INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

HISTOLOGICAL AND BIOCHEMICAL CHANGE IN THE LIVER OF MALE ALBINO RATS TREATED WITH DIFFERENT DOSES OF CYCLOPHOSPHAMIDE

Saif. S. Abed Alhassan *, Abed H. Baraaj

University of Baghdad, College of Science, Biology Department, Iraq-Baghdad

*Corresponding Author Email: saifsalah999@yahoo.com

Article Received on: 07/06/18 Approved for publication: 27/06/18

DOI: 10.7897/2230-8407.09691

ABSTRACT

Cyclophosphamide is a synthetic alkylating agent chemically related to the nitrogen mustards widely used as an anticancer and immunosuppressive drug. This study was to investigate the effect of different doses of cyclophosphamide on histological structure and functions of liver enzyme. Use for this purpose 30 of the strain Sprague Dawley albino rats and divided into three groups, each group consisted of 10 rats; Group 1 (control) was injected with physiological solution (normal saline 0.9%), Group 2 (low dose) received 50mg/kg/b.w/weekly of drug and Group 3 (high dose) received 80mg/kg/b.w/weekly of the drug for 10 weeks. At the end of dosage, duration, the animals were killed, the liver was excised for histological study. The blood was collected for study liver enzyme functions. Histological examination of liver tissues of Cyclophosphamide groups showed histopathological changes that increased with the increased dose compared to control group such as macrophages and lymphocytes were an aggregation in portal area with necrotic hepatocytes. As well as odeoma around blood vessels with fibrosis. In addition, multiple aggregation of mononuclear cells scatters in the Liver parenchyma with necrotic hepatocytes and congested central vein. The results of biochemical tests showed significant ($p<0.05$) increase in the treated groups. The results showed a significant ($p<0.05$) increase in Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkalin phosphatase (ALP). The results of the present study indicated that high and low dose of Cyclophosphamide able to induce histological and biochemical change in the liver.

Key words: Cyclophosphamide**,** Reactive Oxygen Species**,** Phosphoramide, Cytochrome P450

INTRODUCTION

Cyclophosphamide undergoes bioactivation by hepatic microsomal cytochrome P450 mixed function oxidase system to active metabolites that enter the circulatory system. Phosphoramide mustard and acrolein are the two active metabolites of cyclophosphamide.[1] Numerous anticancer drugs are known to the generation of Reactive Oxygen Species (ROS) in cancer cells [2] and these ROS generated lead to oxidative damage in the cell.[3] Through bioactivation of CP, reactive oxygen species are also, formed, which can modify the components of both healthy and neoplastic cells, leading to decreased antioxidative capacity.[4]

Cyclophosphamide antineoplastic effects are related to Phosphoramide mustard, while acrolein is associated with its toxic side effects.[5] Cyclophosphamide has been reported to create genotoxicity and oxidative stress in mice.[6] Numerous studies have shown that CP exposure can disrupt the redox balance of tissues and that these biochemical and physiological disturbances resulted from oxidative stress may be implicated in disorders like hemorrhagic cystitis, testicular gametogenic and androgenic disorders, liver and kidney disorders, inhibition of ovarian steroidogenesis, etc.[7] Therapeutic doses of cyclophosphamide are restricted by the onset of liver and kidney toxicity.[8]

The pathology of cyclophosphamide toxicity includes a substantial reduction in smooth endoplasmic reticulum, increase autophagocytosis and sequestration of glycogen and progressive loss of structural density in the mitochondria.[9] Cell structure and function is essentially disrupted under conditions of oxidative stress and can be discovered in changes in the carbohydrate, lipid, and DNA profile of the affected tissues.[10]This study was aimed to investigate the effect of different doses of cyclophosphamide on histological structure and functions of liver enzymes.

MATERIALS AND METHODS

Preparation of cyclophosphamide drug

Cyclophosphamide (Endoxan) produced by (Baxter international company U.S), was used by injecting 50 mg/kg of body weight/week as low dose and 80 mg/kg of body weight/week as high dose. It injected with animal intraperitonially for 10 weeks.

Experimental Animal

The experimental animals used in this study were male albino rats, Rattus norvegicus weighing (230 – 260 g), and at the age of 3 months. The animals were purchased from Pharmaceutical control of the Ministry of Health in Baghdad. Animals were given food and water *ad libitum*. Rats were maintained in a friendly environment with a 12 h/12 h light-dark cycle at room temperature (22 °C – 25 °C). Rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment.[11]

Experimental Design

In the present investigation, 30 male rats divided into three groups, each group consisted of 10 rats as follows:

Group I (control) Injected orally with normal saline.

Group II (low dose) injected intraperitonially with CP (50mg/kg/b.w) one time/week for 10 weeks.

Group III (high dose) injected intraperitonially with CP (80mg/kg/b.w) one time/week for 10 weeks.

Collection of organ

The male albino rats were anesthetized with diethyl ether for several minutes. The rats were dissected and the liver was excised and washed with normal saline (0.9 % NaCl).

Stains

Using a colored Eosin was prepared according to procedure .[12]
Ehrlich Haematoxylin a colored Haematoxylin was prepared according to the procedure. [12]

Solutions
1. Absolute ethanol… 100%
2. Normal saline 0.9% (NaCl) it was prepared by dissolving 9 g NaCl in 1000 ml distilled water. [12]
3. Neutral Buffered 10 % Formalin the contents mixing together. [12]
4. Mayer's albumin was prepared by shacking 50ml of egg albumin with 50 ml of glycerin and 1gm of thymol and then the mixture was filtered. [12]
The preparation of histological sections was performed on standard methods as follows:

Fixation
The liver and kidney were fixed in formalin (neutral buffered 10% formalin) for 48hr.

Washing
Samples were washed with tap water for three minutes before, the process of dehydration.

Dehydration
The samples were dehydrated by passing through progressively concentration of ethanol (70%, 80%, 90%, 95% for 1hr. Per each concentration and 100%, twice) overnight.

Clearing
The samples were cleared by passing through two changes of xylene for 30 min per each change.

Infiltration
The samples were placed in a mixture of xylene and melted paraffin wax at 60°C with ratio 1:1 for 30 min in electric oven at temperature 60°C and then the samples were left in paraffin wax through two changes for 30 min per each change.

Paraffin embedding
Pure melted paraffin wax was poured out gently in the L shape metal template, and then the tissue sample was transferred to this template and hot needle was passed nearby the sample to remove the foam and bubbles around and left to solidify. The paraffin blocks at metal and kept in the refrigerator.

Sectioning
The paraffin blocks were cut by rotary microtome into sections at 5-6 µ in thickness, and the ribbons of these sections were floated on distilled water for 30 second in water bath at 50°C. The section was picked up with clean slide after place one drop of Mayer's albumin on the slide and spread, then fixing the section by using a hot plate.

Staining
The following steps performed it: Deparaffinizing by putting the slide basket in electric oven at 60°C for 20 min, then passing through three changes of xylene for dewaxed in electric oven too, for ten min per each change.

Rehydration by passing through regressive concentrations of ethanol alcohol 100% for five min, 90%, 80% and 70% for 30 Sec per each concentration, then rinsing in tap water for two min.Hematoxylin and Eosin Stain (H&E) (which stained the nucleus with blue color and cytoplasm pink to red color,staining with Ehrlich's hematoxylin stain for 3–5 min, rinsing with running tap water for 2-3 min., staining with eosin stain for 30 Sec. and dipping in tap water for two min. Dehydration, by passing through progressive concentrations of ethanol alcohol 70%, 80%, 90% for 30 Sec per each concentration and 100% for five min.
Clearing by passing through two changes of xylene for 20 min per each change.

Mounting
Sections were mounted by Canada balsam then covered by cover slides and kept at room temperature to dry.

Microscopic examination
The sections were examined under a light microscope and then photographs were taken from the microscope immediately by Digital camera.

Liver function tests

Estimation serum activity of ALT
Kit from Agappe Company (India) was used in this step to estimate ALT enzyme activity in serum.[13]

Estimation serum activity of AST
Kit from Agappe Company (India) was used in this step to estimate AST enzyme activity in serum.[14]

Estimation serum activity of ALP
Kit from Agappe Company (India) was used method to estimate ALP enzyme activity in serum.[15]

RESULT

Histological examination

Control group animals

The control animals show the normal of central vein and hepatocytes with Kuepffer cell (Figure 1).

Animals treated with CP (50 mg/kg)

Histological section in the Liver of animal treated with low dose of cyclophosphamide (50mg/kg b.w.) Shows macrophages and lymphocytes were an aggregation in portal area with necrotic hepatocytes (Figure 2). As well as there is massively congested of blood vessels with ballooning degeneration in hepatocytes (Figure 3). The microscopic examination also shows odeoma around blood vessels with fibrosis (Figure 4).

Animals treated with CP (80 mg/kg)

Histological section in Liver of animal treated with high dose of cyclophosphamide (80mg/kg b.w.) show a small multiple aggregation of mononuclear cells scatter in the Liver parenchyma with necrotic hepatocytes and congested central vein (Figure 5). In other animal shows deformed architecture and massive necrotic area in the Liver parenchyma with hemosiderin (Figure 6). The liver of animal treated with high dose cyclophosphamide (80mg/kg b.w.) shows granulomatous lesions consisting from the aggregation of macrophages and mononuclear cells in portal area with ballooning degeneration of hepatocytes (Figure 7).
Liver functional Parameters

Administrations of cyclophosphamide show a significant increase in liver enzymes at 10 weeks.

Serum Alanine aminotransferase (ALT)

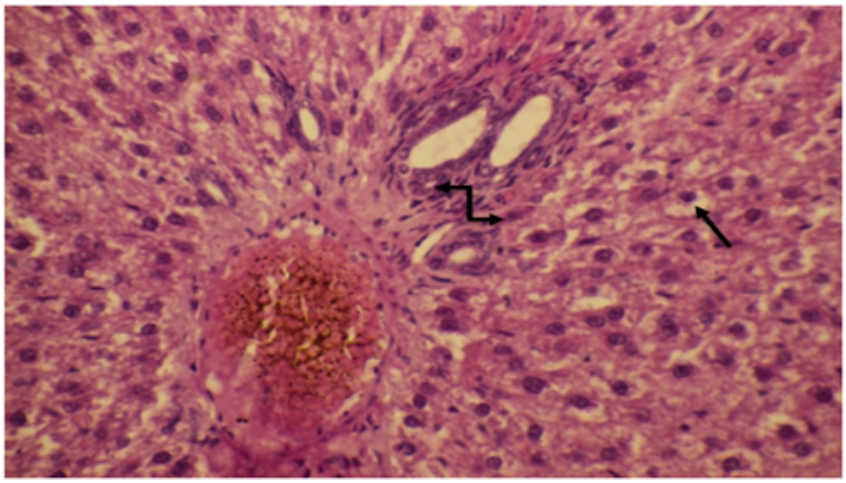
The results in (Table 1) show a significant ($P < 0.05$) increase in ALT of cyclophosphamide low dose group (50 mg/kg b.w.) was (39.262±4.324) IU/L and CP high dose group (80 mg/kg b.w.) was (40.049±5.741) IU/L compared with the control group was (33.830±4.551) IU/L.

Serum Aspartate aminotransferase (AST)

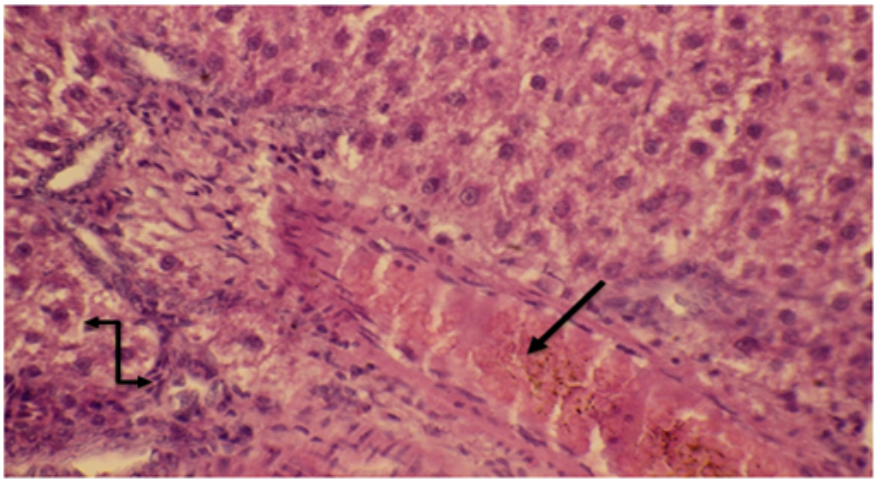
The results in (Table 1) show a significant ($P < 0.05$) increase in AST of cyclophosphamide low dose group (50 mg/kg b.w.) was (58.852±5.992) IU/L and CP high dose group (80 mg/kg b.w.) was (60.373±7.547) IU/L compared with the control group was (47.750±6.990) IU/L.

Serum Alkaline phosphatase (ALP)

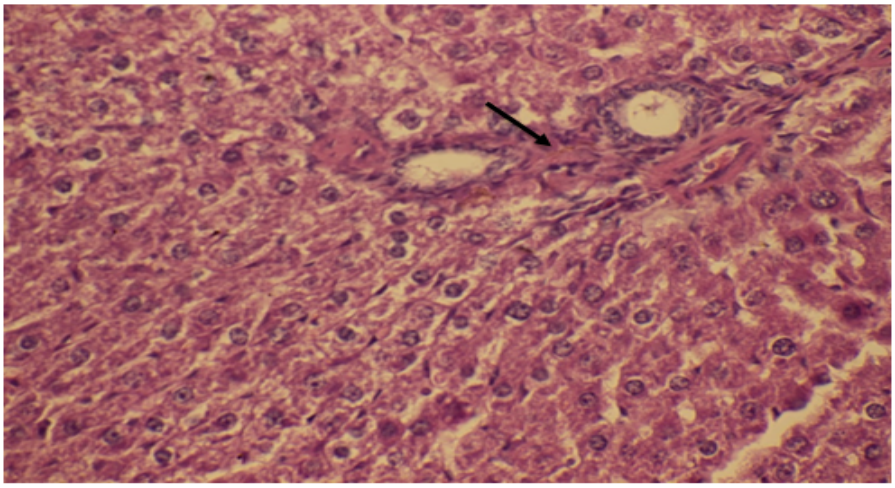
The results in (Table 1) show a significant ($P < 0.05$) increase in ALT of cyclophosphamide low dose group (50 mg/kg b.w.) was (232.25±10.87) IU/L and CP high dose group (80 mg/kg b.w.) was (260.31±12.49) IU/L compared with the control group was (210.02±11.72) IU/L.



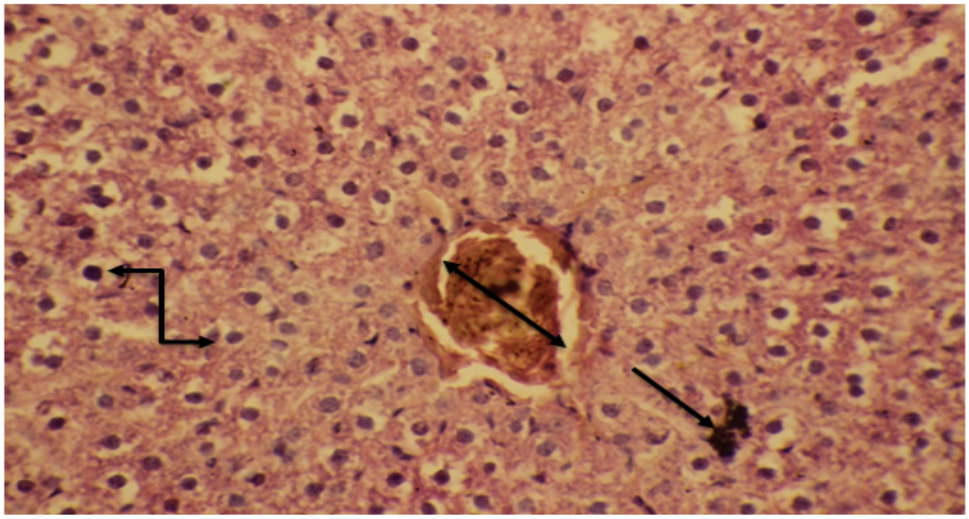
(Figure 2) Cross section in the Liver of animal treated with cyclophosphamide (50mg/kg b.w) Shows macrophages and lymphocytes were aggregation in portal area with necrotic hepatocytes (H&E 40X).



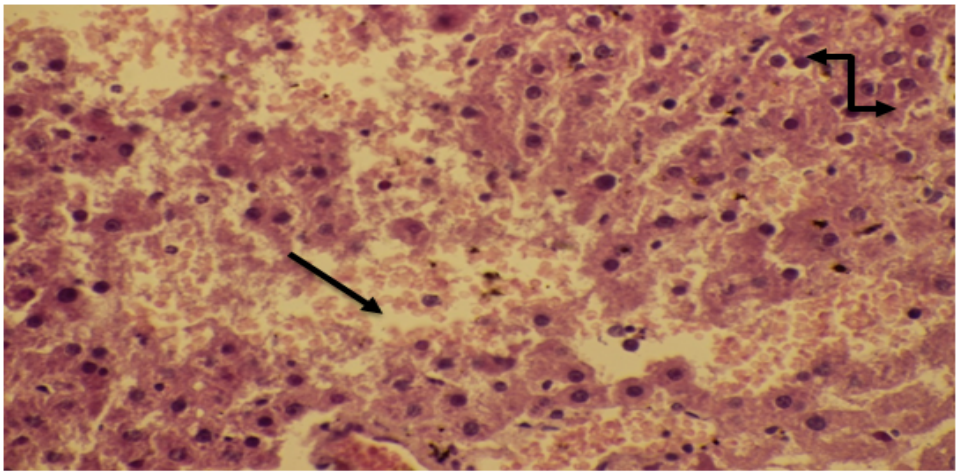
(Figure 3) Cross section in liver animals treated with cyclophosphamide (50mg/kg b.w) Show massive congested of blood vessels with ballooning degeneration in hepatocytes (H&E 40X).



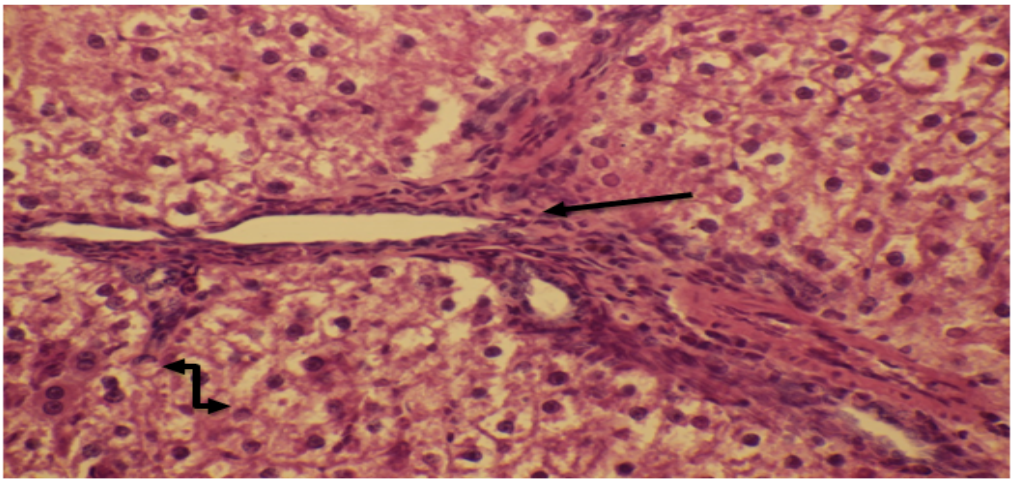
(Figure 4) Cross section in the liver treated with cyclophosphamide (50mg/kg b.w) shows odeoma around blood vessels with fibrosis ↘(H&E 40X).



(Figure 5) Cross section in Liver of animal treated with cyclophosphamide (80mg/kg b.w.) Shows small multiple aggregation of mononuclear cells scatter in the Liver parenchyma ↘with necrotic hepatocytes ↰↘and congested central ↔ vein (H&E 40X).



(Figure 6) Cross section in the Liver of animal treated with cyclophosphamide (80mg/kg b.w.) Shows deformed architecture and massive necrotic area in the Liver parenchyma with hemosiderin (H&E 40X).



(Figure 7) cross section in the Liver of animal treated with cyclophosphamide (80mg/kg b.w.) Shows granulomatous lesions consisting the aggregationation of macrophages and mononuclear cells in portal area with ballooning degenerationation of hepatocytes (H&E 40X).

Table 1: Effect of Cyclophosphamide on liver enzymes

Groups (Treatments)	Mean ± SD (stander deviation)		
	ALT IU/L	AST IU/L	ALP IU/L
Control	33.830±4.551	47.750±6.990	210.02±11.72
Low dose	39.262±4.324	58.852±5.992	232.25±10.87
High dose	40.049±5.741	60.373±7.547	260.31±12.49
LSD	2.18*	3.19*	18.87*
* (p<0.05)			

DISCUSSION

Histological examination

This result in the present study shows noticeable distortion in the architecture in the liver of animals treated with different doses of cyclophosphamide. The toxic properties of CP are the significance of its being metabolized, which results in the generation of very reactive compounds, including free radicals. [16]

The mechanism of production of ROS takes place when CP metabolized by cytochrome P450 in the liver into the reactive aldehydes chloroacetaldehyde and dichloroethyl. [17]

Previous investigation, which report increased levels of nitric oxide in liver of CP-treated rats; this could be credited to acrolein able to activate both ROS and NOx metabolite production with further production of superoxide anion, hydroxyl radical and hydrogen peroxide during CP oxidative metabolism leading to a depressed antioxidant defense mechanism in different tissues. [18]

Increased levels of nitric oxide in liver of CP-treated rats; this could be credited to acrolein able to that decreased glycogen content could be related to the liver damage induced by the released lysosomal hydrolytic enzymes from the hepatocytes following a stimulated lipid peroxidation.[19]

The cyclophosphamide induced hepatic histopathological changes. CP was confirmed to cause infiltration of acute inflammatory cells and congestion of blood vessels, focal degeneration of hepatocytes, fatty degeneration, hyperproliferation around the deformed bile ducts and dysplasia. [20] Metabolism of cyclophosphamide occurs by the hepatic cytochrome P450 system, which results in sinusoidal obstruction syndrome, which induces necrosis, obstruction, and congestion of the hepatic veins. [21]

Liver functions

In the present study showed increases in liver enzyme parameters such as ALT, AST and ALP. Raised serum levels of these enzymes by CP are indicative of cellular damages and loss of functional integrity of the hepatocyte membrane leading to their leakage into the serum.[22]

Serum ALT and AST values have been described to be influenced by chemical agents having a toxic effect on hepatocytes in the liver, and ALT is considered a more important parameter than AST.[23]

Serum Alkaline phosphatase (ALP) is related to the cell membrane and their increase in the serum is a sign of impairment of intrahepatic and extra-hepatic bile flow (cholestasis), Hepatobiliary injury and overproduction or leakage of ALP.[24]

CONCLUSION

High and low doses of drug Cp caused histological injury, to liver and induced alteration functional parameters of liver enzyme such as ALT, AST and ALP. High dose of Cp (80mg/kg) should be not used because of its more negative effects on the liver compared with low dose of Cp (50mg/kg).

RECOMMENDATION

Studying the effect of CP on the different organs of the body and to find out how appropriate it is to the histological changes.

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Cite this article as:

Saif. S. Abed Alhassan and Abed H. Baraaj. Histological and biochemical change in the liver of male albino rats treated with different doses of cyclophosphamide. Int. Res. J. Pharm. 2018;9(6):64-70 http://dx.doi.org/10.7897/2230-8407.09691

Source of support: Nil, Conflict of interest: None Declared