

# The effect of antidepressant drugs on thioacetamide-induced oxidative stress

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**Abstract.** – **OBJECTIVE:** The aim of the present study was to investigate the effect of the serotonin selective reuptake inhibitors (SSRIs) fluoxetine and sertraline and the tricyclic drug imipramine on oxidative stress in the brain and liver caused by thioacetamide in rats.

**MATERIALS AND METHODS:** Drugs were administered orally once daily at doses of 10 and 20 mg/kg for two weeks prior to intraperitoneal injection of thioacetamide (300 mg/kg). Rats were euthanized 24 h after thioacetamide. Reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide were measured in brain and liver. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured in serum and histopathological evaluation of liver injury was performed.

**RESULTS:** The administration of thioacetamide increased MDA by 151.8% and 161.2%, increased nitric oxide by 57.2% and 63.9% and decreased GSH by -40.6% and -67% in the brain and liver, respectively. Thioacetamide markedly increased serum ALT, AST and ALP by 277.8, 80.8 and 121%, respectively. In the brain, MDA was decreased in rats treated with fluoxetine or sertraline. The level of GSH increased by fluoxetine and by the higher dose of sertraline. Nitric oxide in brain was unchanged by fluoxetine, but increased after sertraline at 20 mg/kg. Brain MDA was increased by imipramine, which also decreased brain nitrite level. In the liver, fluoxetine or sertraline treatment increased GSH and nitrite levels. MDA was also increased by either drug. The drugs markedly decreased ALT, but increased ALP in serum. Meanwhile, imipramine decreased liver nitric oxide levels (at the lower dose only -32.9%), markedly increased hepatic GSH, but did not change MDA level. Serum ALT decreased by imipramine (but AST and ALP showed no change). Histopathological and histochemical examinations indicated that thioacetamide-induced liver injury was not decreased after treatment with the antidepressant drugs.

**CONCLUSIONS:** In thioacetamide-treated rats, pretreatment with the SSRIs drugs fluoxetine and sertraline is associated with decreased lipid peroxidation in brain; liver peroxidation, howev-

er, is increased. Imipramine displayed opposite effects. The thioacetamide-induced hepatic damage was not reduced by fluoxetine, sertraline or imipramine.

*Key Words:*

Thioacetamide, Brain, Liver, Antidepressant drugs, Oxidative stress, Rat.

## Introduction

Depression is common among patients with chronic liver diseases especially those caused by hepatitis C virus infection or cholestatic liver disorders<sup>1,2</sup>. It can also complicate antiviral therapy with interferon alpha or interferon-ribavirin combination<sup>1,3-5</sup>. The last few years have witnessed the introduction of several newer antidepressant drugs that are better tolerated than the classic agents such as the tricyclic antidepressants or the monoamine oxidase inhibitors. In this context, the advent of the selective serotonin reuptake inhibitors (SSRIs) has clearly improved therapy of depressive disorders. These agents have largely replaced the tricyclic antidepressants in terms of efficacy as well as their more favorable side effects pattern<sup>6</sup>. In the context of liver disease, depression presents a special clinical problem for many antidepressant drugs are metabolized in the liver and toxicity is then a concern. There were also reports of hepatotoxicity associated with the use of some of these agents (e.g., sertraline, paroxetine, and nefazodone) in patients with no preexisting liver disease<sup>7,8</sup>.

The effect of antidepressant drugs on liver integrity and on progression of liver disease is largely unknown, although experimental evidence suggests a beneficial effect of a number of antidepressant drugs in reducing hepatic injury<sup>9,10</sup>. In humans, these agents appear to be

well tolerated and generally safe in patients with liver disease<sup>1,3,4,11</sup> and there were few studies in which sertraline had been used with success in patients with cholestatic liver disease to decrease pruritus, a troublesome symptom in these disorders<sup>12,13</sup>.

The SSRIs act by inhibiting the reuptake of serotonin, thereby increasing its concentration in the synaptic cleft. The net result is augmenting serotonergic neurotransmission in areas of brain, which is largely believed to account for their mood enhancing effects<sup>6</sup>. This fact is important in liver diseases; since there is evidence that altered central serotonergic neurotransmission contributes to fatigue complicating chronic hepatitis C infection<sup>14</sup>. Serum tryptophan concentrations were reduced in hepatitis C virus-infected patients compared with healthy volunteers or hepatitis B virus-infected patients with comparable liver damage<sup>2</sup>. Moreover, hepatitis C virus-infected patients have reduced serum tryptophan that returned towards normal levels after successful antiviral treatment<sup>15</sup>. The effect of SSRIs is important especially in the context of the hepatic encephalopathy, a serious neuropsychiatric manifestation of both acute and chronic hepatic insufficiency, associated with alterations in brain ammonia and neurotransmitter levels<sup>16-19</sup>. It has been suggested that therapeutic approaches aiming at the normalization of serotonin turnover could be beneficial in the prevention and treatment of early neuropsychiatric symptoms of hepatic encephalopathy<sup>19,20</sup>.

Oxidative stress is important in the development and progression of liver disease and the use of antioxidants is an established treatment modality in this condition<sup>21,22,23</sup>. Oxidative stress has been implicated in the pathogenesis of hepatic encephalopathy<sup>24</sup>. Studies have shown that a number of antidepressant drugs inhibit glial activation-mediated oxidative stress<sup>25,26</sup>. The SSRI fluoxetine suppressed microglial NADPH oxidase activation and decreased reactive oxygen species generation and oxidative stress caused by endotoxin administration<sup>25</sup>. Escitalopram, another SSRI, decreased microglia activation and oxidative stress after transient cerebral ischaemia<sup>26</sup>.

It, therefore, looked pertinent to examine the effect of antidepressant drug administration in the presence of liver damage induced by the administration of the hepatotoxic agent thioacetamide. The administration of thioacetamide has been widely used to cause acute liver failure and encephalopathy<sup>27</sup> and also for inducing hepatic

fibrosis when applied for prolonged time at lower doses<sup>28</sup>. The aim of this study was to: (1) evaluate the effect of thioacetamide on oxidative stress in the brain and liver in rats; (2) investigate the possible modulatory effect of some antidepressant drugs on the neurotoxic and hepatotoxic effects of thioacetamide. Compounds studied included the SSRIs fluoxetine and sertraline as well as the tertiary amine tricyclic antidepressant imipramine.

## Materials and Methods

### 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 doses of drugs employed were based upon the human dose after conversion to that of rat according to Paget and Barnes<sup>29</sup> conversion tables. The dose of thioacetamide was selected based on other studies<sup>27</sup>.

### Drugs and Chemicals

The following drugs were used: thioacetamide (Sigma, St Louis, MO, USA), fluoxetine hydrochloride (Amoun Pharmaceutical Co., Cairo, A.R.E.), sertraline hydrochloride (Pfizer Egypt, Cairo, A.R.E.) and imipramine hydrochloride (Novartis Pharma, Cairo, A.R.E.). All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use.

### Study Design

Rats were divided into 8 equal groups (6 rats each). Rats were treated with fluoxetine (10, 20 mg/kg) (group 1, 2), sertraline (10, 20 mg/kg) (group 3, 4) and imipramine (10, 20 mg/kg) (groups 5, 6) or saline (group 7, control) once daily orally for 2 weeks before the administration of thioacetamide (300 mg/kg, i.p.). In addition, an eighth group of rats (n=6) received only saline (-ve control). Rats were killed 24h after thioacetamide by decapitation under ether anaesthesia. Rats had free access to food and drinking water during the study. At the end of the experiments, blood samples were obtained from the retro-or-

bital vein plexuses, under ether anaesthesia. Rats were euthanized by decapitation, the brains and livers were then removed, washed with ice-cold saline solution (0.9% NaCl) and stored at  $-80^{\circ}\text{C}$  for further determination of biochemical parameters. They were homogenized with 0.1 M phosphate buffered saline (PBS) at pH 7.4, to give a final concentration of 10% w/v for the biochemical assays.

## Biochemical Assessment

### *Determination of Lipid Peroxidation*

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the brain tissues. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea et al<sup>30</sup>, in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm which was measured using UV-VI8 Recording Spectrophotometer (Shimadzu Corporation, Australia).

### *Determination of Reduced Glutathione*

Reduced glutathione (GSH) was determined by Ellman's method<sup>31</sup>. The procedure is based on the reduction of Ellman's reagent by  $-\text{SH}$  groups of GSH to form 2-nitro-s-mercaptobenzoic acid, the nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically. A mixture was directly prepared in a cuvette: 2.25 ml of 0.1 M K-phosphate buffer, pH 8.0; 0.2 ml of the sample; 25  $\mu\text{l}$  of Ellman's reagent (10 mM 5,5'-dithio-bis-2-nitrobenzoic acid in methanol). After 1 min the absorbance was measured at 412 nm and the GSH concentration was calculated by comparison with a standard curve.

### *Determination of Nitric Oxide Level*

Serum nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage et al<sup>32</sup>, where nitrite, stable end product of nitric oxide radical, is mostly used as indicator for the production of nitric oxide.

### *Determination of Serum Liver Enzymes*

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum were measured according to Reitman-Frankel colorimetric transaminase procedure<sup>33</sup>, whereas colorimetric determination of alkaline phosphatase (ALP) activity was done according to the method of Belfield and Goldberg<sup>34</sup>, using commercially available kits (BioMérieux, Marcy l'Etoile, Craponne, France).

metric transaminase procedure<sup>33</sup>, whereas colorimetric determination of alkaline phosphatase (ALP) activity was done according to the method of Belfield and Goldberg<sup>34</sup>, using commercially available kits (BioMérieux, Marcy l'Etoile, Craponne, France).

### *Histological and Histochemical Studies*

At the end of the treatment period, rats were euthanized by decapitation under ether anaesthesia, livers excised, and specimens then fixed in 10% neutral-buffered formal saline for 72 hours. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Other specimens were fixed in Carnoy's fixative and embedded in paraffin. Five  $\mu\text{m}$  thick sections were stained with hematoxylin-eosin and observed under a microscope for histopathological examination. Periodic acid-Schiff (PAS) method was used for glycogen staining. All sections were investigated by light microscope.

### *Statistical Analysis*

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

## Results

### Brain Parameters

#### *Lipid Peroxidation*

The administration of thioacetamide at a single dose of 300 mg/kg induced a significant elevation of brain MDA levels by 151.8% ( $98.2 \pm 4.1$  vs  $39.0 \pm 3.6$  nmol/g.tissue;  $p < 0.05$ ). Fluoxetine given at 10 mg/kg prior to thioacetamide, decreased brain MDA by 25.2% ( $p < 0.05$ ). The higher dose decreased MDA by-16.5%. The effect of fluoxetine at the highest dose was, however, not significant. Sertraline administered at 10 or 20 mg/kg decreased MDA by 20.4 and 18.7%, respectively ( $p < 0.05$ ). Brain MDA was significantly increased by 17.9-84.2% after treatment with imipramine at 10 and 20 mg/kg ( $p < 0.05$ ) (Table I).

#### *Nitric Oxide*

Thioacetamide administration significantly increased brain nitric oxide level by 57.2% ( $39.3$

**Table I.** Effect of fluoxetine, sertraline or imipramine on brain malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione in thioacetamide-treated rats.

GSH	MDA (nmol/g. tissue)	NO ( $\mu\text{mol.g. tissue}$ )	GSH ( $\mu\text{mol.g. tissue}$ )
Saline	39.0 $\pm$ 3.6	25.0 $\pm$ 0.4	3.40 $\pm$ 2.8
Thioacetamide	98.2 $\pm$ 4.1*	39.3 $\pm$ 1.2*	2.02 $\pm$ 2.2*
+ Fluoxetine 10 mg/kg	73.4 $\pm$ 2.6**	34.0 $\pm$ 1.6*	2.58 $\pm$ 1.5**
+ Fluoxetine 20 mg/kg	82.0 $\pm$ 1.8*	35.75 $\pm$ 2.9*	2.71 $\pm$ 2.1**
+ Sertraline 10 mg/kg	78.2 $\pm$ 5.3**	35.0 $\pm$ 2.6*	2.22 $\pm$ 1.1*
+ Sertraline 20 mg/kg	79.8 $\pm$ 6.1**	49.0 $\pm$ 1.2*##	3.58 $\pm$ 2.7**
+ Imipramine 10 mg/kg	115.8 $\pm$ 8.1**	27.7 $\pm$ 2.1 <sup>+</sup>	2.15 $\pm$ 1.3*
+ Imipramine 20 mg/kg	180.9 $\pm$ 12.8**	30.5 $\pm$ 2.3**	2.22 $\pm$ 2.1*

Results are mean  $\pm$  S.E. Six rats were used per each group. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's test.  $p < 0.05$  was considered statistically significant. \* $p < 0.05$  vs saline control group. <sup>+</sup> $p < 0.05$  vs the thioacetamide only group. ##Denotes significant difference between the two doses of the same drug.

$\pm 1.2$  vs  $25.0 \pm 0.4 \mu\text{mol/g}$ ,  $p < 0.05$ ). In thioacetamide-treated rats, nitric oxide level was unchanged by fluoxetine or by the lower dose of sertraline. However, the level of nitric oxide was increased by 24.7% ( $p < 0.05$ ) by sertraline at the dose of 20 mg/kg. The level of nitric oxide was significantly increased by 29.5 and 22.4% after the administration of imipramine at doses of 10 and 20 mg/kg, respectively, compared with the thioacetamide control group (Table I).

### Reduced Glutathione

Significant decrease in the level of GSH by 40.6% was observed after thioacetamide treatment ( $2.02 \pm 2.2$  vs  $3.40 \pm 2.8 \mu\text{mol/g}$ ,  $p < 0.05$ ). The level of GSH was significantly increased in brain by 27.7 and 34.2% by fluoxetine at doses of 10 and 20 mg/kg, respectively ( $p < 0.05$ ). The effect of sertraline at the lower dose of 10 mg/kg was not significant. However, the level of GSH was increased by 77.2% ( $p < 0.05$ ) by sertraline at the dose of 20 mg/kg. The administration of imipramine had no significant effect on brain GSH (Table I).

### Liver Parameters

#### Lipid Peroxidation

The level of MDA was significantly increased by 161.2% by the administration of thioacetamide ( $156.7 \pm 12.5$  vs  $60.0 \pm 5.4 \text{ nmol/g. tissue}$ ;  $p < 0.05$ ). Fluoxetine given at doses of 10 and 20 mg/kg prior to thioacetamide, resulted in further increase in MDA by 59.9 and 28.4% ( $p < 0.05$ ), respectively. Significant increase in MDA

level by 30.6 and 25.4% ( $p < 0.05$ ) was observed after sertraline administration at doses of 10 and 20 mg/kg, respectively. Meanwhile, the administration of imipramine did not significantly alter liver MDA (Table II).

#### Nitric Oxide

In the liver, thioacetamide administration significantly increased nitric oxide by 63.9% ( $29.5 \pm 2.0$  vs  $18.0 \pm 1.2 \mu\text{mol/g}$ ,  $p < 0.05$ ). Nitric oxide level was significantly increased by 82 and 34.2% by fluoxetine at doses of 10 and 20 mg/kg and by 41.7 and 32.2% by sertraline at doses of 10 and 20 mg/kg, respectively. However, the level of nitric oxide was decreased by 32.9% ( $p < 0.05$ ) by the lower dose of imipramine. The effect of imipramine at the highest dose of 20 mg/kg was not significant (Table II).

#### Reduced Glutathione

The level of GSH was reduced by 66.9% after thioacetamide treatment ( $3.83 \pm 0.2$  vs  $11.60 \pm 0.8 \mu\text{mol/g}$ ,  $p < 0.05$ ). Reduced glutathione was significantly increased by 34.2 and 242.8% by fluoxetine at doses of 10 and 20 mg/kg and by 201.8 and 423.5% by sertraline at doses of 10 and 20 mg/kg, respectively ( $p < 0.05$ ). The administration of imipramine at doses of 10 and 20 mg/kg, also significantly increased liver GSH by 41 and 447.8%, respectively (Table II).

#### Serum Liver Enzymes

The administration of thioacetamide resulted in significant increase in serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase by 227.8, 80.9 and 121%, re-



**Table II.** Effect of fluoxetine, sertraline or imipramine on liver malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione in thioacetamide-treated rats.

	MDA (nmol/g. tissue)	NO (µmol.g. tissue)	GSH (µmol.g. tissue)
Saline	60.0 ± 5.4	18.0 ± 2.2	11.60 ± 0.8
Thioacetamide	156.7 ± 12.5*	29.5 ± 2.0	3.83 ± 0.2*
+ Fluoxetine 10 mg/kg	250.6 ± 13.6**	53.7 ± 3.6**	5.14 ± 0.4**
+ Fluoxetine 20 mg/kg	201.3 ± 13.0**	39.6 ± 3.0**#	13.13 ± 1.1**
+ Sertraline 10 mg/kg	204.6 ± 10.9**	41.8 ± 2.3**	11.56 ± 0.9 <sup>+</sup>
+ Sertraline 20 mg/kg	196.5 ± 3.5**	39.0 ± 2.1**	20.05 ± 1.6**#
+ Imipramine 10 mg/kg	134.8 ± 3.9*	19.8 ± 1.2 <sup>+</sup>	5.40 ± 0.3**
+ Imipramine 20 mg/kg	178.3 ± 13.8**	33.5 ± 2.0**	20.98 ± 1.8**#

Results are mean ± S.E. Six rats were used per each group. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's test.  $p < 0.05$  was considered statistically significant. \* $p < 0.05$  vs saline control group. <sup>+</sup> $p < 0.05$  vs the thioacetamide only group. #Denotes significant difference between the two doses of the same drug.

spectively. Serum ALT was significantly decreased by fluoxetine (by 35.7 and 58.8%), sertraline (by 62.5 and 76.5%) as well as imipramine (by 37.1 and 51.5%) given at doses of 10 and 20 mg/kg, respectively. Serum AST was unchanged by antidepressant drugs, but serum alkaline phosphatase was significantly increased by fluoxetine (by 73 and 51.6%) and sertraline (by 33.8-40.4%) given at doses of 10 and 20 mg/kg, respectively (Table III).

### Histopathological Results

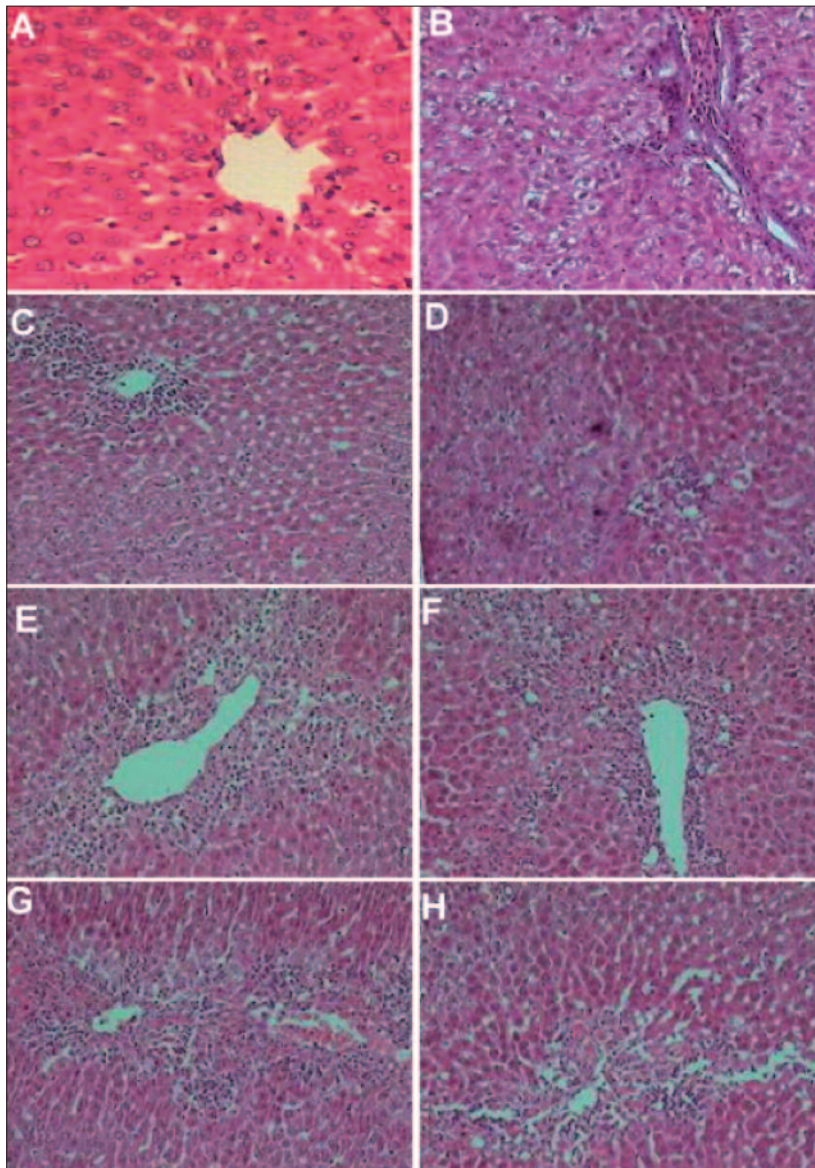
Examination of control liver sections revealed normal hepatic architecture with cords of hepatocytes radiating from the central vein. Each cell exhibited a round vesicular, centrally located nucleus. Some binucleated cells were also seen. Hepatic sinusoids are located in between cell

cord (Figure 1A). Liver sections from rats given thioacetamide only showed loss of hepatic architecture. Hydropic degeneration of the hepatocytes, inflammatory cell infiltration in the portal area and thickness of the interlobular septum were seen (Figure 1B). Sections of liver of rat given fluoxetine at 10 mg/kg showed necrosis of hepatocytes and inflammatory cells infiltration, vacuoles and hydropic degeneration, while many of the hepatocytes appeared normal (Figure 1C). Sections from rats given fluoxetine at 20 mg/kg showed some focal necrosis of hepatocytes only (Figure 1D). Sections from rats treated with sertraline at 10 mg/kg showed inflammatory cells progressively extending from the portal and periportal areas to the parenchyma, with necrosis of hepatocytes (Figure 1E). Sections from rats given sertraline at 20 mg/kg showed focal necrosis of

**Table III.** Effect of fluoxetine, sertraline or imipramine on serum alanine aminotransferase (ALP), aspartate aminotransferase (AST), alkaline phosphatase (ALP), in thioacetamide-treated rats.

	ALT (U/L)	AST (U/L)	ALP (U/L)
Saline	18.0 ± 1.3	52.0 ± 4.0	149.0 ± 10.0
Thioacetamide	68.0 ± 4.0*	94.0 ± 5.0*	329.3 ± 18.9*
+ Fluoxetine 10 mg/kg	43.7 ± 3.9**	92.5 ± 6.6*	569.7 ± 27.0*
+ Fluoxetine 20 mg/kg	28.0 ± 2.9**	94.0 ± 5.8*	499.3 ± 16.8*
+ Sertraline 10 mg/kg	25.5 ± 2.2**	89.8 ± 7.2*	440.8 ± 22.1*
+ Sertraline 20 mg/kg	16.0 ± 1.2**	91.8 ± 10.1*	462.5 ± 18.9*
+ Imipramine 10 mg/kg	42.8 ± 3.0**	98.6 ± 9.6*	338.4 ± 22*
+ Imipramine 20 mg/kg	33.0 ± 2.2**	93.0 ± 7.9*	376.7 ± 24*

Results are mean ± S.E. Six rats were used per each group. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's test.  $p < 0.05$  was considered statistically significant. \* $p < 0.05$  vs saline control group. <sup>+</sup> $p < 0.05$  vs the thioacetamide only group. #Denotes significant difference between the two doses of the same drug.



**Figure 1.** Effect of fluoxetine, sertraline or imipramine on hepatic damage induced by thioacetamide. *A*, Saline-treated rat: normal hepatic architecture. *B*, Thioacetamide only-treated rat showing architectural loss, hydropic degeneration of the hepatocytes, inflammatory cell infiltration in the portal area and thickness of the interlobular septum. *C*, Fluoxetine at 10 mg/kg before thioacetamide administration: necrosis of hepatocytes, inflammatory cells, vacuoles and hydropic degeneration in the hepatic lobule. *D*, Fluoxetine at 20 mg/kg: focal necrosis of hepatocytes. *E*, *F*, Sertraline at 10 or 20 mg/kg before thioacetamide: inflammatory cells extending from the portal and periportal areas to the parenchyma with necrosis of hepatocytes. *G*, *H*, Imipramine at 10 or 20 mg/kg prior to thioacetamide: slight improvement compared to that treated with thioacetamide alone. Focal necrosis of hepatocytes associated with inflammatory cells and vacuoles in the hepatic lobule are still seen (H & E  $\times$  300).

hepatocytes in the periportal area associated with inflammatory cells and vacuolar degeneration in hepatic lobule (Figure 1F). Sections of livers from rats given imipramine exhibited slight improvement compared to that treated with thioacetamide alone. The effect of drug was dose dependent. Focal necrosis of hepatocytes associated with inflammatory cell infiltration and some vacuoles were still present, however, in hepatic lobules (Figure 1 G & H).

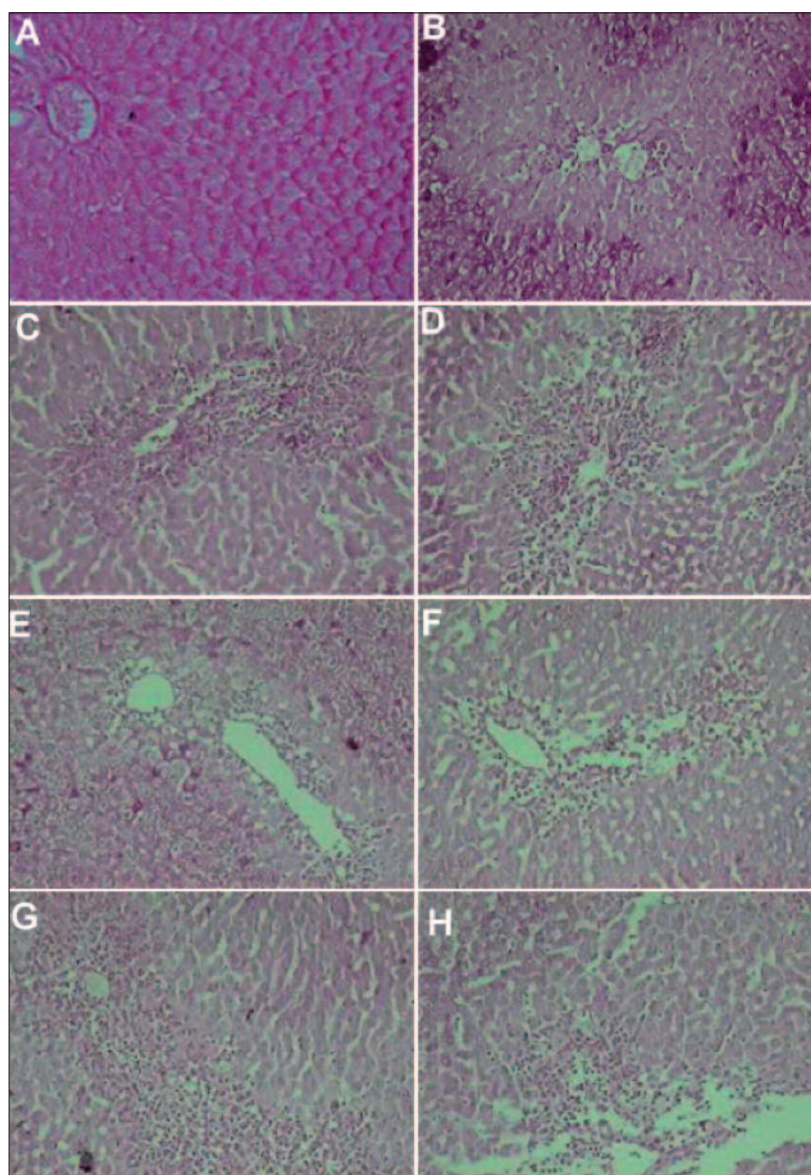
#### **Histochemical Results**

Figure 2A shows normal distribution of glycogen in the hepatocytes (PAS stain). The glycogen particles accumulated at one side of the cyto-

plasm. Examination of liver sections of rats treated with thioacetamide only (Figure 2B) showed heterogeneous stainability of glycogen with the healthy hepatocytes exhibiting strong reaction while the necrotic hepatocytes showing depletion of glycogen. Sections from rats pretreated with fluoxetine showed strong reaction in the normal hepatocytes, faint stainability in some hepatocytes while the necrotic cells were devoid of stainable materials (Figure 2C & D). Histochemical examination of liver from rats given sertraline showed strong reaction in the normal hepatocytes, faint stainability in some hepatocytes while the necrotic cells were devoid of stainable materials (Figure 2 E & F). Sections of liver of



**Figure 2.** Hepatic glycogen distribution (Periodic acid-Schiff's stain). *A*, Normal distribution of Periodic acid-Schiff's reaction in liver cells; the glycogen particles being accumulated at one side of the cytoplasm. *B*, Liver section from rats treated with only thioacetamide showing heterogeneous stainability; healthy hepatocytes exhibiting strong staining and the necrotic cells are devoid of glycogen materials. *C, D*, Liver tissue from rats treated with sertraline at 10 and 20 mg/kg prior to thioacetamide. *E, F*, Liver tissue from rats treated with fluoxetine at 10 and 20 mg/kg prior to thioacetamide. *G, H*, Liver tissue from rats treated with imipramine at 10 and 20 mg/kg prior to thioacetamide (Periodic acid-Schiff  $\times 300$ ).



rats given imipramine showed faint stainability and the necrotic cells were devoid of stainable glycogen materials (Figure 2 G & H).

## Discussion

This study demonstrates an increased oxidative stress in the brain and liver after the administration of a single dose of thioacetamide. Malondialdehyde (MDA) an index of lipid peroxidation activity<sup>35</sup>, is increased and there was significant depletion of reduced glutathione. Thioacetamide administration resulted also in

increased nitric oxide in brain and liver. Thioacetamide evoked liver tissue damage evidenced by increased serum aminotransferases and alkaline phosphatase levels as well as histological damage. The research also indicated that pretreatment with the antidepressant drugs fluoxetine, sertraline and imipramine was capable of differently modulating oxidative stress in both brain and liver.

The findings in the present investigation derives its importance from the evidence that oxidative stress has an important role in development and progression of chronic liver disease<sup>21-23</sup> and in the pathogenesis of hepatic encephalopathy<sup>24</sup>. By modulating oxidative stress, antide-

pressant medications might thus affect the progression and development of several hepatic pathologies.

Changes in brain antioxidant status have been observed following the administration of antidepressant drugs in a number of studies. Fluoxetine has been shown to suppress microglial NADPH oxidase activation and decrease reactive oxygen species generation and oxidative stress caused by endotoxin administration<sup>25</sup>. Escitalopram, another SSRI, decreased microglia activation and oxidative stress in the gerbil hippocampal CA1 region after transient cerebral ischaemia<sup>26</sup>. *In vitro*, imipramine increased mRNA levels of gamma-glutamyl-cysteine synthetase, glutathione-S-transferase and glutathione reductase as well as Cu, Zn superoxide dismutase<sup>36</sup>. *In vivo*, long-term treatment with fluoxetine at the dose of 10 mg/kg, increased GSH in mice hippocampus<sup>37</sup>. In the present work, lipid peroxidation was decreased by the administration of fluoxetine (especially the lower dose) or sertraline, but increased after the tricyclic drug imipramine. Conversely, in the liver, MDA was increased by both fluoxetine and sertraline, but decreased by the lower dose of imipramine. Reduced glutathione showed marked increase in the brain and liver by both fluoxetine and sertraline and in the liver by imipramine. This may be quite significant since GSH has been shown to be reduced in red blood cells of patients with chronic liver disease compared with the controls<sup>38</sup>. GSH is also reduced in the brain in a number of neurodegenerative diseases possibly due to consumption by free radicals<sup>39</sup>. An increasing GSH availability in brain has recently been utilized as an adjunctive treatment in schizophrenia<sup>40</sup>. This might suggest that these drugs can be administered for the treatment of depression in patients with liver disease.

The present study indicated increased nitric oxide in the brain and liver after treatment with thioacetamide. Nitric oxide is an important signaling molecule involved in neurotransmission and in blood flow regulation. Increased nitric oxide production can occur in response to inflammatory cytokines due to the action of inducible nitric oxide synthase (iNOS)<sup>41</sup>. The excessive production of nitric oxide can be deleterious to tissue function because of the ability of nitric oxide to react with biomolecules or with other free radicals e.g., superoxide anion, yielding the highly reactive peroxy nitrite radical capable of evoking the oxidation of important cel-

lular biomolecules<sup>42,43</sup>. The increase in nitric oxide and consequent vasodilatation can benefit tissue function against toxic insults. However, another consequence of the elevated levels of nitric oxide is its cellular toxicity which can cause lipid peroxidation. Studies have implicated increased nitric oxide levels in depression and in the mood elevating action of antidepressant drugs<sup>44-46</sup>. Nitric oxide is also increased in serum of patients with liver cirrhosis<sup>47,48</sup> and correlated with the severity of the disease<sup>48</sup>. In this report, nitric oxide is increased in the brain by sertraline and in the liver by fluoxetine or sertraline. Conversely, it was observed that nitric oxide is decreased in both brain and liver after imipramine treatment. Studies demonstrated variable effects for antidepressants on brain nitric oxide. There has been decreased nitric oxide concentration and nitric oxide synthase activity in brain by imipramine<sup>46,49,50</sup>. Fluoxetine has been reported to have no effect on brain nitric oxide<sup>46</sup>, to increase mRNA expression of iNOS (NOS2)<sup>51</sup> or to inhibit endotoxin-induced release of nitric oxide<sup>50</sup>. Meanwhile, sertraline inhibited the generation of nitric oxide from INF- $\gamma$  activated microglia<sup>52</sup>. Whether these reflect different effects on neurotransmitter release is not clear. The SSRIs share the common property of inhibiting the reuptake of serotonin at synaptic terminals<sup>53</sup>. There is also an evidence suggesting inhibition of noradrenaline and dopamine reuptake by fluoxetine<sup>54,55</sup> and of dopamine reuptake by sertraline<sup>56</sup>. The tricyclic drug imipramine, on the other hand, inhibits both noradrenaline and serotonin reuptake<sup>57</sup>.

## Conclusions

The administration of thioacetamide evoked oxidative stress in the brain and liver. Pretreatment with the SSRIs fluoxetine and sertraline decreased lipid peroxidation in the brain; liver peroxidation, however is increased. The opposite effects were observed in rats pretreated with the tricyclic antidepressant imipramine. The thioacetamide-induced hepatic damage was not prevented by the antidepressants. The clinical significance of these findings is yet to be established in view of the fact that antidepressant drugs are used for the treatment of depression in patients with hepatitis C viral infection, especially those treated with interferon-alpha/ribavirin combination therapy.



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