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Effects of Single Dose of Intraperitoneal Ketamine on the Liver of Adult Male Albino Rats: Histological and Immunohistochemical Study

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Abstract

Background: Ketamine has sedative, dissociative anesthetic and analgesic properties. Ketamine and its metabolites induced hepatic damage with chronic use for treatment of chronic pain. This study examined the effects of single intraperitoneal ketamine doseon adult male albino rats' liver by applying histological and immunohistochemical methods.

Methods: After institutional animal research ethical committee approval, thirty healthy adult male albino rats were equally divided into three groups. Group I received Intraperitoneal (IP) saline in a similar volume to IP ketamine given in group II and III. Group II and Group III had 120 and 160 mg. Kg^{-1} body weight ketamine single intraperitoneal dose respectively. After 3 days' rats were killed by decapitation and parts of their livers were processed and stained with Haematoxylin and Eosin (H and E) and Mallory trichrome for light microscope examination. Immunohistochemical examination for cyclooxygenase II enzyme (Cox II) was performed and the optical density of the reaction was calculated. Also, blood samples were taken from tail veins to measure Aspartate Transaminase (AST) and Alanine Transaminase (ALT) levels.

Results: H and E examination revealed normal hepatic architecture in rats' liver that received normal saline (Group I). Hepatocytes were mildly affected in Group II. Marked affection of hepatocytes in group III with loss of normal hepatic architecture. Mild increase in connective tissue appeared after Mallory trichrome stain in group II, while more increase in group III was seen in the portal area. Also, liver enzyme Aspartate Transaminase (AST) level increased significantly in group II and III compared to group I. In addition, there was a significant increase in Alanine Transaminase (ALT) level only in group III when compared to group I and II.

Conclusion: It appears that single intraperitoneal doses of 120 mg and 160 mg ketamine can cause variable histological changes and damage in rats' liver.

Keywords: Ketamine • Liver damage • Histological changes

Introduction

Ketamine is a non-competitive antagonist of the N-Methyl-D-Aspartate (NMDA) receptors [1,2]. It has a sedative, dissociative anesthetic and analgesic property. Also, long term ketamine infusion is being used for treatment of chronic pain [1-4]. Ketamine is used in providing analgesia in the intra and post-operative periods. It is metabolized in the liver by microsomal enzymes into metabolites I and II. Ketamine and its metabolites have been reported to damage hepatocytes and other liver cells in chronic conditions [5-7].

Dundee JW, et al. observed increase in liver enzymes on the 3-4th postoperative days after the administration of intraoperative intravenous ketamine infusion [8]. In addition, chronic ketamine abuse can produce toxicity to the gastrointestinal and urinary tract [9,10]. Gastrointestinal changes include epigastric pain, hepatic dysfunction and impaired gall bladder activity [1,11,12]. Furthermore, hepatotoxicity has been observed in patients receiving ketamine for chronic pain treatment [3,13].

In chronic ketamine use, hepatotoxicity was attributed to hepatocytes damage caused by metabolite I and II [6]. Also, ketamine inhibited mitochondrial complex I and oxygen consumption [14]. Most of the reports about ketamine toxicity described liver changes after repeated doses of ketamine over a long period during treatment of chronic pain [1-3]. To our knowledge, none of these reports described the effects of single ketamine dose on the liver by applying histological and immunohistochemical methods. Therefore, we put our hypothesis that single dose of ketamine can cause considerable degree of liver injury. The primary outcome of this study was to examine the effects of single Intraperitoneal (IP) ketamine doses (120 and 160 mg. Kg^{-1}) on the liver of adult male albino rats by applying histological and immunohistochemical methods. The secondary outcome was to study the effect of such doses on liver enzymes Aspartate Transaminase (AST) and Alanine Transaminase (ALT) Levels.

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Materials and Methods

Drugs and chemicals

Ketamine was purchased from TROIKAA PHARMACEUTICALS LTD as ketamine hydrochloride injection, USP 50 mg/ml in 10 ml vial.

Animals: After Institutional ethical committee approval for animal research

of Faculty of Medicine, Assiut University, Assiut, Egypt, thirty adult healthy Wistar male albino rats weighing 150 to 180 gm were used in this study. This manuscript adheres to ARRIVE (Animal Research: Reporting of in vivo Experiments) guidelines. Rats were purchased from the animal house, Faculty of Medicine, Zagazig University and were housed under standard laboratory conditions at temperature 21 ± 2 . They were maintained on standard laboratory food and water ad libitum throughout the period of the experiment.

Experimental design

Rats were divided into three equal groups (Ten rats each group):

Group I (Control group): Each rat received an injection of sterile Intraperitoneal (IP) normal salinein a similar volume to IP ketamine given in group II and III.

Group II: Received ketamine in a dose of 120 mg. Kg^{-1} body weight single intraperitoneal injection.

Group III: Rats received ketamine in a dose of 160 mg. Kg^{-1} body weight single intraperitoneal injections.

We have chosen such ketamine dose based on previous study performed by Kalkan YILDIRAY, et al. [6] administered intraperitoneal ketamine injection in rats up to 100 mg. Kg^{-1} twice daily for two weeks to investigate its long term hepatic effects, while in this study we used single intraperitoneal dose of 120 mg. Kg^{-1} and 160 mg. Kg^{-1} to study the hepatic effects of such doses [6].

Histological study

Three days after injection, rats were decapitated and the liver of each animal was dissected, excised, cut into smaller pieces (1 cm3) and processed for light microscope examination to prepare paraffin blocks. Liver specimens were immediately placed in 10% buffered formalin. After 10 min when the tissue was hardened, specimens were fixed in 10% buffered formalin for 24 hrs and processed to prepare 5 µm sections stained with Haematoxylin and Eosin and Mallory trichrome [15].

The immunohistochemical staining for the localization of CoxII was carried out by Avidin-Biotin peroxidase Complex method [16] following the manufacturer's instructions (Dako Company, Wiesentheid/Bavaria, Germany, Biotin blocking system and code X0385).

Image analysis: Sections stained with immunohistochemical reaction were examined by image analyzer computer system to measure the mean area percentage of collagen fibers and optical density of Cox II expression. The data were obtained using image analyzer computer system using the software Leica Qwin 500 (Leica Microsystems Imaging Solutions Ltd, Cambridge, UK) at Pathology Department, Cairo University. The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the Olympus microscope (CX41) and controlled by the Leica Qwin computer software. The image analyzer was first calibrated automatically to convert the measurement unit (pixels) produced by the image analyzer program into actual micrometer units for each section.

Biochemical study

After three days blood samples were obtained from the tail vein to measure liver enzymes [8,17]. Blood samples were collected into tubes containing Ethylene Diaminetetraacetic Acid (EDTA) and centrifuged at 2000 g for 10 min. Plasma was separated and then stored at 2 until analyzed for liver enzymes Aspartate Transaminase (AST) and Alanine Transaminase (ALT).

Statistical analysis: Data are presented as mean and standard deviation. All results were analyzed with SPSS version 20 for Windows (Chicago, IL, USA). Data presented as mean and SD. Data were analyzed by using analysis of variance (ANOVA) test and post hoc Tukey's test to compare between groups.

Results

Examination of H and E stained tissue samples showed normal hepatic architecture in Group I (Figure 1a). While, in group II hepatocytes were mildly affected. Some hepatocytes with small dark pyknotic nuclei and intact basophilic cytoplasm were observed. Congested blood sinusoids and proliferation of bile ducts were noticed (Figure 1b).

In group III, hepatocytes were markedly affected with loss of normal hepatic architecture. Some hepatocytes appeared with pyknotic nuclei. Prominent Kupffer cells, congested blood sinusoids and subcapsular hemorrhages were also detected (Figure 1c-e). Bile duct proliferation noticed around the congested portal vein with pre-portal cellular infiltration and fibrosis (Figure 1).

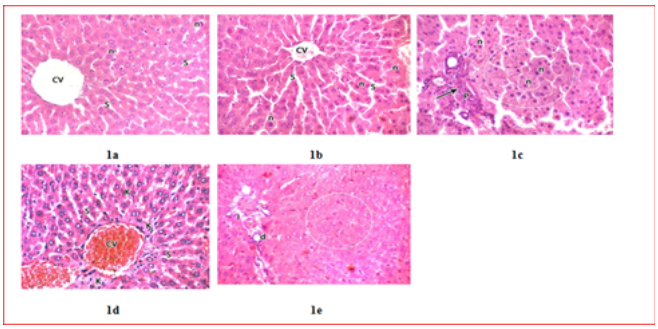

Figure 1. Hematoxylin and Eosin stained (H & E) sections of the three groups [group I (control group); group II (120 mg. Kg^{-1} intraperitoneal ketamine); group III (160 mg. Kg^{-1} intraperitoneal ketamine). 1a) H and E stained sections of Control Group shows normal liver architecture with tightly packed cords of hepatocytes with vesicular nuclei (n) radiating from Central Vein (CV) and separated by blood sinusoids (s) (H & E X400). 1b) H and E stained sections of Group II shows hepatocytes with darkly stained nuclei (n) radiating from Central Vein (CV) and separated by dilated congested blood sinusoids (s) (H & E X400). 1c) H and E stained sections of group III showing loss of normal liver architecture (circle), hepatocytes with darkly stained nuclei (n) and fibrosis (arrow) in portal area (p) (H & E X400). 1d) H and E stained sections of group III showing prominent kupffer cells (k), congested central veins (cv) and blood sinusoids (s) (H & E X400) and 1e) H and E stained sections of group III showing complete loss of normal liver architecture (circle), Bile duct proliferations (d) are noticed around the congested portal vein with preportal cellular Infiltration (I) and fibrosis. (H & E X400).

Mallory trichrome revealed mild increase in the connective tissue in group II, but notable increase in group III was seen in the portal area. The optical density of the reaction increased in group II and III in comparison to the control group (Figure 2a-c).

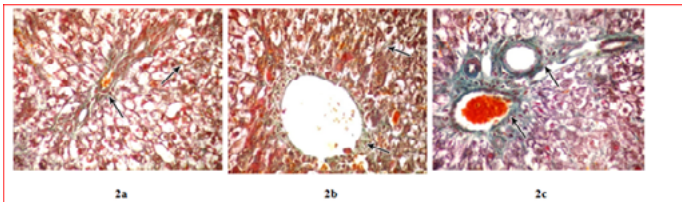

Figure 2. Mallory trichrome stained sections of the three groups [group I (control group); group II (120 mg. Kg^{-1} intraperitoneal ketamine); group III (160 mg. Kg^{-1} intraperitoneal ketamine)]. 2a) Mallory trichrome stained sections of control group showing normal collagen fibers distribution (arrow) (Mallory trichrome X400). 2b) Mallory trichrome stained sections of group II showing mild increase in the collagen fibers (arrow) (Mallory trichrome X400) and 2c) Mallory trichrome stained sections of group III showing notable increase of collagen fibers in the portal area (arrow) (Mallory trichrome X400).

Immunohistochemical stained sections of Cox II (Figure 3) revealed mild reaction in the cytoplasm of few cells of group I (Figure 3a), moderate reaction in the cytoplasm of some cells of group II (Figure 3b) and marked reaction in the cytoplasm of most cells of group III (Figure 3c). Mean optical density of immune reaction of Cox II was significantly higher in group II and III compared to group I (Figure 4).

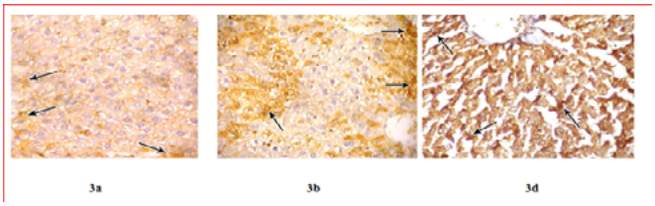

Figure 3. Immunohistochemical stained sections of the three groups [group I (control group); group II (120 mg. Kg^{-1} intraperitoneal ketamine); group III (160 mg. Kg^{-1} intraperitoneal ketamine)]. 3a) Immunohistochemical stained sections of group I showing mild immune reaction in the cytoplasm of few for Cox II (arrow) (Immunoperoxidase technique for Cox II, X400). 3b) Immunohistochemical stained sections of group II showing moderate immune reaction in the cytoplasm of some cells for Cox II (arrow) (Immunoperoxidase technique for Cox II, X400). 3c) Immunohistochemical stained sections of group III showing marked immune reaction in the cytoplasm of most cells for Cox II) (arrow) (Immunoperoxidase technique for Cox II, X400).

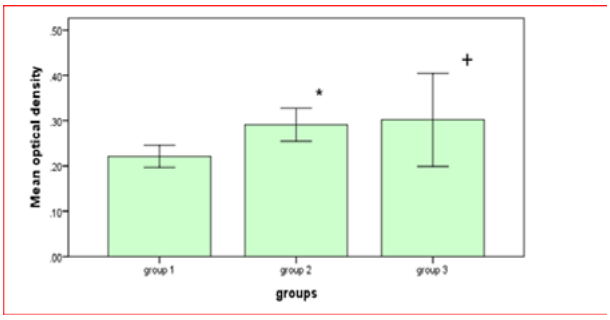

Figure 4. Mean and SD Optical density of immune reaction to COX II of the three groups [group I (control group); group II (120 mg. Kg^{-1} intraperitoneal ketamine); group III (160 mg. Kg^{-1} intraperitoneal ketamine)]. Analysis of Variance (ANOVA), followed by Tukey's test *P<0.05 (group I vs. group II), +P<0.05 05 using Tukey's test (group I vs. group III), No significant difference between group II and III.

Mean level of liver enzyme Aspartate Transaminase (AST) increased significantly in group II and III when compared to group I (Figure 5). In addition, there was a significant increase in mean Alanine Transaminase (ALT) level only in group III when compared to group I and II (Figure 6).

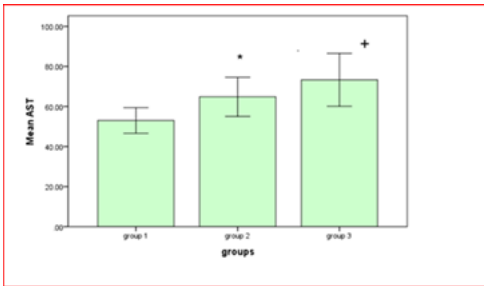

Figure 5. Mean and SD of Aspartate aminotransferase (AST) ($U.L^{-1}$) of the three groups [group I (control group); group II (120 mg. Kg^{-1} intraperitoneal ketamine); group III (160 mg. Kg^{-1} intraperitoneal ketamine)]. Analysis of Variance (ANOVA), followed by Tukey's test *P<0.05 (group I vs. group II), +P<0.05 05 using Tukey's test (group I vs. group III), No significant difference between group II and III.

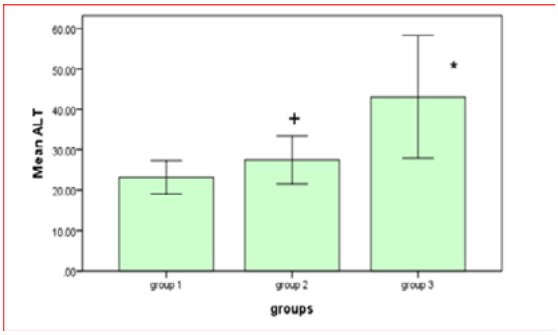

Figure 6. Mean and SD of Alanine Aminotransferase (ALT) ($U.L^{-1}$) of the three groups [group I (control group); group II (120 mg. Kg^{-1} intraperitoneal ketamine); group III (160 mg. Kg^{-1} intraperitoneal ketamine)]. Analysis of Variance (ANOVA), followed by Tukey's test *P<0.05 (group I vs. group III), +P<0.05 05 using Tukey's test (group II vs. group III), No significant difference between group I and II.

Discussion

The results of this study demonstrated that single dose of intraperitoneal ketamine of 120 mg. Kg^{-1} and 160 mg. Kg^{-1} caused significant hepatic damage in adult male albino rats. Several human and animal reports have detected different hepatotoxic effects during long term administration of ketamine [5,6,10,14,17-19]. In addition, vague abdominal pain and epigastric pain with or without vomiting have been observed in ketamine abusers [20,21].

Such hepatic damage in this study varied from dark stained nuclei of some hepatocytes and dilated congested sinusoids in group II to loss of normal liver architecture and dark stained nuclei together with cellular infiltration, congested central veins and sinusoids, periportal fibrosis and bile duct proliferation in group III. This dark stained pyknotic nuclei can be explained as the first stage of apoptosis in which nuclear changes proceed cytoplasmic changes. Similarly, Wai MSM, et al. revealed fatty degeneration, fibrosis and rise of liver enzymes in animals treated with increasing doses of ketamine. Such effects were more severe if animals were also received alcohol [22]. Moreover, Kalkan YILDIRAY, et al. [6] observed apoptotic cells in rats in chronic ketamine hepatotoxicity. They explained apoptotic cell death to occur due to mitochondrial degeneration in hepatic cells [6]. Apoptosis in chronic ketamine hepatotoxicity is correlated to the generation of Reactive Oxygen Species (ROS) which promote lipid peroxidation, alter calcium homeostasis with subsequent apoptosis activation [6,19].

In addition, vascular changes appeared in group II and were more prominent in group III of this study. Such changes were in the form of congested central veins, congested dilated sinusoids, prominent kupffer cells and cellular infiltration. There is growing evidence from animal models that kupffer cells may be involved in various liver diseases such as viral hepatitis, steatohepatitis, alcoholic liver disease, intrahepatic cholestasis, activation or rejection of the liver during liver transplantation and liver fibrosis. Furthermore, it may have a role in hepatocytes destruction [23]. Others have suggested that ketamine related vascular vasodilatation can be explained either by nitric oxide synthesis-cyclooxygenase mediated mechanism or by the of reduction of calcium in vascular smooth muscle cell [5,6,10].

Also, cholangiopathy, dilated common bile duct, obstructive jaundice, biliary tract abnormalities and cirrhosis have been detected in chronic ketamine users [10,18,24,25]. The mechanism by which ketamine produces biliary dilatation and duct proliferation is unknown, but may be related to N-Methyl-D-Aspartate (NMDA) receptor blockade in smooth muscle [26]. In addition, Ketamine may have an effect through dorsal motor nucleus of the vagus nerve, which possesses fibers that project to the gall bladder [26]. In this work, dose dependent increase in the optical density and in the immunoreactions of Cox II enzyme appeared in group II and III.

Chuang SM, et al. [27] found that Ketamine treatment groups had interstitial fibrosis and accelerating macrophages infiltration in bladder tissue. Ketamine also initiated the up-regulations of Cyclooxygenase-II (Cox-II) and Nitric Oxide Synthase (iNOS and eNOS) expressions. Such up-regulated enzymes might play an important role to ketamine-induced alterations in micturition patterns and ulcerative cystitis [27]. In addition, it has also been reported that ketamine stimulated the expression of NF-XB which was a necessary transcription factor for Cox-II gene activation and its translocation from cytoplasm to nucleus. They also found that CoxII inhibitor reduces the intensity of fibrosis and bladder dysfunction in ketamine treated rats [28].

In this study mild elevation of AST appeared in rats that received 120 and 160 mg. Kg^{-1} ketamine while mild increase ALT occurred in rats which had 160 mg. Kg^{-1} ketamine. Similarly, it has been reported that short period of ketamine infusion [8] as well as its prolonged use can cause increase in liver enzymes [17,22]. In addition, transient liver function abnormalities have been

observed in patients receiving intermittent ketamine infusions for chronic pain management [7,10,29]. Noppers IM, et al. [7] ended his study because of the hepatotoxic effects of ketamine that has been developed in three patients out of thirteen while using ketamine for chronic pain management. All the three patients had elevated liver enzymes and two of them suffered itchy rashes, one of whom had petechiae [7]. The exact mechanism for liver injury is not clear, but it may be due to reduced hepatic oxygen delivery and in cased lipid peroxidation with free radical formation [10].

Conclusion

It is clear from this study that ketamine can cause variable histological changes and damage in the liver of adult male albino rats after single dose intraperitoneal of 120 mg. Kg^{-1} and 160 mg. Kg^{-1}. Also, ketamine should be used cautiously in patients with liver impairment.

Acknowledgement

None.

Conflict of Interest

None.

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