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POSSIBLE ANTIOXIDANT AND GLUTATHIONE REPLENISHING EFFECTS OF PAROXETINE IN LIVER HOMOGENATES OF RATS EXPOSED TO CHRONIC RESTRAINT MODEL

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Abstract-

Background and Aim: Depressed mood could affect the progression and severity of several diseases e.g. hypertension, myocardial infarction, gastritis, peptic ulcer etc. Liver is one of the major organ that could be affected by chronic exposure to stress because stress may result in hepatic inflammation in particular due to accumulation of reactive oxygen species (ROS). The present study was done to investigate the potential antioxidant effect of paroxetine, as a selective serotonin reuptake inhibitor (SSRI), to protect against chronic restraint stress-induced oxidative damage in the liver.

Methods: Thirty wister albino rats were divided into 3 groups. Group-1 was control, non-stressed non-treated group. Group-2 was exposed to chronic restraint model by placing them in wire mesh cages exactly fit to their size for 6 hours daily for 21 days. Group-3 were also exposed to chronic restraint model for 21 days while they were administered by paroxetine 1 mg/kg/day ip during the restraint period. At the end of the study, liver transaminases (ALT and AST) were determined by commercial kits. The hepatic levels of GPx, catalase and TBARS were also determined by spectrophotometric methods. Glutathione repletion ability by hepatic cells with and without paroxetine treatment was also determined in all tested groups.

Results: The results showed a significant (p<0.05) increase in serum levels of ALT & AST and liver levels of GPx and catalase enzymes while levels of TBARS were significantly (p<0.05) reduced in paroxetine-treated group compared with non-stressed non-treated control rats. Glutathione repletion ability was also significantly (p<0.05) increased in treated group to a level comparable to the control non-stressed non-treated values.

Conclusion: Paroxetine could possess a protective effect to liver tissue of chronic restrained rats. This hypothesis may help its use to reduce oxidative stress caused by exposure to chronic stress.

Keywords- Paroxetine, Chronic restraint model, wister rats, anti-oxidant enzymes, TBARS, glutathione repletion, liver

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Introduction

Paroxetine is one of the selective serotonin reuptake inhibitors (SSRIs). It is commonly used in the treatment of patients with depressive disorders since the late 1990s [1]. It is generally preferred over tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) due to lesser adverse effects, good compliance, and comparable efficacy with these older drug groups [2].

Chronic stress exposure is associated with neurodegeneration and a marked change in anti-oxidant enzymes. These enzymes are known to have an important role in the prophylaxis against damage of any cell in the body by oxidative stress. Generation of free radicals and reactive oxygen species (ROS) is an ordinary result of metabolic processes that occur regularly in human body. However, in case of exposure to chronic stress, they are produced in a very high levels that lead to toxic effects on intracellular components such as DNA, proteins and lipids [3]. These toxic effects result in abnormalities in the signaling processes in the body and increase the incidence of apoptosis of cells of vital organs [4]. Liver and other body's systems are exposed to damaging effects by oxidative stress associated with depressed mood and disorders of important mediators of central nervous system [5-7].

Depressed mood in human and exposure of animals to chronic restraint model are associated with an increase in incidence of lipid peroxidation and production of ROS [8,9]. Newer generations of antidepressant drugs were found to have the ability to combat the oxidative stress with their abilities to normalize or even increase the activities of potent antioxidant markers [10,11].

The aim of the present study is to investigate the possible protective

effect of paroxetine, as an SSRI used in treatment of depressed mood, on the liver of albino wister rats exposed to chronic restraint model.

Material and Methods

Animals

Albino Wister rats (150-200 g) were purchased from the animal house (Bilharzial Center of Research, Ain Shams University, Cairo, Egypt), housed in controlled environmental conditions. They were left for one week for acclimatization. They were housed in cages with a natural light-dark cycle and fed on a standard pellet diet and water ad libitum.

Chemicals

Paroxetine HCl (Glaxo SmithKline, U.K.) was purchased as an odorless, off-white powder whose molecular weight is 329.4, N-(l-naphthyl)ethylenediamine dihydrochloride (ICN Biomedicals Inc., Egypt) and diethyl maleate (Merck Biochemicals, Egypt). Serum alanine & aspartate transaminases (ALT, AST) spectrum diagnostic kits were obtained from Biodiagnostics, Cairo, Egypt. All other chemicals were purchased from Sigma chemicals co.

Animal Grouping

Albino Wister rats were divided into 3 groups (N= 10 rats in each group). They were classified as follows:

Group-1: non-stressed non-treated control group which received normal diet and administered only by deionized water ip.

Group-2: non-treated group but exposed to chronic restraint model and administered only by saline ip as a solvent of paroxetine

Group-3: Paroxetine-treated group: was administered paroxetine dissolved in saline (1 mg/kg b.wt./day ip) for 21 days. This dose was selected according to many studies [12,21,22].

Ethics

All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act.

Chronic Restraint Stress Procedure

Each rat of both Group-2 & Group-3 were placed in a wire mesh restrainer 6 hours daily for 21 days. At the end of the restraint period, rats were moved to their cages.

At the end of the experimental period, the animals were weighted, anesthetized with ether. Blood samples were collected from the abdominal aorta as follows: the rat was fixed on a wooden plate and the abdominal cavity was opened, then the abdominal aorta was explored at its bifurcation after gentle traction of the viscera using soft tissue. Blood was centrifuged at 5000 rpm for 10 minutes for serum separation, and kept at -80°C until measurements of bot ALT & AST.

Biochemical Measurements

Serum levels of alanine and aspartate transaminases (ALT & AST) were measured using biochemistry automatic analyzer (Hitachi 7600).

Determination of Glutathione Peroxidase [GPx] Enzyme Activity

Glutathione peroxidase (GPx) activity in the liver homogenates was measured by the method described by Rotruck, et al [13]. The activity was expressed based on inhibition of GSH.

Determination of Catalase Enzyme Activity Catalase (CAT) activity in the liver homogenates was assayed colorimetrically as described by Sinha, et al [14] using dichromate- acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed 1:3 (v:v). Spectrophotometric readings were recorded at 620 nm and the amount of hydrogen peroxide hydrolyzed was calculated for the catalase activity.

Hepatic lipid peroxidation Hepatic lipid peroxidation was quantified by measuring TBARS according to the method described by Fraga, et al [15].

Liver tissue from each rat was homogenized in nine volumes of 50 mmol/L Tris-HCl buffer (pH 7.4) containing 180 mmol/L KCl, 10 mmol/L ethylenediamine tetra-acetic acid, and 0.02% butylated hydroxytoluene. To 0.2 mL of tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid, 1.5 mL of 0.9% thiobarbituric acid, and 0.6 mL of distilled water were added and vortexed. The reaction mixture was placed in a water bath at 95°C for 1 hr. After cooling on ice, 1.0 mL of distilled water and 5.0 mL of butanol/pyridine mixture (15:1, v/v) were added and vortexed. After centrifugation at 10,000 × g for 10 minutes, absorbance of the resulting precipitate was deter- mined at 532 nm. The TBARS concentration was calculated using 1,1,5,5-tetraethoxypropane as standard.

Determination of Glutathione Repletion Ability by Hepatic Cells of Tested Rats

Diethyl maleate (DEM), a carbonyl compound that rapidly depletes cytosolic glutathione was used to compare glutathione-replenishing abilities of hepatic cells of all tested groups. Briefly, hepatocyte monolayers cut by microtome were exposed to 500 uM DEM for 30 min to achieve glutathione depletion. The media containing DEM were then removed and the cells were washed with Hanks-buffered saline (HBS) before the media were replaced with DEM-free RPMI-1640 media. After 1, 2 and 4 hrs., the plates were washed 3 times after removal of culture media. Wash was done using HBS before 1 ml of trichloroacetic acid (6.5%) was added to precipitate cellular proteins. The cellular matter was then scraped from the plates and transferred to Eppendorff's tubes. After centrifugation, the acidic supernatant was analyzed for glutathione using a procedure based on the method of Saville [16]. The remaining pellets were dissolved in 0.5 ml of 0.5 M sodium hydroxide, and incubated at 40°C for 1 hr.; protein was then determined using the Bradford method [17]. Glutathione levels were expressed as μmol/mg protein. Determination of intracellular reduced glutathione levels. Glutathione concentrations were determined by the method of Saville [16]. One milliliter of 6.5% trichloroacetic acid in 0.5 mM EDTA was added to each dish, and then the cells were scraped with a rubber policeman, collected into 1.5 ml microcentrifuge tubes, and centrifuged at 13,000 rpm (Beckman microcentrifuge) for 5 min. The supernatants were transferred to new tubes, the protein pellets were dissolved in 0.5 ml of 0.1 M NaOH each, and the fractions were frozen for al least 24 hrs. before performance of the assay. The thiol determination was carried out with freshly prepared reagents on supernatant fractions in 96-well microplates. Fifty microliters of thawed supernatant was mixed with 50 ul of 10 mM sodium nitrite solution prepared in 0.96% sulfuric acid, and the mixture was incubated for 5 min at room temperature. Ten microliters of 0.5% ammonium sulfamate was added, and the mixture was incubated for 5 min. Then 100 μL

of a solution consisting of 1 part 0.5% mercuric chloride and 4 parts 3.4% sulfanilamide in 0.4 M hydrochloric acid was added, and the mixture was incubated for 5 min. The color reaction was developed after a 5-min incubation with 40 ul of substrate solution containing 0.2% (N-(l--naphthyl) elhylenediamide dihydrochloride in distilled water. The absorbance at 540 nm was read in a spectrophotometer; the blank solution consisted of 50 µL of 6.5% trichloroacetic acid processed as described for the supernatant fractions. The standard curve was generated from triplicate samples of serial dilutions of reduced glutathione, and the results were expressed in nmoles of glutathione per milligram of protein.

Protein Determination

The protein content of the liver homogenates was determined by spectrophotometry according to the method of Bradford method [17]. The aim was to express catalase and glutathione peroxidase enzyme activity as Unit/mg tissue protein and the TBARS concentration as nmol/mg tissue protein.

Data Analysis

Results are expressed as mean ± SD [Standard Deviation]. Statistical analysis was performed by analysis of variance followed by Tukey's post hoc using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, USA). Differences with $p<0.05$ were considered to be statistically significant.

Results

Chronic restraint of rats of Group-2 significantly ($p < 0.05$) reduces the levels of serum ALT & AST. While, paroxetine administration, in a dose of 1 mg/kg/day ip for 21 days, significantly ($p <0.05$) restores them to levels comparable to control non-stressed, non-treated Group-1 [Fig-1].

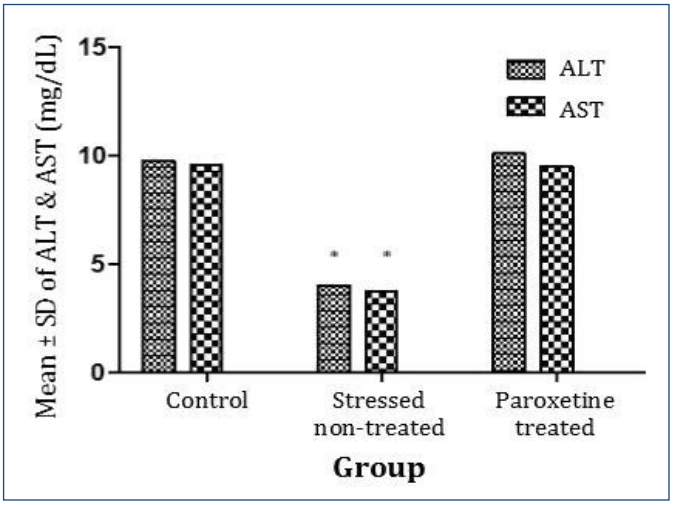

Fig. 1- Effect of treatment with paroxetine on the serum level of alanine and aspartate aminotraminase (ALT & AST) enzymes of rats compared to both Group-1 & Group-2.

**Significant ($p <0.05$) reduction in levels of both enzymes compared to control non-stressed Group-1 and paroxetine-treated stressed Group-3.*

A significant ($p <0.05$) decrease in the activities of these enzymes was demonstrated in stressed non-treated rats. Paroxetine-treated group showed that their activities were significantly ($p <0.05$) increased compared to stressed non-treated group and reached levels comparable to that recorded with control Group-1.

This suggests that paroxetine owns a possible antioxidant action in case of chronic restrained albino rats with a significant ($p <0.05$) decrease in their liver transaminases [Table-1].

Table 1- Mean ± SD changes in the activities of liver CAT, GPx in rats of all tested groups expressed as unit/mg tissue protein of liver homogenates.

	Control group	Stressed non-treated group	Stressed paroxetine-treated group
Glutathione peroxidase Unit[b]/mg tissue protein	12.33± 1.5	0.55± 0.01*	10.96± 0.65**
Catalase Unit[a]/mg tissue protein	65.43± 3.2	3.12± 0.42*	57.65± 4.5**

[a] = Moles of hydrogen peroxide consumed per minute.

[b] = µg of glutathione consumed per minute.

**p <0.05, significant reduction in activity of both enzymes in stressed non-treated group versus control group.*

***p, 0.05, significant increase in activity of both enzymes by paroxetine administration versus stressed non-treated group.*

There is significant (*$p <0.05$) reduction in glutathione levels in stressed non-treated rats compared to control group. However, treatment of chronic restrained rats with paroxetine for 21 days significantly (**$p <0.05$) is likely to protect hepatic cells against acute depletion of glutathione in comparison to Group-2 suggesting that ip paroxetine administration would enhance glulathione replenishing ability in hepatic cells of chronic restrained rats [Fig-2].

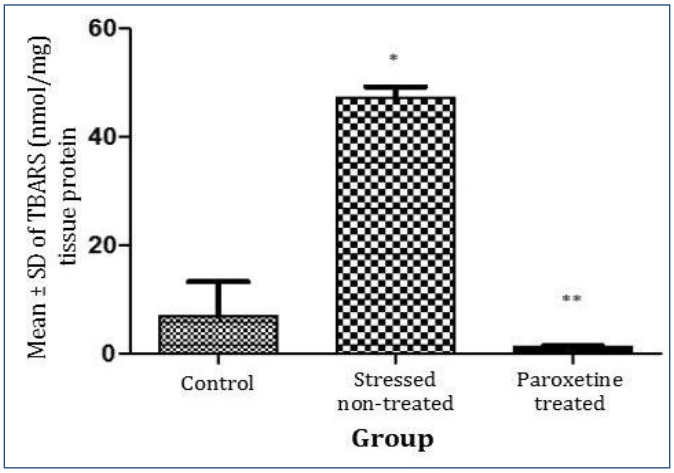

Fig. 2- Effect of 21 days administration of paroxetine on thiobarbituric acid-reactive substance (TBARS) in nmol/mg tissue protein of the liver tissue homogenates of all tested albino rats

Results are expressed as mean ± SD (n = 10 albino rats/group).

A significant (*p <0.05) decrease in TBARS levels of paroxetine-treated Group-3 in comparison to the stressed non treated Group-2.

*$p <0.05$= significant increase in TBARS levels in Group-2 compared to the control non-stressed non-treated rats Group-1.

**$p <0.05$ = significant decrease in TBARS levels in paroxetine-treated Group-3 compared to the stressed non-treated rats Group-2.

The [Table-2] shows changes in glutathione levels expressed as nmol/ mg protein before and 4 hrs. after exposure to 0.5 mM diethyl maleate (DEM). Data were obtained from hepatic cells isolated from either control, stressed non-treated or stressed+paroxetine-treated group.

Table 2- Effect of treatment with paroxetine on Glutathione repletion ability of hepatic cells expressed nmoles of glutathione per milligram of protein

Groups	Change in glutathione level in hepatic cells		
	Before exposure to DEM	4 hrs. after exposure to DEM	% change in glutathione level
Control	54.5 ±2.4	55.4 ±3.2	1.65%
Stressed non-treated	53.5± 3.4	2.1 ±0.03*	-96.07%
Stressed paroxetine-treated	51.2 ± 3.1	55.7 ±3.7**	8.79%

** p <0.05= significant reduction in hepatic glutathione replenishing ability in Group-2 compared to the control non-stressed non-treated rats Group-1.*

*** p <0.05 = significant increase in hepatic glutathione replenishing ability in paroxetine-treated Group-3 compared to the stressed non-treated rats Group-2.*

Discussion

Exposure of albino wister rats, in the present study, to chronic restraint model significantly decreases serum levels of liver transaminases (ALT & AST) and reduces activities of CAT and GPx anti-oxidant enzymes in hepatic homogenates of these stressed rats. It also decreases hepatic replenishing ability of glutathione. Ip Administration of paroxetine for 21 days reverses all these changes to almost the levels of control non stressed group.

Intraperitoneal (ip) administration of paroxetine for 28 days for anhedonic rats, either due to acute dexamethasone administration or due to exposure to chronic mild stress (CMS) model of anhedonia, reversed the anhedonic effect. These results provide a strong evidence of antidepressant effect of paroxetine as already well-known. Its antidepressant effect against anhedonia-induced by glucocorticoids could be related to suppression of oxidative stress that is associated with dexamethasone administration [18].

There is a reported relationship between depression and immune system. Results of previous studies revealed that depressed mood is often associated with an activation of the immune system functions with a marked secretion of inflammatory cytokines [19]. This increase in immune functions was thought to be related to damaging actions of exposure to oxidative stress. As it was found that activation of immune system results in stimulation of marked synthesis of ROS, instability of anti-oxidant enzymes and down-regulation of their synthesis [8]. Proper control of the functions of immune system leads to a positive impact on the synthesis and activities of the antioxidant enzyme mitochondrial manganese superoxide dimutase (SOD2) [20]. Chronic paroxetine administration to stressed adult rats produces protective effects against apoptosis and oxidative stress in their hippocampus [21]. These findings could be applied to the results of the present study in relation to the possible hepatoprotective effect of paroxetine in chronic restrained rats by its preservation of antioxidant enzymes and a significant reduction in hepatic TBARS, as a marker of lipid peroxidation.

Male Sprague-Dawley rats were exposed to chronic unpredicted stress with administration of Paroxetine (1.8 mg/kg once daily) by an intragastric gavage. The study recorded any change in rat behaviors, activites of some anti-oxidant enzymes : superoxide dismutase and catalase and lipid peroxidation in the form of malondialdehyde level in the serum in addition to the expression of serotonin transporter (5-HTT) in the hippocampus and norepinephrine transporter (NET) in the pons. Results demonstrated that pparoxetine produced a significant reduction in depression-like behaviors and in the malondialdehyde level. Additionally, it increased the activities of superoxide dismutase and catalase anti-oxidant enzymes with an increase in 5-HTT and NET expression. The study concluded that the antidepressive effect of paroxetine could be related partly to overcoming oxidative stress disorders and increasing the expression of 5-HTT and NET that elevated mood of depressed rats [22].

In conclusion, the results of the present study would provide a suggestion that paroxetine possesses an anti-oxidant action that helps in protection of livers of chronic restrained rats.

Conflicts of Interest: The author reports no conflicts of interest in this work.

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