the hepatic lobule. **(B)** section of liver tissue from a rat that received CCl_4 and trazodone (5 mg/kg) showing that the protein content of liver tissue was still much less than normal compared with control levels. **(C)** section of liver tissue from a rat that received CCl_4 and trazodone (10 mg/kg) showing improvement of protein content level in hepatocytes, although the areas around the central vein still suffered from protein deficiency. **(D)** section of liver tissue from a rat that received CCl_4 and trazodone (20 mg/kg) showing normalization of protein content level.

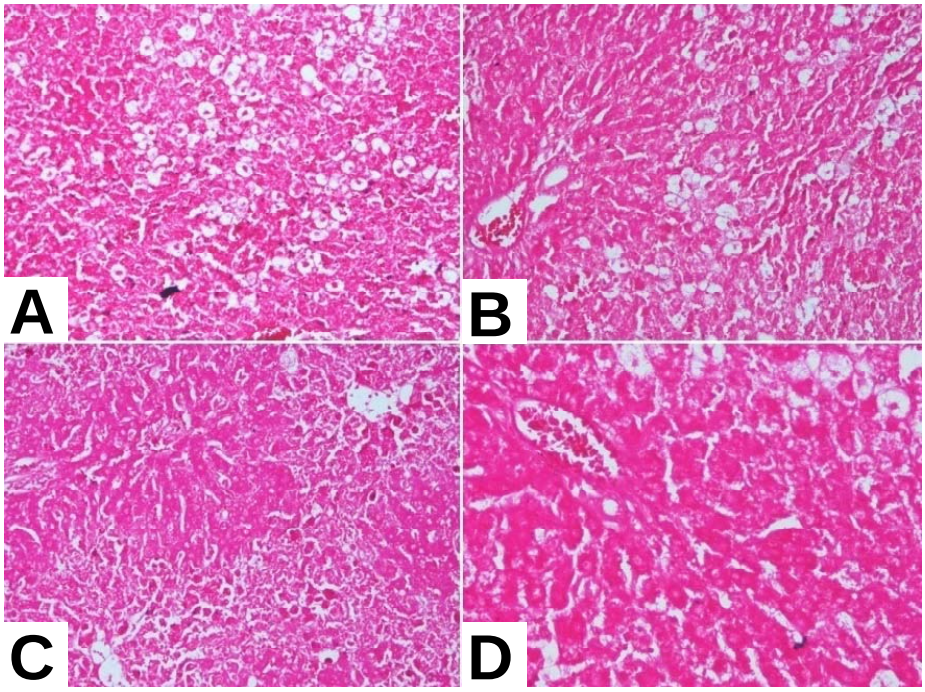

Figure 4. Mucoploysaccharide stain of liver tissue sections from rats treated with CCl_4 and various doses of trazodone. (A) section of liver tissue from a rat treated with CCl_4 alone showing marked decrease in mucopolysaccharide content over the hepatic lobule that appeared completely distorted. **(B)** section of liver tissue from a rat that received CCl_4 and trazodone (5 mg/kg) showing marked decrease in mucopolysaccharide content specially in cells suffering from vacuolar degeneration. **(C)** section of liver tissue from a rat given CCl_4 and trazodone (10 mg/kg) showing mild improvement of mucopolysaccharide content. **(D)** section of liver tissue from a rat treated with CCl_4 and trazodone (20 mg/kg) showing marked increase in mucopolysaccharide content level.

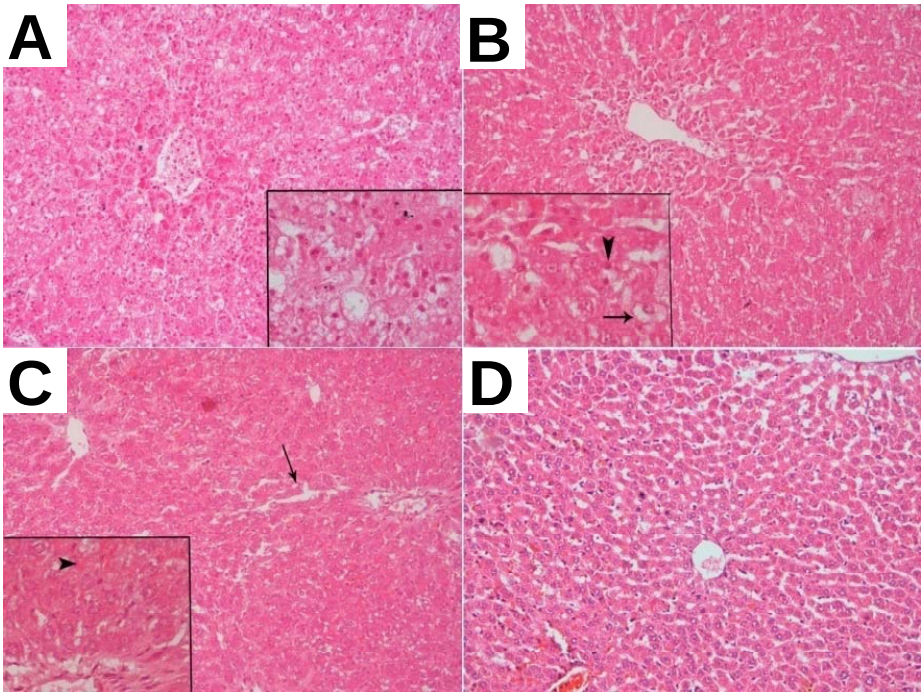

Figure 5. H&E stain of liver tissue sections from rats treated with CCl_4 and various doses of nefazodone. (A) section of liver tissue from a rat treated with CCl_4 and nefazodone (5 mg/kg) showing vacuolar degeneration of variable degrees and pyknotic nuclei in most of the cells. **(B)** section of liver tissue from a rat that received CCl_4 and nefazodone (10 mg/kg) showing noticeable improvement although there was cellular infiltrate around the central vein (arrow head) and vacuolar degeneration in a few cells. **(C)** section of liver tissue from a rat given CCl_4 and nefazodone (20 mg/kg) showing marked improvement of liver tissue except for slight dilatation of some blood sinusoids (arrow) and congestion in others (arrow head). **(D)** section of liver tissue from a rat treated with silymarin showing normal liver tissue architecture.

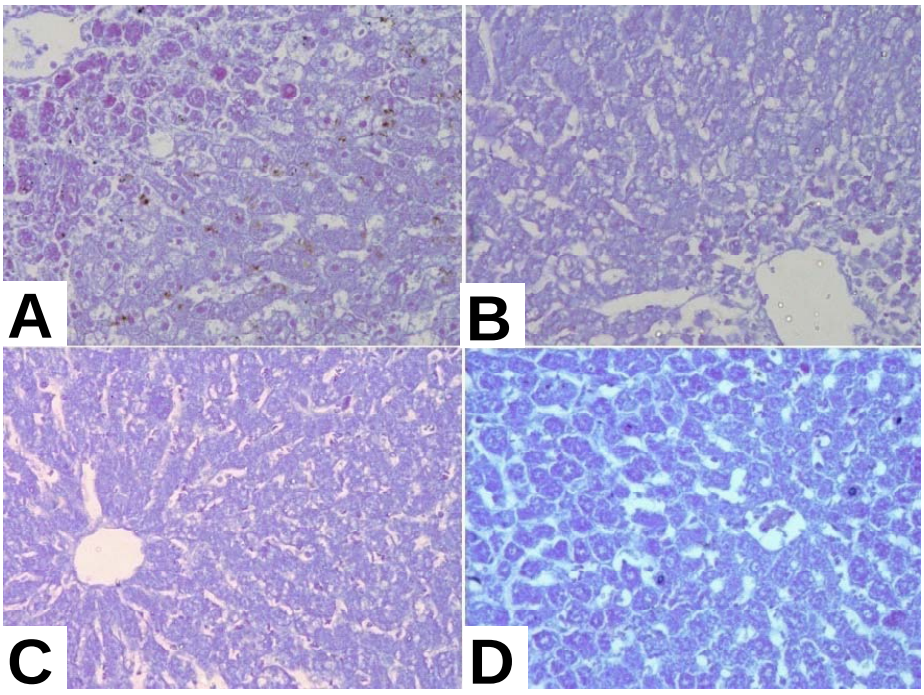

Figure 6. Protein stain of liver tissue sections from rats treated with CCl_4 and various doses of nefazodone. (A) section of liver tissue from a rat treated with CCl_4 and nefazodone (5 mg/kg) showing marked decrease in protein content in most of the hepatocytes mainly due to vacuolar degeneration. **(B)** section of liver tissue from a rat given CCl_4 and nefazodone (10 mg/kg) showing mild increase in protein content. **(C)** section of liver tissue from a rat that received CCl_4 and nefazodone (20 mg/kg) showing marked increase in protein content in most of the hepatocytes. **(D)** section of liver tissue from a rat treated with silymarin showing normal protein content of hepatocytes.

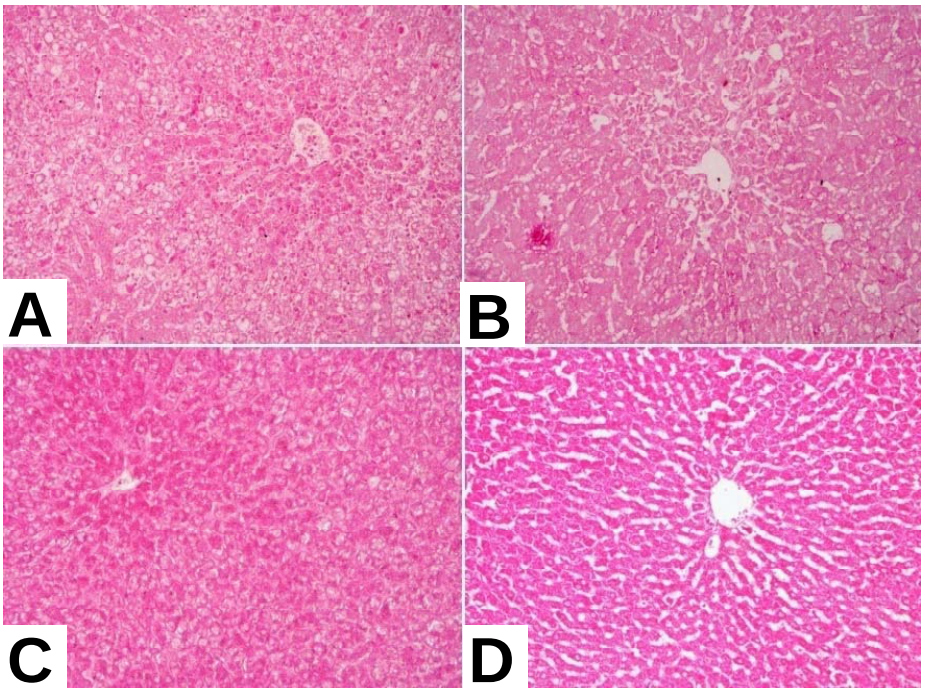

Figure 7. Mucoploysaccharide stain of liver tissue sections from rats treated with CCl_4 and various doses of nefazodone. (A) section of liver tissue from a rat treated with CCl_4 and nefazodone (5 mg/kg) showing that the mucopolysaccharide content in most of the hepatocytes was still very low compared to control levels. **(B)** section of liver tissue from a rat that received CCl_4 and nefazodone (10 mg/kg) showing mild increase in stain density. **(C)** section of liver tissue from a rat treated with CCl_4 and nefazodone (20 mg/kg) showing marked increase in mucopolysaccharide content. **(D)** section of liver tissue from a rat given silymarin showing normal mucopolysaccharide content of hepatocytes.

Table 4. Effect of trazodone and nefazodone on DNA content of hepatocytes (% cells) in carbon tetrachloride-treated rats

Treatment groups	< 1.5C	DNA index	1.5C-2.5C	DNA index	2.5C-3.5C	DNA index	3.5C-4.5C	DNA index	> 4.5C	DNA index
Vehicle	22.5 ± 0.13	0.63	60.0 ± 0.27	0.99	16.67 ± 0.08	1.46	0.83 ± 0.12	1.93	0	0
CCl_4 (control)	0	0	1.96 ± 0.08[a]	1.02	9.80 ± 0.30[a]	1.57	25.5 ± 0.19[a]	2.07	62.74 ± 0.34[a]	2.89
CCl_4 + trazodone 5 mg/kg	1.89 ± 0.15[a,b]	0.06	0[a,b]	0	1.89 ± 0.13[a,b]	1.60	13.21 ± 0.21[a,b]	2.09	83.02 ± 1.45[a,b]	3.34
CCl_4 + trazodone 10 mg/kg	0	0	3.57 ± 0.13[a,b]	1.06	14.27 ± 0.12	1.62	25.0 ± 0.12	2.04	57.14 ± 1.17[a]	3.09
CCl_4 + trazodone 20 mg/kg	0	0	0[a,b]	0	20.75 ± 0.13[b]	1.57	24.53 ± 0.23[a]	2.07	54.72 ± 1.04[a]	3.02
CCl_4 + nefazodone 5 mg/kg	2.9 ± 0.13[a,b]	0.64	1.5 ± 0.01[a]	1.91	7.24 ± 0.19[a]	0.63	11.30 ± 0.23[a,b]	1.32	77.06 ± 0.25[a,b]	0.94
CCl_4 + nefazodone 10 mg/kg	0	0	5.21 ± 0.11[a,b]	1.79	18.75 ± 0.16[b]	1.38	20.0 ± 0.14[a]	0.63	56.04 ± 0.29[a]	0.94
CCl_4 + nefazodone 20 mg/kg	2.2 ± 0.08[a,b]	0.64	0[a,b]	0	26.87 ± 0.15[a,b]	1.40	18.46 ± 0.06[a,b]	1.35	52.47 ± 0.26[a]	1.01

Data are means ± S.E. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's multiple range test. $p < 0.05$ was considered statistically significant. [a] $p < 0.05$ *vs.* vehicle control group. [b] $p < 0.05$ *vs.* CCl_4 control group.

3.4.1. *Effect of trazodone*

Treatment of rats with CCl_4 + trazodone 5 mg/kg showed that 1.88% of the examined hepatocytes contained DNA at < 1.5C, 1.96% of the examined cells contained a diploid DNA value of 2C, 9.8% of the examined cells contained a 3C DNA value (low proliferating index) and 25.49% of the examined cells were in the 4C area. After treatment with trazodone 10 mg/kg, 0.0% of the examined cells contained DNA at < 1.5C, 3.57% contained a diploid DNA value of 2C, 14.28% of the examined cells contained a 3C DNA value (medium proliferating index) and 25% of the examined cells were in the 4C area. Following treatement with trazodone at 20 mg/kg, 0.0% of the examined cells contained DNA at < 1.5C, 0.0% of the examined cells contained a diploid DNA value of 2C, 20.75% of the examined cells contained a 3C DNA value (high proliferating

index) and 24.52% of the examined cells were in the 4C area. These results indicated that treatment with 5, 10, and 20 mg/kg trazodone resulted in a low, medium and high proliferating index, respectively, while the group treated with only CCl_4 showed decreased DNA values (hypoploidy) (Table 4).

3.4.2. *Effect of nefazodone*

After treatment with CCl_4 along with 5 mg/kg nefazodone, 2.9% of the examined hepatocytes contained DNA that was < 1.5C, 1.5% of the examined cells contained diploid DNA value of 2C, 7.24% of the examined cells contained DNA of a 3C value (low proliferating index) and 11.30% of the examined cells were in the 4C area. The group treated with CCl_4 + nefazodone 10 mg/kg showed that 0.0% of the examined cells contained DNA at < 1.5C, 5.21% contained a diploid DNA value of 2C, 18.75% of the examined cells contained a 3C DNA value (medium proliferating index), and 20% of the examined cells were in the 4C area. Following treatment with CCl_4 + nefazodone 20 mg/kg, 2.2% of the examined cells contained DNA at < 1.5C, 0.0% of the examined cells contained diploid DNA value of 2C, 26.87% of the examined cells contained a DNA value of 3C (high proliferating index) and 18.46% of the examined cells were in the 4C area. Thus treatment with nefazodone resulted in a dose-dependent increase in proliferating index in contrast to the CCl_4 control group that exhibited decreased DNA values (hypoploidy) (Table 4).

4. Discussion and Conclusion

The present study provided evidence that in the CCl_4 model of hepatic toxicity, the systemic administration of nefazodone or trazodone, two drugs that are in use in pharmacotherapy of depression, was associated with a decrease in the extent of hepatic damage. In CCl_4-treated rats, the serum levels of hepatocellular enzymes ALT and AST decreased after the administration of either drug in a dose-dependent fashion, which is an indication of improved liver function and preserved liver architecture in the face of the toxic insult to the liver. Plasma levels of alkaline phosphatase, a cell wall enzyme induced by stagnation of bile flow, also showed a marked and significant decrease. Histopathological and histochemical investigations were in agreement with the biochemical findings and indicated that cellular perturbations evoked by the hepatotoxin in the form of decreased protein and mucopolysaccharide content in hepatocytes were ameliorated by either drug. Thus in circumstances of acute toxic insult to the liver, these two drugs did not exacerbate liver injury, but in fact lessened the development of hepatocellular injury. These observations might be unexpected in view of the ability of the two drugs to cause hepatotoxicity in humans, that appeared to be more evident and more serious in the case of nefazodone, leading to its withdrawal from the US market in 2004 (*21*).

Both trazodone and nefazodone enhance serotonin synaptic transmission by blocking postsynaptic 5-HT_2 receptors and by inhibiting the reuptake of 5-HT (*11,12,14-16*). In the brain the increase in serotonergic neuronal activity is thought to have a major role in the alleviation of symptoms of depression (*13*). Most serotonin (95%), however, is found in the gut in the mucosal enterochromaffin cells and within the enteric nervous system, where it is involved in the control of smooth muscle tone and motility (*32*). Serotonin is also stored in platelets and it is this aspect of serotonin which has been implicated in liver regeneration (*9,10*), but also in aggravation of liver injury (*33,34*).

Selective serotonin reuptake inhibitors as well as trazodone significantly reduce the concentration of 5-HT in platelets and inhibit platelet aggregation (*35-38*). This is one mechanism which might underlie the propensity of these drugs to cause gastrointestinal bleeding events alone or especially in combination with NSAIDs and aspirin (*39,40*) and also a mechanism which is likely to explain their cardiovascular benefit (*35*). Since these drugs reduce platelet serotonin, then a possible explanation for their beneficial effect upon experimental liver injury may be by lessening the vasoconstrictor effect of platelet-derived serotonin in the hepatic microcirculation. Studies have shown that serotonin provokes constriction of sinusoids and decreased sinusoidal and central venous perfusion after endoportal application (*41*). Alternatively, the inhibition of the serotonin transporter by these agents (*42*) will likely result in increased extracelllular serotonin. In mucosal preparations of the rat ileum, blockade of the serotonin transporter mechanism by fluoxetine increased both the stimulated serotonin release as well as background levels of 5-HT in the mucosa (*43*). Serotonin might then act in a paracrine way by binding to presynaptic 5-HT_4 receptors to stimulate the release of acetylcholine and calcitonin gene-related peptide release from nerve terminals between the submucosal intrinsic primary afferent neurons and adjacent interneurons (*44*) or to affect hepatic stellate cell functions, the principal cell involved in liver fibrogenesis. Hepatic stellate cells express 5-HT receptors and the serotonin antagonist ketanserine was capable of suppressing the proliferation of hepatic stellate cells as well as increasing their rate of apoptosis (*45*). *In vitro*, serotonin acted as a potent hepatocyte comitogen and induced DNA synthesis in primary cultures of rat hepatocytes (*8*). Serotonin is also involved in liver regeneration induced by estrogen in rats (*10*). Inhibitors of serotonin reuptake might also alleviate hepatic injury by preventing metabolic derangement due to CCl_4. In the present study, histochemical investigations revealed that the decrease in intracellular protein and mucopolysaccharide

content in hepatocytes evoked by CCl_4 was reduced by treatment with trazodone and nefazodone. Indeed, these agents appear to have significant effects on glucose regulation and hepatic glycogen distribution (*46,47*).

Glutathione (GSH) is an intracellular tripeptide (glycyl-glutamic acid-cysteine) common in all tissues, that provides a major antioxidant defense mechanism and which is important in protecting the liver against toxic injury (*48*). In the present study, blood reduced glutathione was significantly decreased in CCl_4-treated rats. A dose-dependent increase in reduced glutathione was registered after treatment with trazodone. It was noted, however, that after treatment with nefazodone even at the highest dose examined *i.e.*, 20 mg/kg, blood glutathione did not show a further decrease beyond that caused by the toxic agent alone. Moreover, blood glutathione was increased by the lower doses of the drug which did not substantially reduce hepatic damage, thereby indicating in this case that the protective effect is unrelated to glutathione levels in blood. This, however, does not exclude the possibility that toxicity reported in many clinical instances with higher doses of the drug (*19*) might be caused by depletion of liver glutathione.

Nitric oxide production can be estimated from determining the concentrations of nitrite and nitrate end products (*27*). Nitric oxide is generated by inflammatory cytokines due to the action of inducible nitric oxide (iNOS) and iNOS overexpression was observed after the administration of CCl_4 (*49,50*). In the present study, nitrite/nitrate concentration in serum was markedly raised in CCl_4-treated rats, in accordance with other studies (*51*). Treatment with trazodone or nefazodone significantly protected against the CCl_4-induced increase in serum nitric oxide level, possibly due to a decrease in the inflammatory response caused by the drugs.

Serotonin antagonists and reuptake inhibitors such as trazodone and nefazodone might also alleviate hepatic injury *via* central mechanisms such as enhanced serotonergic neurotransmission or through vagal-mediated mechanisms. Brain neuropeptides have been shown to modulate liver injury. For example, the intracisternal administration of thyrotropin-releasing hormone stimulated hepatic DNA synthesis (*52*), enhanced liver blood flow (*53*) and inhibited hepatocellular necrosis and the elevation of serum alanine aminotransferase level induced by CCl_4 (*6*). This latter effect was abolished by hepatic branch vagotomy, atropine, and indomethacin and also by the nitric synthase inhibitor N(G)-nitro-L-arginine methyl ester (*6*). In contrast, intracisternal injection of corticotropin releasing factor was found to exacerbate the development of CCl_4-induced acute liver injury. This effect was mediated through the sympathetic-noradrenergic nervous systems (*7*). There is also evidence to suggest that increased serotonin content in cerebral cortex and brain stem induced hepatic proliferation through sympathetic stimulation (*54*). The present study showed that treatment with CCl_4 resulted in 0.0% of the examined cells containing DNA were < 1.5C, which meant a decrease in DNA content (hypoploidy) compared to normal rats. Meanwhile, treatment with either trazodone or nefazodone resulted in a dose-dependent increase in proliferating index in contrast to the CCl_4 control group that exhibited decreased DNA values. In a previous study, it was demonstrated that fluoxetine, a prototype selective serotonin reuptake inhibitor, led to the amelioration of liver injury caused by CCl_4 in the rat (*55*), suggesting a common mechanism(s) for drugs potentiating serotonergic neurotransmission in protecting against liver injury.

Cases of hepatotoxic reactions due to trazodone and nefazodone have been reported in the literature (*17-19*). Hepatotoxicity has also been observed after treatment with monoamine oxidase inhibitors and tricyclic antidepressants and among the serotonin reuptake inhibitors, paroxetine has the largest number of cases of hepatotoxicity recorded (*56,57*). In other instances *e.g.* in alcohol-dependent subjects (*58*) or in the case of chronic hepatitis C with interferon/ribavirin therapy (*2*), nefazodone was not associated with hepatotoxicity. In the past few years, there have been attempts at explaining nefazodone hepatotoxicity. *In vitro*, nefazodone (and not trazodone) inhibited mitochondrial respiration in isolated rat liver mitochondria and in intact HepG2 cells. The target was mitochondrial complex I (*59*). Inhibition of bile acid transport may be another mechanism of nefazodone-induced hepatotoxic reactions, an effect which is not shared by trazodone (*60*). It has been suggested that the low oral bioavailability of nefazodone of ~ 20% and short half-life of ~ 1 h due to extensive first pass metabolism mediated by P4503A4 in the small intestine and liver and the resultant high daily dose (200-400 mg/day) increase the total body burden to reactive metabolite exposure which might exceed a threshold needed to cause toxicity (*61*). The optimum therapeutic dosage of nefazodone appears to be between 300 and 600 mg/day (*14*) and doses in the range of 200-600 mg/day were associated with acute hepatic reactions (*19*). Liver toxicity with markedly elevated serum transaminases have also been observed following a dose of 150 mg nefazodone twice daily for 20 weeks in an alcoholic patient. Symptoms resolved completely after discontinuation of nefazodone (*18*). Nefazodone is extensively metabolized in the liver and plasma concentrations of the drug are increased in severe hepatic impairment and in the elderly, especially in elderly females (*15*), which requires lowering the dose in patients with liver disease. Notably, in most instances of nefazodone or trazodone hepatotoxicity, drugs were administered for a prolonged time (6-12 months)

before the onset of symptoms (*17-20*). The present study, however, examined only the short term effect of trazodone or nefazodone administration.

Trazodone and nefazodone undergo bioactivation in human liver microsomes to a reactive quinone-imine and an epoxide intermediate, and these react with glutathione, which might represent a rate-limiting step in the initiation of trazodone- or nefazodone-mediated hepatotoxicity (*62,63*). Toxicity due to nefazodone or trazodone can thus be interpreted in terms of as an imbalance between a detrimental affect of the drug itself on hepatocytes and the beneficial effect of increased serotonin levels in the periphery and centrally. This is due to overwhelming metabolites and exceeding the capacity of the liver to deal with *e.g.* an increased rate of liver regeneration. With lower doses the beneficial effects maintaining hepatic integrity and promoting regeneration are likely to prevail.

In summary, the present study has shown that both nefazodone and trazodone, two drugs which block postsynaptic 5-HT_2 receptors and inhibit the reuptake of 5-HT, lessened hepatocellular injury in rats caused by administration of CCl_4. The involvement of enhanced central serotonergic neurotransmission or peripheral serotonergic mediated mechanisms is suggested.

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