Histological and Physiological Study of the Effects of Exogenous Melatonin on Liver of Female Rats Exposed to Formalin in Drinking Water

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Abstract

Aim: The presented study was established to evaluate the impact of formalin (FA) on the blood levels of superoxide dismutase (SOD), lipid profile, total serum protein and serum albumin in female rats. **Materials and methods:** The animals were assigned randomly into groups of six; control group, and treated other five groups as follow, first group exposed to formalin at 200ppm (F200 group), the second exposed to formalin at 400ppm (F400 group), third group exposed to formalin at 200ppm plus melatonin at 10mg/kg (F200M group), fourth group exposed to formalin at 400ppm plus melatonin at 10mg/kg (F400M group), and the fifth exposed to melatonin at 10mg/kg (M group). Samples of blood from the rats were collected at day one, day and day 30 of the experimental period that was continued for 30 days. **Results:** Using immune-based techniques, the SOD activity, it has been here-resulted in significant ($p<0.05$) after-formalin-exposure-decreases in the blood samples of female rats from F200 and F400 groups, especially, when compared with the control group. The total cholesterol levels revealed significant ($p<0.05$) increases in the blood of the tested animals of the F200 and F400 groups. These increases affected the rates of LDL, VLDL, and triglycerides in the blood of the studied rat-females of both F200 and F400 groups when compared with the control animals. Interestingly, low levels of HDL were noticed in the blood of the animals of the F200 and F400 when comparisons with control animals. The concentrations of the LDL, HDL, and triglycerides in the serum of the formalin-exposed females showed significant ($p<0.05$) low levels when compared with control animals. For day-30 collection, albumin concentrations revealed significant ($p<0.05$) elevation in the blood of the F200 and F400 animals.. Histological examination revealed that the liver of female rats exposed to 200ppm or 400 ppm of formalin for 30 days have been adversely affected. Whereas, the histological examination of the liver of animals treated with melatonin and formalin were less affected due to formalin exposure. **Conclusions:** These results demonstrate the formalin high toxic effects on the hepatic tissues can be overcome by the use of exogenous melatonin.

Keywords: *Antioxidant, formaldehyde, hormones, melatonin.*

Introduction

Environmental contaminants cause direct toxic effects such as endocrine disruption, that are altering the normal hormonal environment and reproduction due to exposed to exogenous chemicals. They alter the metabolism, synthesis, and gene expression of hormones, creating critical situations for the development of the body[(1)]. Formaldehyde (FA) is one of the substances that caused pollutant in many routs .FA can generate irritation toxicity that affects body organs such as eye and respiratory tract with bronchoconstriction and fluid accumulation in these tracts [(2)]. FA is a potent sensitizer and a carcinogenic effect.

666 According to the foregoing, it became clear that there are many ways to be exposed to formalin and the risks it poses to public health, to show the health effects of the wrong use of formalin in food preservation that randomly imported into the country.

Also the study examined the role of melatonin in preventive or resistance the harmful effects of formalin on experimental animals. The presented study was established to evaluate the impact of formalin (FA) on the blood levels of superoxide dismutase (SOD), lipid profile, total serum protein, serum albumin, follicle stimulating hormone (FSH), luteinizing hormone (LH), and estrogen (E2) in female rats. [3]

Materials and Methods

Animals: The present study was conducted at Science Collage, University of Wasit from November, 2018 to April 2019 . A total number of 36 adult albino rats, weighting 175-250 g and 10-14 weeks age were used in the current study.

Experimental Design: The present study was conducted on 36 adult female rats . Animals were randomly divided in to six equal groups ; each group consisted of 6 adult female rats as in the following :

1-Control group : orally dosed with distilled water .

2-Group 2: orally dosed from formalin 200ppm for 30 days

3- Group 3:orally dosed from formalin with 400ppm for 30 days

4- Group 4:orally dosed from formalin with 10mglkg from melatonin for 30 days.

5-Group 5:dosed from formalin 400ppm with 10mg\ kg of melatonin for 30 days

6-Group 6:10mg/kg dosed of melatonin only for 30 days

Sample Collection: At the end of the tests all the animals were killed. The rats were first weighed before sacrifice and then anesthetized by cotton loaded with anesthesia of diethyl ether in a specific box. A midline abdominal incision was used to open the abdominal cavity to take the sample which includes: Cardic puncture for blood collection was entitled [4]. Liver tissue samples were collected.

Blood analyses :cholesterol Gen 2, and triglycerides TRIGL were measured relying on the kits used and their protocols.

Histological techniques:

The tissue (liver organs) have been extracted from both classes in the following phases and ready for histological research using hematoxylen and eosin stain[5].

Statistical Analysis

Data is evaluated with a mean ± SD. Comparisons of classes were rendered utilizing the SPSS (Statistical System for Social Sciences) with the study of variation (ANOVA). The minimum value maximum was found to be $P<0.05$. Last big check (LSD). The gap between measures was decided for (ANOVA) SPSS [6] .

Results and Discussion

Symptoms in female rats after treated with formalin in both concentration 200,400ppm During the last (30) days of the experiment ,which is by administering dose of formalin treatment at the concentration of (200,400) ppm. some symptoms and signs were observed on the animals of the group. This includes hair colour shifting from white to yellow and also general weakness of the body which is the weakness of teeth that begins to break down or gets yellow. These animals were perished in relation to the second , third, fourth and fifth group ,but the second and third group have more symptoms than the fourth ,fifth group , because the fourth and fifth groups have formalin and also Melatonin, while the sixth group is treated with melatonin only . predation is noticed among them ,binge eating and strength in body. The current study showed a reduction on serum SOD activity. It was recorded a reduction in SOD activity and increase in lipid peroxidation in experimental animals after exposure to formaldehyde . Oxidative stress derived secondary toxic actions might be induced by FA. SOD lowered by formaldehyde due to elevations of some free radicals. Irreversible toxicity may be performed for long time exposure to FA. The catalase, by FA, GSH reduced and no recovery levels were seen in which secondary toxicity induced via oxidative stress was conducted. SOD was significantly higher in group had low dose of formaldehyde with melatonin (GIV) than those of high dose (GV), and compared with those groups had FA only, this result may be the role of antioxidant capacity of melatonin as indicated by previous researchers who concluded the role of melatonin in partially prevent

the liver damage against HCHO intoxication. Broad-spectrum antioxidant effects of melatonin can be seen to eradicate free radicles [7].

Current study showed that exposure to F200 and F400 results in significant increase in cholestrol, TG, LDL-c, VLDL significantly in treated female rats. High rates of LDL and VLDL increases the incidence chances of cardiovascular disease considered to be a major risk factor for chronic disease such as coronary heart disease and hypertension and some types of cancer , while the HDL ratio decreased significantly, which is a result of the previous studies , as it was found by previous researchers that formalin increases the oxidation pressure that leads to high levels of fat. Formalin substance increases the pressure of oxidation as the generation of free radicals that attack the fat of the cellular membranes increases , leading to the generation of malondydehyde , which in turn leads to the destruction of vessels and tissues [8] . HDL decreases in the treated animals with formalin at 200 or 400 can be expected in which certain damages may have been introduced to those pathway components leading to the disturbances in the metabolisms of those lipids showing lower levels of HDL in the blood of the tested rat females.This increase may be due to the effect of formalin on the permeability of the hepatic cell membranes or it may be due to the blockage of the hepatic canal. Bile , which leads to reduced or stopped cholesterol release in the duodenum. LDL is oxidized by the oxygen free radicals in the arteries and converted in to foamy lesions or atherosclerosis [9]

Current study showed that exposure to F200 with 10mglkg from melatonin and F400 with 10mglkg from melatonin results in significant decrease in cholestrol, TG, LDL-c, VLDL and increase of HDL significantly in F200 and F400 groups, whereas these values were normal with 10mglkg from melatonin compare with control group. On the other hand melatonin removes the oxygen free radicals and prevents oxidative changes. LDL is generally a blood lipid disorder and plasma lipid alteration among the risk factors for cardiovascular disease .More attention has recently been paid to melatonin to improve lipid profiles. The mean effects of melatonin on plasma lipids and lipid peroxide level have been reported in previous studies. Studies were also conducted on this hormone on the physiological changes resulting from high-calorie and fat diets. The use of melatonin accompanied by zinc acetate leads to a significant decrease in the level of cholesterol in the blood and TG , LDL .Melatonin probably improves the features of fats from by reducing the oxidation of fats and other necessary proteins by free radicals according to the results of some studies, there are several mechanisms that can be suggested for this action of melatonin : Interactions with LDL-c receptors , inhibition of biosynthesis of cholesterol and LDL-c accumulation , analgesia of lipase protein and reduces lipoprotein activity of the accumulation of TG rich in very LDL , VLDL molecules and decreases in intestinal cholesterol absorption or inhibition of transport of fatty acids through metabolism receptors [10].

Table (1): The effects of formalin, melatonin and formalin with melatonin on serum SOD concentrations mmol/L in mature female rats.

Table 1: Lipid, sex-hormone, and superoxide dismutase profiles in the study groups at day one of sample collection.

Group	Cholesterol	HDL	LDL	SOD	Triglycerides	VLDL
Control	54.86	24.319	19.56	247.77	58.66	11.731
F200	55.01	24.242	19.42	243.78	56.75	11.351
F400	54.4	23.964	19.01	243.51	57.13	11.426
F200M	55.13	23.601	20.16	246.67	56.82	11.364
F400M	54.29	23.202	19.88	249.52	56.06	11.21
M	54.96	24.084	19.41	246.53	57.42	11.482
LSD	N.S.	0.5289	N.S.	3.143	1.011	0.2023

LSD: Least significant difference; N.S.: Non-significant

Table 2: Lipid, sex-hormone, and superoxide dismutase profiles in the study groups at day 15 of sample collection.

Groups	Cholesterol	HDL	LDL	SOD	Triglycerides	VLDL
Control	55.18	24.5	18.87	247.06	59.07	11.814
F200	59.86	20.74	25.84	201.05	66.39	13.279
F400	70.34	18.18	37.59	181.93	72.82	14.563
F200M	58.82	22.28	24.66	238.73	59.42	11.884
F400M	59.89	19.89	27.44	214.98	62.76	12.552
M	51.21	26.29	14.44	266.51	52.43	10.487
LSD	1.19	0.756	1.266	2.826	1.307	0.2614

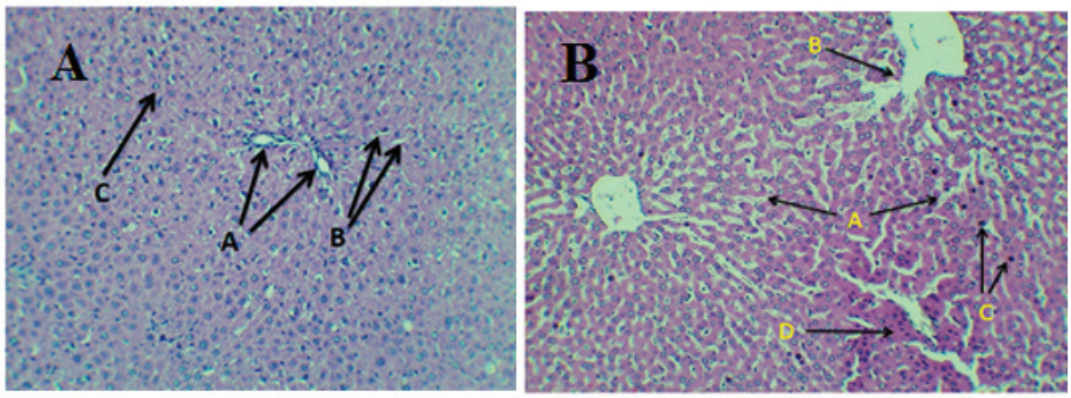

Figure 1 : A- Images microscope represent liver of rat control central vein (A), sinusoidal spaces (B hepatocytes (C).100X(H&E) , B- Images microscope represent liver of rat exposed to formalin 200 ppm at day 30 of the experiment appeared loss of sinusoidal spaces(A), rupture of central-vein (B), pycnotic nuclei(C) and infiltration of lymphocytes(D). 200X (H&E)

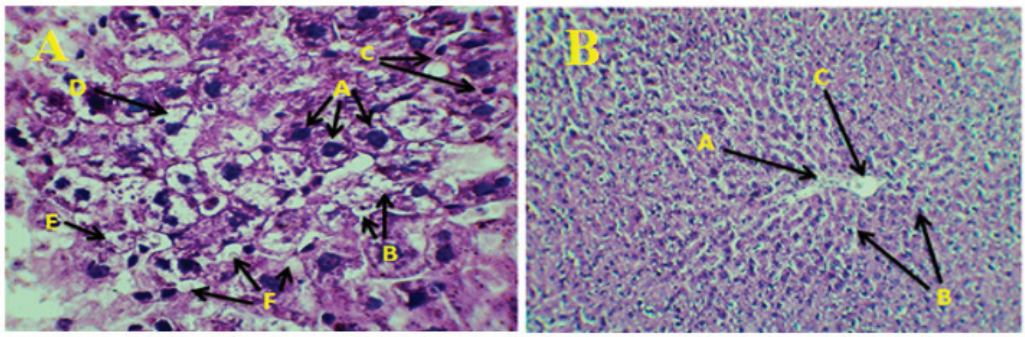

Figure 2 : A-Images microscope represent liver of rat exposed to formalin 400 ppm at day 30 of the experiment appeared pycnotic nuclei(A), degeneration of hepatocytes with numerous vacuolations (B), infiltration of lymphocytes(C), absent nuclei of hepatocyte (D), nuclear-material clumping(E) and necrosis of hepatic cells(F). 400X (H&E), B- Images microscope represent liver of rat exposed to formalin 200 ppm and melatonin 10 mg/kg at day 30 of the experiment less severe central vein congestion (A), less dilated sinusoidal spaces (B) and less impacted endotheliallining(C).200X(H&E)

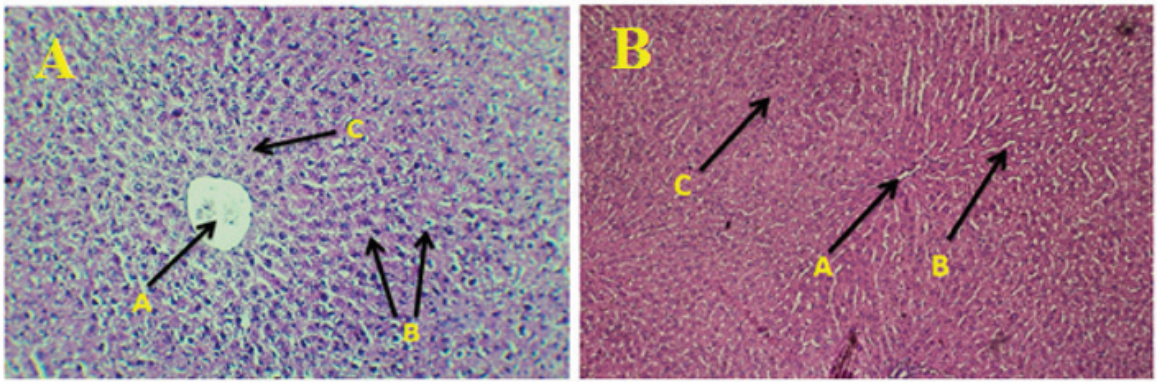

Figure 3 : Images microscope represent liver of rat exposed to formalin 400 ppm and melatonin 10 mg/kg at day 30 of the experiment less severe central vein congestion (A), less dilated sinusoidal spaces (B) low levels of infiltration of inflammatory cells(C) and no found hemorrhages . 20X(H&E) B- Images microscope represent liver of rat exposed to melatonin for 10mg/kg at 30 days of the experiment normal central vein (A), normal sinusoidal spaces (B) normal hepatocytes (C).40X(H&E)

The liver of the control group, the liver tissue revealed normal central vein with liver cords of hepatocytes radiating from the central vein and separated by blood sinusoid plus hepatocytes had rounded nuclei indicating normal (without changes) tissues (Fig.1,A) For both F-200 and F-400 groups (Fig. 1,B and Fig2, A) respectively, at day-30 sample collection time, loss of normal architecture, degeneration of hepatocytes with numerous vacuolations, presence of pleomorphic nuclei with different sizes and degeneration levels, loss of sinusoidal spaces, central-vein rupture, hepatic-tissue based scattered inflammatory cells, blood-filled-central-vein dilation, dropped-out-degenerated hepatocytes, mostly displayed pycnotic, absent nucleoli, and nuclear-material clumping. [11] mentioned that FA is extremely soluble and reactive in its contact with mucus or other nucleophilic-group-containing cellular macromolecules, such as amino acids and DNA. FA may interfere-react with DNA, and it is accountable for the genotoxic and carcinogenic results of FA via the construction of bonds between DNA and FA related adducts. This fact are explain the effect of FA on the nucleus.

For the F-200 10mg/kg at day 30 of the experiment (Fig.2, B), the protective treatment of melatonin against the exposure to 200PPM of FA showed a decrease in the histological changes compared to F-200 group that received FA alone, in which less dilation of central vein, less dilation of sinusoidal spaces, less congestion in the central vein, less infiltration of lymphocytes, and less inflammatory cell infiltrations and hepatocyte degenerations were seen in the melatonin treated group compared with those from the F-200 group.

In the case of the F-400 with 10mg/kg group at day- 30 time-point, the histological findings revealed that the exposure to formalin and melatonin resulted in less severe central vein congestion, some restoration of the liver architecture with less impacted endothelial lining, less dilated sinusoidal spaces with close-normal thickness of tunica media, low levels of infiltration of inflammatory cells, smaller sinusoids, low numbers of hemorrhages (Fig.3,A)

(12) concluded that M fought the induced oxidative stress produced by the exposure to FA that increased the tissue toxicity generating lower infiltration of neutrophils and decreasing the 8-hydroxydeoxyguanosine (8-OHdG) levels, which can be useful for the protection against the exposure to FA, and this agrees with the current investigation results.

For the groups that received 10mg/kg melatonin only, the findings of the liver tissues showed normal tissue structure, architecture, compartments (Fig.3,B) Our study is comparable with [13] who mentioned that the melatonin play important role to improvement the histological structures of kidney and liver of mice and give it the normal characteristics for these organs. [14]

mentioned the fact that melatonin is nowadays there has been a certain knowledge of the importance of melatonin in human biology and diseases, but many of its roles and consequences tend to be overlooked. Melatonin is a powerful free radical scavenger, stronger than vitamin E. The extremely toxic hydroxyl radical and other oxygen-based radicals are modified by melatonin effectively. Melatonin also has antioxidant effects: it improves the concentrates of various antioxidant enzymes such as the SOD, glutathione reductase, and glutathione peroxidase. In addition, melatonin suppresses NO synthase, a pro-oxidant enzyme.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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