Hepatopathy and Reno-cardiopathy Induced by Ritalin in Rats

Nadia A. Abdelmajeed and Amani M. Manaa

Biochemistry Department, Girls college of Education, king Abdulaziz University Jeddah, Kingdom of Saudi Arabia P.O.Box 50098 Jeddah 21523

Abstract: The aim of the present investigation was to illustrate the pathological oxidative toxic effects of ritalin induced tissue damage on different organs of rats. Animals were divided into two groups; G1: normal control (not received any drug), G2: Ritalin treated group. Ritalin administered orally using a single dose of 1 mg/100gm body weight. The pathological toxic effects of this drug on different tissue organs (liver, kidney and heart) were studied after three different experimental periods (after 10, 20 and 30 days) just after drug administration. The results showed that ingestion of Ritalin induced significant increase in the activity of xanthin oxidase (XO, free radical producing enzyme), coupled with elevated level of nitric oxide (NO) in liver, kidney and heart of Ritalin -treated rats versus normal animals, indicating oxidative tissue damage. The deterioration of these biomarkers was in line with induction of malondialdehyde (MDA, index of lipid peroxidation) in kidney, and decreased in adenosine triphosphatase (ATPase) and lactate dehydrogenase (LDH) activities in cardiac tissue. The tissue injury induced in liver of rats under the effect of Ritalin was documented by a depletion in the activity of liver sorbitol dehydrogenase (SD) with elevation in liver serum marker enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT). In addition, the pronounced increased levels of serum biomarkers of kidney function, creatinine and uric acid as well as in serum index enzyme of heart, creatine phosphokinase (CPK) in Ritalin treated rats in relation to normal animals, indicating the adverse toxic effects of the used drug on kidney and heart tissues. The current investigation also demonstrates that ingestion of Ritalin to rats led to a decrease in level of hemoglobin (Hb) compared with normal animals. The toxic effect of the tested drug on the histomorphology of the studied organs was also conducted. The degenerative necrotic alterations observed in biochemical parameters and reflected by histopathological pictures were severe in the three studied periods.

Key words: Ritalin, tissue damage, markers enzymes, histomorphology.

INTRODUCTION

The use of stimulants in patients diagnosed with attention deficit hyperactivity disorder (ADHD) has been indispensable[1]. Stimulants provided short-term symptom relief of hyperactivity, inattention and impulsivity, and also provide longer-term benefits in social and academic function and drug-abuse liability[2]. The prescription of Ritalin (methylphenidate, MPH) has dramatically increased[3-4] and has been used across a wider age of patients. Ritalin is a non-catecholamine sympathomimetic drug, and its pharmacological action is related to enhancement of extracellular levels of dopamine in the striatum by blocking dopamine transporters[5]. The high prevalence of ADHD[6-7] and the increased therapeutic use of Ritalin raises some concerns regarding its possible potential toxicity[8].

Previous clinical and experimental studies reported that administration of Ritalin causes brain oxidative damage, DNA damage[9-10], liver necroinflammatory disorder and tumors[11-12], myocardial infarction and cardiovascular disorder[13-15]. Also, it was found that the acute or chronic administration of Ritalin in young rats increased the production of free radical in some brain areas[16].

In this study, the oxidative toxic effects of Ritalin on rat liver,kidney and heart was investigated. This can be achieved through measuring some biochemical oxidative stress markers in these studied organs. Histomorphological studies were also assessed on these organs.

MATERIALS AND METHODS

Chemicals: All chemical reagents were of analytical grades purchased from Sigma Chemical Co. (St. Louis, Mo, USA), Merk (Germany) and BDH (England). Diazepam drug was obtained from Swiss Hofman Laroch Limited Company.

Corresponding Author: Nadia A. Abdelmajeed, Biochemistry Department, Girls college of Education, king Abdulaziz University Jeddah, Kingdom of Saudi Arabia P.O.Box 50098 Jeddah 21523
**Animals:** 60 adult male albino rats (100-120gm) were obtained from animal house, King Fahed Center for Medicinal Research, King Abdul-Aziz University, Jeddah. The animals were housed in cages under standard hygienic condition and were fed with rat chow and water ad libitum. In order to optimize drug absorption, all animals were fasted for 1 hour prior to drug administration.

**Experimental Design:** Rats were divided into two groups, normal healthy group (group 1) and drug treated group (group 2), each of 30 rats. Ritalin drug induced tissue damage was administered orally using a single dose of 1mg / 100gm body weight\(^{[17]}\). The effect of this drug toxicity on different tissue organs (liver, kidney and heart) was studied after three different experimental periods (after 10, 20 and 30 days) commenced just after of drug ingestion. After each studied period the blood samples were collected from some animals into sterilized tubes for serum separation and into tubes containing heparin for hemoglobin determination. Serum was separated by centrifugation at 3000× g for 10 minutes and used for biochemical serum analysis. After blood collection, rats of each experimental period were sacrificed under ether anesthesia and the liver, kidney and heart samples were collected, minced and homogenized in either ice cold distilled water or 10 % to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 minutes at 10000 g. at 4°C and the supernatants were used for different biochemical tissue analysis.

**Biochemical Analysis:** All the following biochemical parameters were measured spectrophotometrically.

**Tissue Analysis:** Nitrite concentration (an indirect measurement of NO synthesis) was assayed using Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) in acidic medium\(^{[19]}\). Lipid peroxidation was determined by measuring the formed MDA (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method\(^{[19]}\). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, the product of lipid peroxidation was measured at 532 nm. MDA concentration was calculated using extinction coefficient value(s) of MDA-thiobarbituric acid complex (1.56×10\(^3\) /M/cm) . XO (EC 1.1.3.22) activity was determined by the reduction of nitroblue tetrazolium (NBT) in the presence of xanthine, forming formazan. The enzyme activity was calculated using the extinction coefficient of reduced NBT (7.5 cm2/µmol at 540nm)\(^{[20]}\). The activity of the enzyme is expressed as nmol uric acid /min/mg protein. ATPase was determined using the method of Tsakiris and Deliconstantinos\(^{[21]}\). LDH activity was evaluated in a reaction mixture containing trios buffer (50Mm, pH7.5), sodium pyruvate (0.6mM) and NADH (0.18 mM). The rate of NADH consumption is determined at 340nm and is directly proportional to the LDH activity\(^{[22]}\). Sorbitol dehydrogenase (SD, EC 1×1×1×14) was measured by the method of Bergmeyer\(^{[23]}\). The reaction mixture contained the following in the final concentration of: 0×107 M Triethanolamine buffer (pH 7×4), 300 Mm and 0.4Mm NADH. The decrease in the optical density was recorded at 340nm.

**Serum Analysis:** ALT and AST activities were determined according to the method described by Bergmeyer et al.\(^{[24]}\). Gamma Glutamyl Transferees (GGT) was measured by the method described by Shaw et al.\(^{[25]}\), uric Acid (UA) by the method described by Bulgar and Johns\(^{[26]}\), creatinin (Crea) by the method of Larsen\(^{[27]}\) and creatine phosphokinase (CPK) by the method described by Rosalki\(^{[28]}\). GGT was assayed by the method of Schmidt and Schmidt\(^{[29]}\).

**Blood Analysis Hb:** was estimated in the whole heparinized blood by cyanmethaemoglobin method\(^{[30]}\).

**Histological Evaluation:** Representative slices of liver, kidney and heart tissues were taken from the eviscerated animals and fixed in 10% formalin. For light microscopy examination, the tissues were embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H&E).

**Statistical Analyses:** Data were analyzed by comparing values of different treatment groups with the values of individual controls. Results were expressed as mean ± S.D. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) coupled with post-hoc (LSD) and followed by Bonferoni as a post ANOVA test.

**RESULTS AND DISCUSSION**

Table 1 shows the levels of oxidative tissue injury markers in different tissue organs in normal and Ritalin treated groups after 10,20 and 30 days of drug administration. The results show that administration of Ritalin to rats led to pronounced stimulation in the activity of xanthin oxidase (XO) accompanied with increase in nitric oxide (NO) level in liver, kidney and heart of Ritalin -treated rats compared with normal rats. The induction of NO was coupled with increased level of malondialdehyde (MDA, index of lipid peroxidation) in kidney , and depletion in adenosine triphosphatase (ATPase) and lactate dehydrogenase (LDH) activities in cardiac tissue. The present results...
also revealed alteration in the levels of liver function indices in Ritalin treated rats after the three studied different periods versus normal animals. This was indicated by a decrease in the activity of sorbitol dehydrogenase (SD) in liver tissue (Table 1) and increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) (Table 2). Marked elevation of serum markers of kidney function, creatinine and uric acid levels and increase in serum marker enzyme of heart, creatine phosphokinase (CPK) in Ritalin treated animals versus normal ones were also observed (Table 2). Table 2 also shows that administration of Ritalin to rats caused a decrease in level of hemoglobin (Hb) in Ritalin treated animals in relation to normal animals. Figures 1, 2 and 3 show the histomorphological pictures of liver, kidney and heart of normal and Ritalin treated animals respectively. The pictures show severe degenerative changes in the liver, kidney and heart muscle fibres in rats treated with Ritalin. The degenerative necrotic alterations observed in biochemical parameters and reflected by histopathological pictures were severe in the three studied periods.

Discussion: Our findings demonstrate that, administration of ritalin drug to rats induces oxidative damage in different organs of rats ensured by marked stimulation in the activity of the free radical producing enzyme, XO, with concomitant increase in NO level in liver, kidney and heart in comparison with normal animals. The alteration of this oxidative stress indices was coupled by increased MDA (index of lipid peroxidation) in kidney and decrease in ATPase and LDH activities in heart tissue indicating tissue oxidative damage. These results are coped with Martinsa et al.\[9\] stated that administration of Ritalin induces oxidative tissue damage of brain of young rats. Also, Gomesa et al.\[10\] found that the acute or chronic administration of Ritalin in rats increased the production of free radical in some brain areas\[10\]. These results may suggest that Ritalin exerted its toxic effect on body vital organs through its potential role in inducing oxidative stress in liver, kidney and heart of Ritalin-treated rats.

XO is one of the enzymes of oxidase system, responsible for the oxidation of hypoxanthine to xanthine and the later to uric acid. It has the principle role in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that have the crucial role in the development of liver, kidney and cardiovascular diseases\[31-33\]. XO catalyzes the reduction of nitrite to NO which exerts various pathological effects on body tissues\[31,34\]. It is generally accepted that overproduction of nitric oxide is associated with oxidative stress, which is involved in the pathogenesis of cardiovascular diseases, degenerative diseases, or chronic inflammation\[35\]. The toxicity of NO is accelerated by reacting with superoxide radical to give powerful secondary toxic oxidizing species, such as peroxynitrite (ONOÖ) which has deleterious influences on cellular structure and causes lipid peroxidation\[36-38\]. Induction of lipid peroxidation and production of endogenous toxic aldehyde, such as MDA induces many damaging effects, on the cell membrane and cytosol. The effects on cell membrane may include an increase in permeability and inactivation of membrane-bound enzymes\[39-40\].

On the other hand, ATPases is essential for the regulation of ionic content and membrane excitability of myocardial cells, impaired of its function would lead to the coronary artery vasospasm, arrhythmias, ischemic damage and cardiac failure\[41-43\]. This contention is in concert with the some findings that certain cardiovascular diseases, such as cardiomyopathy, and hypertension, associate with decreased ATPase activity\[44\].

The present study revealed that the tissue damage induced in liver in response to Ritalin ingestion was ensured by a reduction in the activity of hepatic SD parallel with increase in liver serum marker enzymes, AST, ALT and GGT. The alteration in these marker enzymes activities might be attributed to the leakage of these enzymes from liver cytosol into the blood stream as a result of tissue injury. Such result was supported by liver histopathological picture which showed severe necrotic degenerative change of hepatocytes. Also, the marked increases of serum creatinine and uric acid levels in Ritalin treated animals were confirmed by damaged in kidney tissue showed in histomorphological pictures and observed by necrotic degenerative changes of kidney tubules and glomeruli during the three studied different periods, implying renopathy. The oxidative cardiac tissue damage induced by toxic effect of Ritalin in rats was supported by obvious increase in the activities of diagnostic serum marker enzymes, CPK and a decrease in LDH of heart tissue in Ritalin treated rats compared to normal rats and confirmed by the histopathological picture which demonstrated necrotic lesion. These findings confirm the onset of myocardial lesion and leaking out of the marker enzymes from heart to blood\[44\].

The current study also revealed that ingestion of Ritalin to rats led to reduced level of Hb in Ritalin treated animals compared with normal animals. The reduction in Hb level in rats under the effect of ritalin might be attributed to the decrease in the Hb molecules induced by the toxic effect of ritalin. Decreased Hb level induced a state of anemia which might lead to a number of biochemical abnormalities and impaired cell-mediated immunity with increased susceptibility to infection\[45-46\].
Table 1: Levels of some markers of oxidative stress in different organs of rats in normal and Ritalin treated groups after three different periods.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XO</td>
<td>3.05 ± 0.194</td>
<td>14.8 ± 2.35*</td>
<td>24.78 ± 2.38*</td>
<td>36.70 ± 2.19*</td>
</tr>
<tr>
<td>NO</td>
<td>16.83 ± 1.20</td>
<td>26.16 ± 2.88**</td>
<td>35.10 ± 2.77*</td>
<td>36.26 ± 3.04*</td>
</tr>
<tr>
<td>SD</td>
<td>35.5 ± 1.82</td>
<td>7.39 ± 0.67*</td>
<td>4.04 ± 0.34*</td>
<td>2.80 ± 0.29*</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XO</td>
<td>1.96 ± 0.26</td>
<td>15.09 ± 1.28*</td>
<td>22.87 ± 1.58*</td>
<td>37.47 ± 2.40*</td>
</tr>
<tr>
<td>NO</td>
<td>5.92 ± 0.72</td>
<td>21.36 ± 1.85*</td>
<td>33.18 ± 2.00*</td>
<td>35.77 ± 2.17*</td>
</tr>
<tr>
<td>MDA</td>
<td>12.72 ± 1.71</td>
<td>35.27 ± 2.36**</td>
<td>43.66 ± 2.23*</td>
<td>65.39 ± 3.50*</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XO</td>
<td>1.94 ± 0.15</td>
<td>11.02 ± 1.3*</td>
<td>16.65 ± 1.01*</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>12.61 ± 1.53</td>
<td>35.11 ± 2.05**</td>
<td>43.62 ± 3.11*</td>
<td>51.66 ± 2.72*</td>
</tr>
<tr>
<td>LDH</td>
<td>5.04 ± 0.16</td>
<td>2.65 ± 0.01*</td>
<td>2.36 ± 0.12*</td>
<td>1.65 ± 0.011*</td>
</tr>
<tr>
<td>ATPase</td>
<td>5.08 ± 0.35</td>
<td>3.00 ± 0.02**</td>
<td>2.00 ± 0.16*</td>
<td>1.31 ± 0.012*</td>
</tr>
</tbody>
</table>

Data are expressed as mean± SD of 6 rats in each group, XO and SD are expressed in n mol/ min/mg protein, NO is expressed in umol/g tissue, LDH and ATPase are expressed in u mol/min/mg, MDA is expressed in nmol/g tissue. *P< 0.0001, **P < 0.001, when compared with normal group.

Table 2: Levels of blood functional markers of different organs in normal and Ritalin treated rats after three different periods.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (u/l)</td>
<td>17.75 ± 0.59</td>
<td>309.66 ± 6.88*</td>
<td>335.33 ± 3.82*</td>
<td>395.00 ± 4.09*</td>
</tr>
<tr>
<td>ALT (u/l)</td>
<td>22.75 ± 1.74</td>
<td>85.00 ± 2.44*</td>
<td>91.50 ± 1.92*</td>
<td>104.50 ± 1.37*</td>
</tr>
<tr>
<td>GGT (u/l)</td>
<td>13.68 ± 0.92</td>
<td>42.53 ± 2.20*</td>
<td>47.90 ± 1.92*</td>
<td>51.28 ± 1.05*</td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>2.75 ± 0.19</td>
<td>5.40 ± 0.24*</td>
<td>6.63 ± 0.95*</td>
<td>10.35 ± 0.83*</td>
</tr>
<tr>
<td>Creat (mg/dl)</td>
<td>0.59 ± 0.005</td>
<td>5.93 ± 0.59*</td>
<td>8.96 ± 0.57*</td>
<td>11.66 ± 0.51*</td>
</tr>
<tr>
<td>CPK (u/l)</td>
<td>33.63 ± 7.9</td>
<td>126.5 ± 4.37*</td>
<td>141.66 ± 11.53*</td>
<td>148.16 ± 8.32*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>16.35 ± 0.61</td>
<td>11.55 ± 0.38**</td>
<td>10.30 ± 0.55***</td>
<td>9.05 ± 0.50**</td>
</tr>
</tbody>
</table>

Data are expressed as mean± SD of 6 rats in each group. *P< 0.001, **P < 0.01, when compared with normal group.

Fig. 1: Effect of Ritalin on Liver Tissues. Fig A-light microscopic picture of normal liver showing normal hepatocytes. Fig B - liver of rats treated with Ritalin after 10 days of drug administration showing loss of liver normal architecture and necrotic degenerative hepatocytes in many areas. (figs C and D) - Liver of rats received Ritalin after 20 days and 30 days of drug ingestion respectively showing severe degenerative change in hepatocytes (H&E ×400).
Fig. 2: Effect of Ritalin on Kidney Tissues. Fig A-light microscopic picture of normal kidney showing normal tubules and glomeruli. Fig B-kidney of rats treated with ritalin 10 days, just after of drug administration, showing necrosis of glomeruli, shrinkage of epithelial lining of some tubules and degeneration of other ones. (Figs C and D) -kidney of rat treated with Ritalin 20 days and 30 days, just after of drug administration respectively, showing severe necrotic changes in glomeruli and tubules (H&E ×400).

Fig. 3: Effect of Ritalin on heart Tissues. Fig A-light microscopic picture of normal heart showing normal muscle fibers. Fig B - Heart of rat treated with ritalin 10 days after drug administration showing infarction of some muscle fibres. (Figs C and D) -Hearts of rats treated with Ritalin 20 days and 30 days after drug administration respectively showing disorganization of muscle fibres (Fig C) and severe degenerative damage of most muscle fibres (Fig D) (H&E ×400).

The changes in both biochemical indices as well as in histopathological pictures of liver, kidney and heart were severe in the three studied periods after Ritalin ingestion. From the current results, it could be suggested that Ritalin drug may exert toxic potential action on the vital body organs as liver, kidney and heart.
REFERENCES


