Research Article

Methylphenidate Hydrochloride Overdose Induces Liver Damage in Rats: The Evaluation of Histopathology and Some Antioxidant Enzymes Biomarkers

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Abstract | Background: The drug of choice for the treatment of attention deficit hyperactivity disorder is Methylphenidate hydrochloride (MPH) or Retalin (the commercial name) . **Objective:** The effects of MPH on adult male rats have been evaluated in this study. **Materials and methods:** receiving an oral dose (2 daily MPH doses), represented by Group 1 (5 mg/kg/day in a 5% glucose solution), Group 2 received daily dosages of methylphenidate in escalating amounts (5, 8, 12, and 16 mg/kg/day in a 5% of sucrose solution), as well as Group 3 as control animals (5% glucose solution). Group 1 resembled the therapeutic doses administered to humans, while Group 2 simulated the wrong use of some of the human addicts. 30 days after the last Ritalin dose administration, the rats were all sacrificed, and blood and liver tissues were collected for biochemical and histological analysis . **Results:** Comparison to the Control Group, the ratio of ALT in the blood serum significantly increased in groups 1 and 2 of MPH-treated rats, meanwhile, the ratio of AST remained unchanged significantly in Group 1. In the liver, MDA and GSH-PX rates were significantly increased (P≤0.05) in 1 and 2 groups compared to the 3 groups (the control), while SOD level significantly rises only in Group 2, also did not appear MPO values any Significant alteration throughout the experiment. While, the histology alterations revealed different levels of significant damage in the liver, represented in group 1 by slight damage, mainly , congestion of the central vein, hepatocytes degeneration, nuclei pyknotic, sinusoids dilation, and slight inflammation, while group 2 revealed severe damage mainly , lost the typical polymeric shape, hepatocytes alteration, hepatocytes vacuoles, necrotic foci, inflammation cells aggregation, sever dilated and congestion of sinusoids and nucleus pyknotic. **Conclusion:** These findings demonstrated that the MPH overdose induced free radical-mediated oxidative stress, through an increase of antioxidant enzymes and histological alterations in the liver.

Keywords | Methylphenidate hydrochloride, Liver Damage, Rats, Histopathology, Antioxidant Enzymes

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INTRODUCTION

Anxiolytics, opioid analgesics, and stimulants such as methylphenidate hydrochloride and alprazolam in particular have seen a considerable rise in abuse over the past ten years, around the globe, although the European Medicines Agency's Committee for Medicinal Products concluded in 2009 that there was a lack of information regarding methylphenidate hydrochloride side effects in long-term overdoses EMA, (2009). However, these drugs

have a high danger of being abused alone, and peculiar cases have shown their harmful effects, till now few studies investigated the reference of the physiological and histopathological change. The primary concern over these drugs lies in their chronic use, anyway, despite the lack of studies focused on this subject, adequate data regarding reference to the physiological and histopathological changes of the adverse effects of MPH have been reported (Schweren, de Zeeuw and Durston, 2013). MPH (commercially called Ritalin) is one of the most commonly used medications for treating attention deficit hyperactivity disorder (Mannuzza et al., 2008). Furthermore, it can be used for the treatment of depression (May and VandenBerg, 2015), for improving memory (Costa et al., 2012), lessening the desire for sleep and enhancing feelings of pleasure (Keane, 2008). According to earlier research, Ritalin induces tactile hallucinations that are comparable to marijuana as well as the study suggests that neurodegeneration and behavior changes following MPH usage may be related to the production of ROS and inflammation (Berridge et al., 2006). MPH addiction is generally unappreciated due to the disease's neuropathological complications (O'Brien, 2003). Studies on rodents assisted to understand the histological, physiological, and biochemical responses to MPH. Research on rats showed that a long term overdose of MPH causes alteration in the histological, biochemical, metabolism, and behaviours (Brandon and Steiner, 2003). Molecular and cellular evidence suggests that exposure to acute MPH doses can cause alterations in gene expression and cellular functions (Carlezon and Konradi, 2004). A few studies mentioned the effect of MPH on vital organs, liver, kidneys, and brain (Loureiro-Vieira et al., 2017). Therefore, the current study aims to evaluate the consequences of MPH overdose in a rat model. Subsequently providing a better insight into the mechanism of action and the side effect of the drug.

MATERIALS AND METHODS

Experimental design

Fifteen mature male rats weighing an average of 240 ±10.4g were randomly divided into three equal groups (5 rats each). Animals were housed under a controlled environment (twelve-hour light-dark cycle at 28 ± 2 °C temperature), with water and food available *ad libitum*.

The first group (vehicle control) receiving 2 ml of 5% glucose solution via oral gavage. Group 1 treated with 5 mg/kg/day MPH suspended in a 5% glucose solution. group 2 receiving an increasing dose of 5, 8, 12, and 16 mg/kg/day of MPH in a 5% glucose solution for 30 days. The doses started with 5 mg/kg/day (days 0 to 7), followed by 8 mg/kg/day (days 8 to 14), 12 mg/kg/day (days 15 to 21) and 16mg/kg/day (days 22 to 30), MPH doses in the current study were adopted according to (Reagan-Shaw, Nihal and Ahmad, 2007).

At the end of the experiment, all rats were hypnotized with chloroform, and blood samples were collected from the heart, and the liver was isolated and fixed with neutral buffered 10% formalin.

Evaluation of plasma enzymes biomarkers

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Creatine kinase-MB, and Total Creatine kinase levels were measured after blood sampling from the heart were taken and placed in EDTA-containing tubes. According to the manufacturer's instructions, enzyme biomarkers were assessed using the Horiba ABX Pentra C400 Clinical Chemistry Analyzer.

Evaluation Histbiochemical programs

Our tests were designed to measure the levels of myeloperoxidase MPO, glutathione peroxidase GSH-Px, superoxide dismutase SOD, and malondialdehyde MDA for qualitative structural assessment of the liver, the liver samples were homogenized at a ratio of 1:10 (w:v) with ice-cold 150 mM KC1. The remaining homogenates were kept at -70°C until tissue GSH-Px and SOD examination were conducted, and the MDA contents of the homogenates were depending on the fluorometric method which mentioned by (Wasowicz, Nève and Peretz, 1993), SOD level was measured based on (Weydert and Cullen, 2009). GSH-Px rates was evaluated according to (Capela et al., 2007), while the MPO activity was evaluated depending on a procedure identical to that mentioned by (Teixeira-Gomes et al., 2016).

Histological analysis

The histological procedures were according to (Dores-Sousa et al., 2015), who included specimens of the liver of five rats of the (1. 2 and 3) groups were fixed in neutral buffered (10%) formalin, and then dehydration with different-grade concentrations of ethanol solutions, Xylene cleaning and paraffin embedding. The manual rotator microtome was used to produce sections with a thickness of 5-6 micrometers. All sections were stained with hematoxylin and eosin for routine histological evaluation, which was analyzed and images by a light microscope connected to a digital camera.

Statistical analysis

Values were expressed as mean ±SD. One-way ANOVA was performed using SPSS software. Differences were considered to be significant at (P≤ 0.05), (SPSS, 2010).

Abbreviation term	Meaning
ALT	Alanine Aminotransferase enzyme
AST	Aspartate Aminotransferase enzyme

CK-MB	Creatine Kinase - MB
Total- CK	Total Creatine Kinase
MPO	Myeloperoxidase
GSH - Px	Glutathione Peroxidase enzyme
SOD	Superoxide dismutase enzyme
MDA	Malondialdehyde

RESULTS

Behaviourally, the rats showed decreases hyperactivity after the treatment with Methylphenidate, and No animal deaths were recorded during the experiment. While, to identify liver and heart damage in the experimental groups of male rats, serum biomarkers levels were measured (AST, ALT, CK-MB, and Total-CK) enzymes. Our results revealed significant changes (P≤0.05) in groups 1 and 2 following exposure for 30 days in a row compared with a control group. Generally, Group 2, recorded the higher significantly values (P≤0.05) (45.4 ± 2.33, 140± 8.3, 730± 12.54 and 735± 10.6) respectively, than those of control group (30± 1.11, 105± 4.8, 373± 10.2 and 383± 11.4) and group 1 (36± 2.1, 122± 5.43, 589± 9.7 and 570± 8.76) respectively, in all parameters Figure 1. (a, b, c, and d).

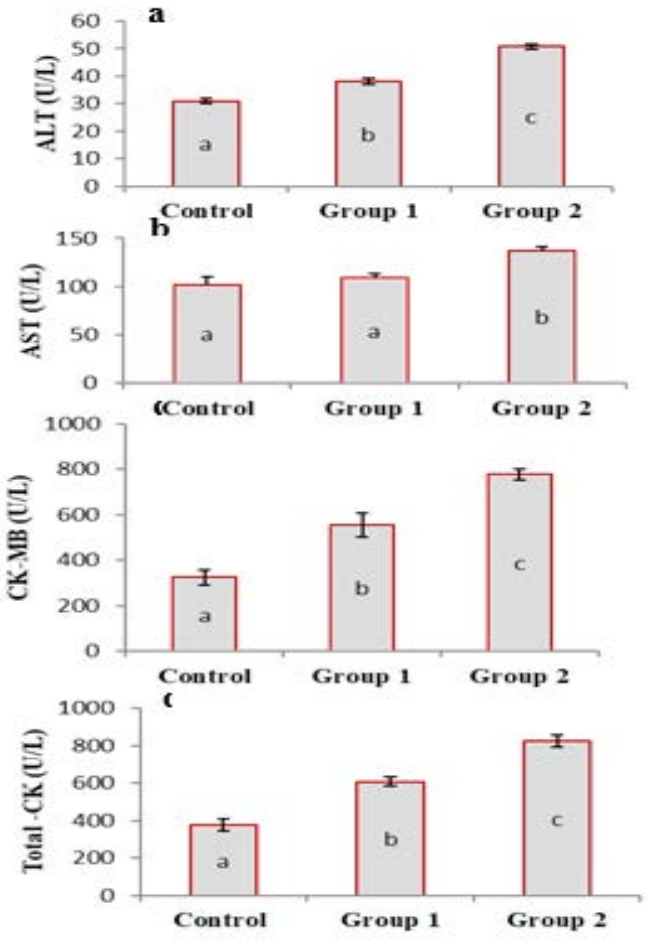

Figure 1: Serum biochemical biomarkers of control, group 1 and group 2 rats. (a) ALT, (b) AST, (c) CK-MB, and (d) Total- CK serum levels. P≤0.05 highly significant.

Figure 2. (a, b, c, and d), summarizes the levels of MDA, SOD, GSH-PX, and MPO in liver tissue, accurding to our results observed, a significant excess (P≤0.05) in the MDA of groups 1 (647± 11.1) and 2 (663± 10.5) compared to the control (433± 8.95), while the SOD enzyme recorded a significant excess (P≤0.05) in group 2 (32.5± 1.7) compare with control group (23.8± 1.6) and group 1 (25.3± 2.1). GSH-PX level appeared strong tendency for a significant excess (P≤0.05) among the groups 2, 1 and control, which recorded (3.85±0.21, 2.74± 0.14 and 1.68± 0.11) respectively, while the MPO level did not seem to be any noticeable differences (P≥0.05) between the experimental groups, (7.21± 3.7, 6.35± 3.92 and 5.68± 3.87) in group 2, 1 and control respectively.

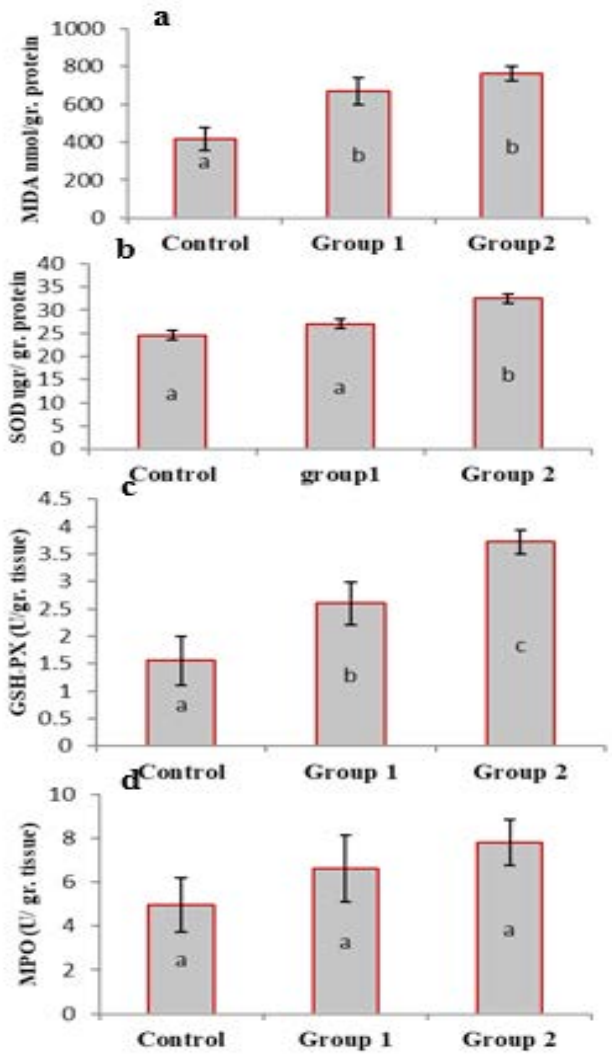

Figure 2: Changes in (a) MDA, (b) SOD, (c) GSH-Px, and (d) MPO in liver tissue as biomarkers of control, group 1 and group 2 rats. P≤0.05 highly significant.

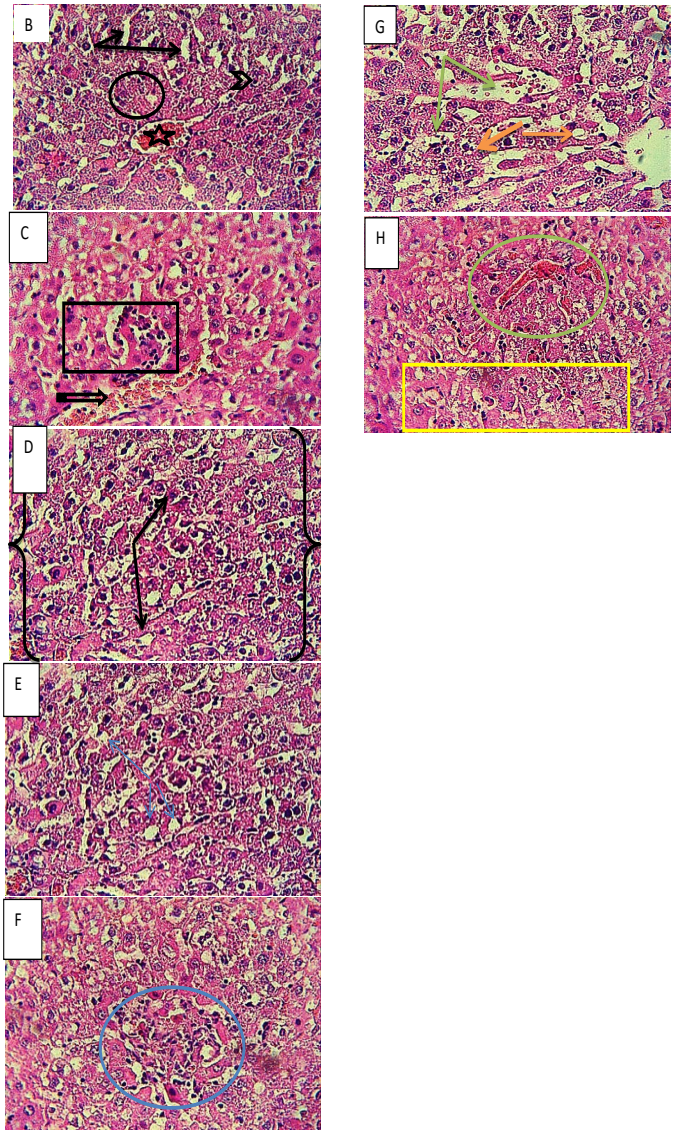

Figure 3: Liver Sections of testes groups, (A) control group showing (bow) normal hepatic parenchyma, exhibiting well defined hepatocytes have polyhedric in shape, (arrows) hepatocytes, (arrows head) hepatocytes nucleus, transversally sectioned sinusoid, and (star) hepatic portal vein . (B, C, and D) 5 mg/kg/day group, showing (star) congestion of central vein, (circle) fat degeneration of hepatocytes, (arrow head) karyopyknosis, (arrows) dilated of sinusoids, (thick arrow) capillaries hemorrhage, and (square) slightly chronic inflammation represented by lymphocytes. (E, F, G, and H) 5, 8, 12, and 16 mg/kg/day group showing, (blue arrows) vacuolization, (green arrows) severe dilated congestion of sinusoids, (blue circle) necrotic foci and aggregation of inflammation cells, and (orange arrows) severe coagulative necrotic vasculitis, (green circle) congestion of sinusoids (a rectangle) many altered cells exhibit nuclear pleomorphisms, (H and E 400X).

Histological examinations of liver

In the control group, the liver tissue revealed normal histological structure, the hepatocytes normally polygonal, with oval-shaped nuclei, and normal size and shape of the central vein (Figure 3. A), while group 1 represent during 28 days after dosing with oral methylphenidate hydrochloride by (5 mg/kg/day), appeared moderate histological changes of the central vein congestion, congestion among of sinusoids, and cytoplasmic vacuolation (Figure 3. B, C, and D), while the group 2 represent 28 days after dosing with ascending doses every seven days of the methylphenidate hydrochloride (5, 8, 12, and 16 mg/kg/day), reflected more sever histo-pathological conditions was represented by hepatocytes coagulative necrosis, chronic inflammation of lymphocytes infiltration, capillaries hemorrhage, karyopyknosis and central vein congestion (Figure 3. E, F, G, and H).

DISCUSSION

Methylphenidate Hydrochloride is the main primary prescribed for the treatment of attention deficit hyperactivity, but its abuse is, led to serious great concerns regarding possible long-term exposure consequences, (Loureiro-Vieira et al., 2017). According to our study, Ritalin in each of group 4mg/kg/day and group 4-16 mg/kg/day, induced disturbances in the level of measured blood serum enzymes as well as liver tissue enzymes compared to the control, also its caused histopathological effects in the liver tissues, represented in group 1 by the vein congestion, congestion among of sinusoids, and vacuolation cytoplasmic, while the group 2 reflected more sever histopathological conditions by hepatocytes necrosis, inflammation infiltration cells, hemorrhage, and central vein congestion. These results agree with the results (Loureiro-Vieira et al., 2018), which found that Methylphenidate Hydrochloride induced enzymatic disorders in blood serum (AST, ALT, CK-MB, and total – CK) enzymes. however a psychostimulant agent, methylphenidate (MPH) abuse can cause serious liver damage (Ahmadinasab, H. et al., 2022). While, (Alireza Akhavan Rezayat et al., 2020) confirmed that the MPH group appeared Hormonal disorders and histological characteristics diseases in male rats. One study confirmed this a substantial rise ($P \leq 0.05$) in AST and ALT enzymes in eachof the experimental groups compared to control group was recorded, while the rat's liver tissue showed fibrosis around the arteries, moderate to weak fibrosis, and infiltration of inflammatory cells around the arteries in rats subjected to various dosages of Ritalin for 30 days (Sahar et al., 2018). Our results are confirmed that the different doses of Ritalin, caused damaged the liver tissue and the amount of enzyme alterations connected to the physiological and biochemical function of the liver tissue, our results may lead us to conclude that the drug should be used with caution or prevented from using it in patients with various liver diseases. The study's findings for (Lotfi et al., 2016) and (Soares et al., 2015) indicated that frequent use of Ritalin, even at modest doses, has an adverse effect

on the testes, kidney, and endocrine gland axis, resulting in impairment to their function. Although the results of the study are different in terms of the type of tissue tested, they are identical in terms of the harmful effects of amphetamine. SPSS (2010) mentioned that In the rats treated with MPH, the ratio of GSH and reduced glutathione to oxidized glutathione (GSH/GSSG) levels appeared a significant rise (P≤0.05), with decreased GSSG in the heart tissues, While histology showed severe damage, including vascular congestion, interstitial edema, and the presence of a fibrin-like substance, The MPH therapy produced large necrotic regions with cellular infiltration and disarray in the kidneys. According to reports (Sharma and Couture, 2013) and (Alam and Ikram 2018), administering methylphenidate to mice causes liver necrosis, while the SGPT enzyme concentration was increased and the SRECEIVED enzyme concentration was appeared decrease. Methylphenidate metabolized extensively in the liver Faraone, (2018). Our histological results were consistent with a study (Ghaji et al., 2021) About the harmfulness of acute dose of anesthetic drugs on liver tissue.

CONCLUSION

The present study demonstrated that in treatment groups (4 mg /kg / day group) and (4 – 16 mg/kg/day group) there are elevated levels of ALT, AST, CK-BM, and Total-CK enzymes in the blood serum, also showed increasing levels of the MDA, SOD, GSH, and MPO. The histological study also appeared tissue damage in the liver in the treatment groups.

RECOMMENDATIONS

Through the results of our study, it was noted that Ritalin has adverse effects physiologically and histologically when used long-term and excessive dose taken by the patient, so we recommend cautious use by and the recommended dose by the specialist doctor in this field.

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CONFLICT OF INTEREST

No authors have disclosed any conflicts of interest.

AUTHORS CONTRIBUTION

Each author attests that the work is original and hasn't been published before and isn't currently being considered for publication somewhere else.

ETHICAL ENDORSEMENT

All institutional and national policies on the handling and application of laboratory animals were adhered to.

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