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Evaluation of histological changes induced by prednisolone and cyclophosphamide in some organs of male albino mice

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Abstract

Prednisolone is a synthetic corticosteroid used to treat various diseases. It is known to be used to treat many conditions such as autoimmune diseases and asthma. Cyclophosphamide is a type of nitrogen mustard therapy that works by alkylation of DNA and is used as an immunosuppressant in rheumatoid arthritis and the treatment of many cancers as well. Due to the wide use of these two drugs, the study aimed to evaluate the histological changes in the liver, kidneys, and small intestine of mice. Seventy-five adult mice aged 8-12 weeks were used which were divided into three groups, the first group was orally dosed with 0.1 mg/kg prednisolone, the second group was orally dosed with 0.1 mg/kg cyclophosphamide, and the third group received orally distilled water for 30 days daily. After 24 hours of the last treatment, the animals were sacrificed and the organs (liver, kidney, small intestine) were taken out and placed in 10% formalin solution until histological techniques were performed. The results of the study showed a statically significant difference at P>0.05 of histological changes in the studied organs represented by necrosis, fibrosis, cell degeneration, congestion, and hemorrhage of blood vessels and inflammatory cells when compared with the control group, and that the highest significant difference for these changes was at grade 1 and 2. Our study confirms that these drugs cause histological changes that differed in severity between organs as well as within a single organ when compared to the control group and that cyclophosphamide causes more histological changes than prednisolone.

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Introduction

After cytokine stimulation of the hypothalamus-pituitary-adrenal, corticosteroids are naturally formed in the adrenal cortex of vertebrates (1,2). It has an essential role in controlling various biological processes involving immune responses, metabolism, reproduction, cell development, and proliferation (3,4). *Prednisolone* is a synthetic steroid named 11,17,21 trihydroxypregna-1,4-diene-3,20-dione that is chemically clarified. It is used to treat many inflammatory diseases, for example, rheumatoid arthritis, systemic lupus erythematosus, and many other inflammatory diseases (5). The corticosteroid action connects the intracellular glucocorticoid receptor, which is then transferred to the nucleus where the receptor-ligand complex binds to specific glucocorticoid-response elements on DNA, thus activating genes that mediate glucocorticoid responses (6,7). These have potent anti-inflammatory effects and reduce the causes of swelling in joints and other organs and the pain (8). Cyclophosphamide is the most widely used to remedy cancer, autoimmune disease, marrow, and blood transplantation (9,10). It is a medication that has a broad extent of clinical applications, especially in autoimmune disorders such as rheumatoid arthritis and vasculitis, and cancer treatment (11). It is an alkylating nitrogen agent composed of $C_7H_{15}Cl_2N_2O_2P$ (6). The target organs are the

causes of different side effects on the gastrointestinal, liver, lungs, heart, urinary bladder, and reproductive system (12). Moreover, Chemotherapy alters the fecal microbiota in patients, reducing species richness and total bacterial capacity (13). Some studies dealt with the use of different concentrations of cyclophosphamide for different periods to know its toxic effects on cells and identify its harmful effect on different tissues. Bhat *et al.* (14) found many histological changes in the liver when rats were dosed for 7 and 28 days with low and high concentrations of cyclophosphamide.

Materials and methods

Healthy male Swiss albino mice were used for the study. Seventy-five adult male albino mice (*Mus musculus*) with an average weight of 25±2 g and ages between 8-12 weeks. Animals were housed in polycarbonate boxes and maintained under standard laboratory conditions in the animal house of the Department of Biology, Mustansiriyah University. Kept for adaptation for ten days before starting the experimental under the controlled temperature conditions of 25°C and 12 hrs light /12 hrs dark cycle. The animals were provided with pellets and tap water for feeding and drinking.

Ethical approve

Informed consent according to the Declaration of Helsinki was obtained from the ethics committee of the College of Science, Mustansiriyah University Ref. No. BCSMU/0721/0001.

Study groups

The animals were divided into three groups of 25 animals at random. The G1 group, which considers as control, received orally distilled water only for 30 days. According to Shafi (15) G2 group which orally given 0.1mg/Kg of Prednisolone 20 mg (Julphar Company, Gulf pharmaceutical industries, Ras AL Khaimah, UAE) administration for 30 days while the G3 group which orally given 0.1 mg/Kg of Cyclophosphamide 50 mg, Baxter, mfg. Lic. No.186) administration for 30 days. After 24 hours of the last administration, the mice were sacrificed by chloroform for several minutes. The organs were removed, and some small specimens were taken for histological techniques.

Histological examination and grading

In 10% formalin solution, the liver, kidneys, and small intestinal tissues were preserved for 24 hrs and washed with 70% ethanol, tissues were then dehydrated using 70% to 100% alcohol series and embedded in paraffin. Blocks of paraffin were cut at 5 µm using a rotating microtome, spread on glass slides, and then dried. Slides were observed under a light microscope following staining with hematoxylin and eosin stain and mounted with mount media according to Suvaran *et al.* (16), and a percentage scale determined histopathological grading.

Statistical analysis

The Chi-square test values of $P<0.05$ were regarded as statistically different in the descriptive analysis. SPSS (Version 25) was used to conduct the statistical analysis.

Results

Effect of Prednisolone and Cyclophosphamide on the liver tissue

Microscopic finding of the liver was summarized in (Table 1).

Table 1: Histopathological grading of liver tissues in prednisolone, cyclophosphamide, and control groups

Category	Group	0 (normal) N	%	1 (mild) N	%	2 (moderate) N	%	3 (severe) N	%	P- value
Necrosis	G 1	25	100	-	-	-	-	-	-	0.01
	G 2	21	84	3	12	1	4	-	-	
	G 3	6	24	10	40	5	20	4	16	
Fibrosis	G 1	25	100	-	-	-	-	-	-	0.04
	G 2	9	36	8	32	7	28	1	4	
	G 3	2	8	7	28	9	36	7	28	
Cellular inflammation	G 1	25	100	-	-	-	-	-	-	0.05
	G 2	17	68	5	20	3	12	-	-	
	G 3	4	16	12	48	6	24	3	12	
Degeneration in epithelium cells	G 1	23	92	2	8	-	-	-	-	0.01
	G 2	0	-	16	64	7	28	2	8	
	G 3	0	-	8	32	11	44	6	24	
Vascular congestion and Hemorrhage	G 1	25	100	-	-	-	-	-	-	0.05
	G 2	11	44	8	32	5	20	1	4	
	G 3	7	28	8	32	6	24	4	16	

P-value: $P>0.05$ between control and tested groups, each group total number=25 mice.

Normal morphological findings were seen in the control of all the liver tissues except 8% showed degeneration in epithelium cells (Figure 1). Salient microscopical findings in this study, as observed in (Figure 2-7) statistical analysis, were an increase in grade 1 of G2 in all categories. On the other hand, statistical analysis was an increase in grade 1 of G3 were necrosis, cellular inflammation, and vascular congestion and hemorrhage, while increased in grade 2 were fibrosis, degeneration in epithelium cells, when treated mice with 0.1 mg/kg body weight of cyclophosphamide and prednisolone for 30 days.

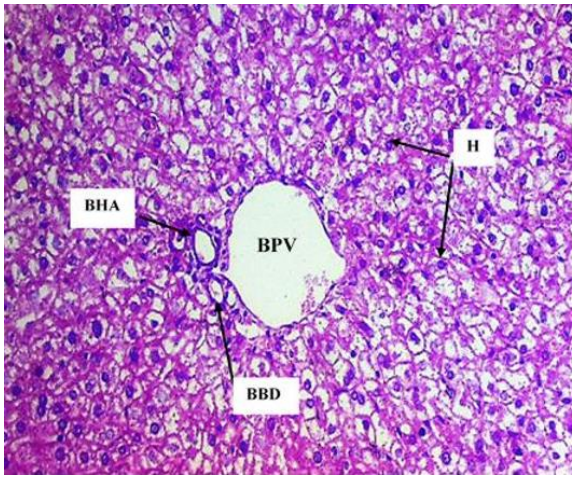

Figure 1: Crosse section in the normal mice liver architecture shows appearance of hepatocytes (H), a branch of portal vein (BPV), branch of the hepatic artery (BHA), and a branch of the bile duct (BBD) (H&E, X10).

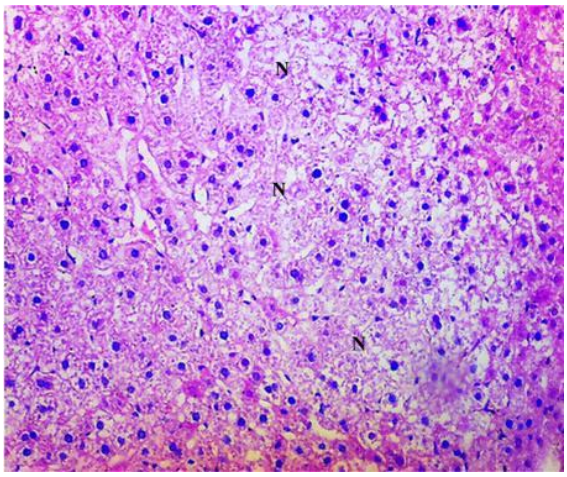

Figure 2: Cross section in the liver of the treated mice shows the necrotic zone (N) in the liver lobe (H &E, X10).

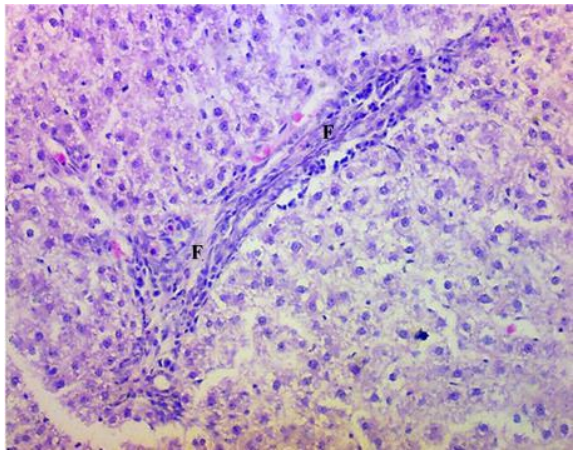

Figure 3: Cross section in the liver of the treated mice shows periportal and septal fibrosis (F) with the aggregate of inflammatory cells (H &E, X10).

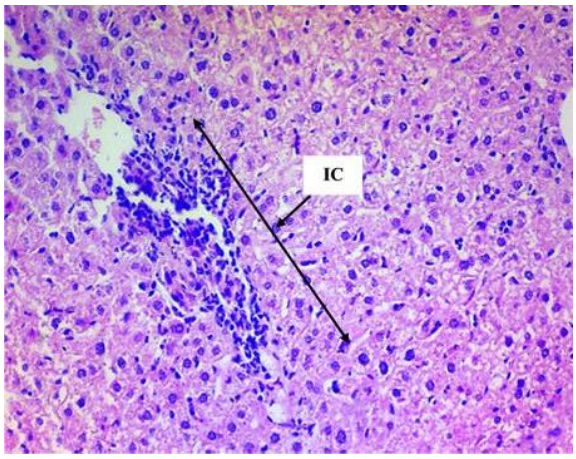

Figure 4: Cross section in the liver of the treated mice shows the aggregate of inflammatory cells (IC) (H &E, X10).

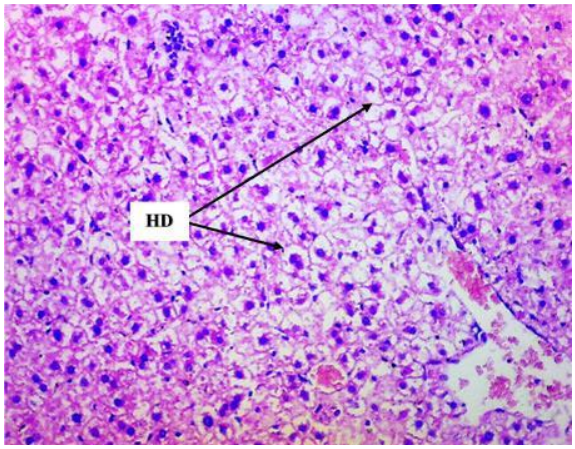

Figure 5: Cross section in the liver of the treated mice shows hydropic degeneration (HD) in hepatocytes (H &E, X10).

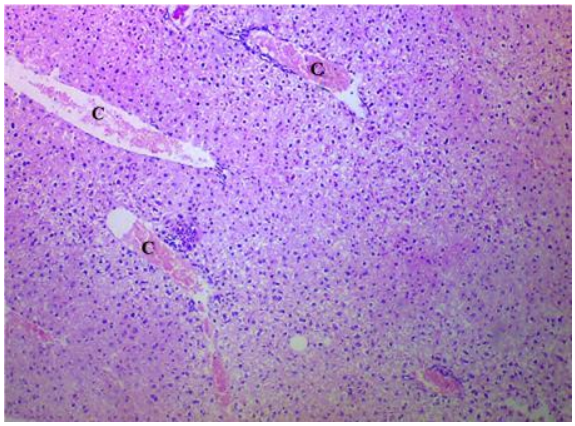

Figure 6: Cross section in the liver of the treated mice shows congestion of blood vessels (C) (H &E, X4).

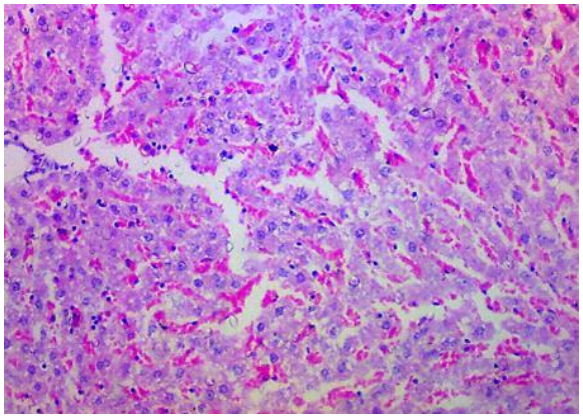

Figure 7: Cross section in the liver of the treated mice shows haemorrhage in sinusoids (red colour) (H &E, X10).

Effect of prednisolone and cyclophosphamide on the kidneys tissue

Microscopic examination of the kidney tissue revealed the glomeruli and renal tubules (Figure 8). From table 2 and figure 9-13, it is found that kidney damage significantly occurs in treated groups compared to the control group. Our study found that treated mice with 0.1 mg/kg body weight of cyclophosphamide and prednisolone for 30 days statistical analysis of G2 and G3 were an increase in grade 1 of all categories.

Effect of prednisolone and cyclophosphamide on the small intestinal tissue

Gross examination of the small intestine obtained from the control group revealed normal appearance in all sections (Figure 14). Our study observed histological alterations in small intestinal tissue between the prednisolone, cyclophosphamide, and controls group in table 3 and figure 15-18 statistical analysis were an increase in grade 1 of G2 in all category. On the other hand, statistical analysis was an increase in grade 1 of G3 were necrosis, cellular inflammation, degeneration in epithelium cells, and vascular congestion and hemorrhage, while fibrosis increased in grade 2 when treated mice with 0.1 mg/kg body weight of cyclophosphamide and prednisolone for 30 days.

Table 2: Histopathological grading of kidney tissues in prednisolone, cyclophosphamide, and control groups

Category	Group	0 (normal) N	%	1 (mild) N	%	2 (moderate) N	%	3 (severe) N	%	P- value
Necrosis	G 1	25	100	-	-	-	-	-	-	0.01
	G 2	24	96	1	4	-	-	-	-	
	G 3	18	72	3	12	2	8	2	8	
Fibrosis	G 1	25	100	-	-	-	-	-	-	0.04
	G 2	11	44	9	36	3	12	2	8	
	G 3	4	16	12	48	7	28	2	8	
Cellular inflammation	G 1	25	100	-	-	-	-	-	-	0.05
	G 2	15	60	7	28	3	12	-	-	
	G 3	3	12	14	56	5	20	3	12	
Degeneration in epithelium cells	G 1	25	100	-	-	-	-	-	-	0.01
	G 2	-	-	17	68	7	28	1	4	
	G 3	-	-	10	40	9	36	6	24	
Vascular congestion and Hemorrhage	G 1	25	100	-	-	-	-	-	-	0.05
	G 2	20	80	4	16	1	4	-	-	
	G 3	3	12	14	56	5	20	3	12	

P-value: P>0.05 between control and tested groups, each group total number=25 mice.

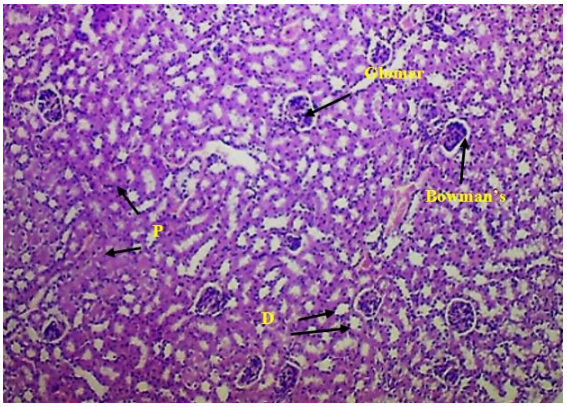

Figure 8: Cross section in the normal mice kidney architecture shows appearance of the renal corpuscles (glomerulus and Bowman's space), PCT = proximal convoluted tubule, and DCT = distal convoluted tubule (H&E, X4).

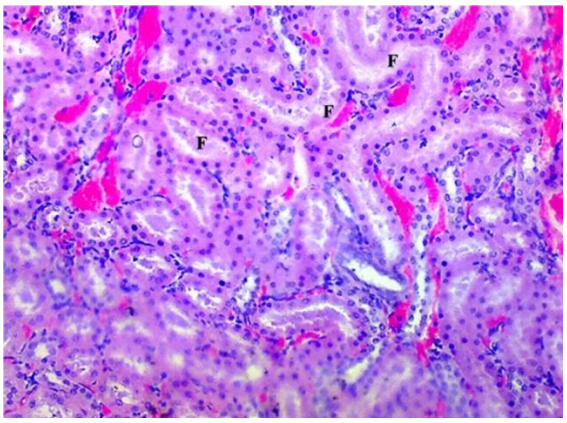

Figure 9: Cross section in the kidney of the treated mice shows necrosis epithelial cells and hemorrhage between convoluted tubules (H &E, X10).

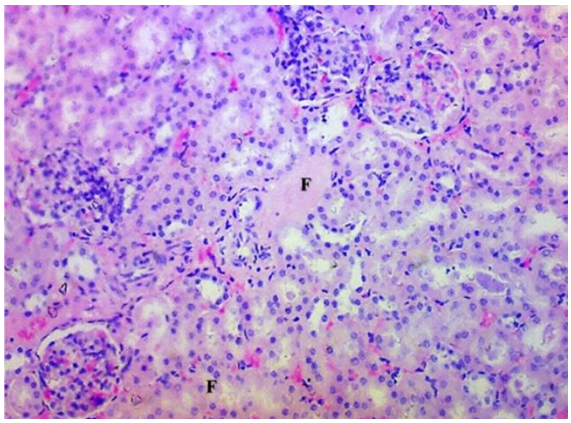

Figure 10: Cross section in the kidney of the treated mice shows fibrin (F) deposition between tubules (H &E, X10).

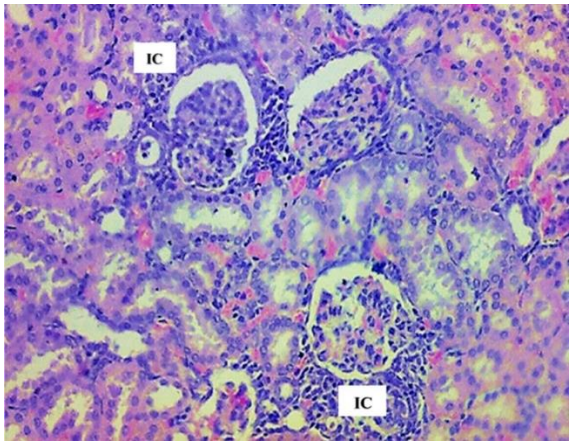

Figure 11: Cross section in the kidney of the treated mice shows inflammatory cells infiltration around Bowman's capsule (IC) (H &E, X10).

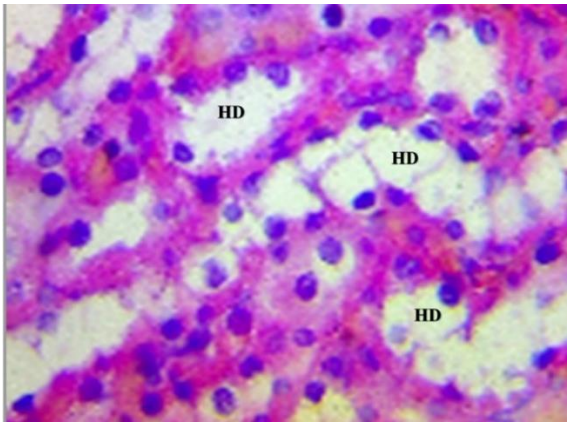

Figure 12: Cross section in the kidney of the treated mice shows hydropic degeneration (HD) in epithelial cells of the collecting tubule (H &E, X40).

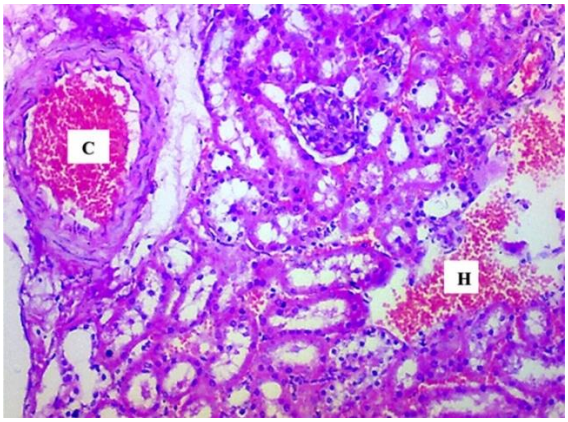

Figure 13: Cross section in the kidney of the treated mice shows congestion of blood vessel (C) and haemorrhage (H) in the interstitial space between the tubules (H &E, X10).

Table 3: Histopathological grading of small intestine tissues in prednisolone, cyclophosphamide, and control groups

Category	Group	0 (normal) N	%	1 (mild) N	%	2 (moderate) N	%	3 (severe) N	%	P- value
Necrosis	G 1	25	100	-	-	-	-	-	-	0.03
	G 2	24	96	1	4	-	-	-	-	
	G 3	22	88	2	8	1	4	-	-	
Fibrosis	G 1	25	100	-	-	-	-	-	-	0.04
	G 2	5	20	13	52	5	20	2	8	
	G 3	2	8	9	36	10	40	4	16	
Cellular inflammation	G 1	25	100	-	-	-	-	-	-	0.04
	G 2	23	92	1	4	1	4	-	-	
	G 3	4	16	12	48	7	28	2	8	
Degeneration in epithelium cells	G 1	24	96	1	4	-	-	-	-	0.05
	G 2	2	8	17	68	4	16	2	8	
	G 3	-	-	15	60	6	24	4	16	
Vascular congestion and Hemorrhage	G 1	25	100	-	-	-	-	-	-	0.01
	G 2	21	84	3	12	1	4	-	-	
	G 3	4	16	13	52	7	28	1	4	

P-value: P>0.05 between control and tested groups, each group total number=25 mice.

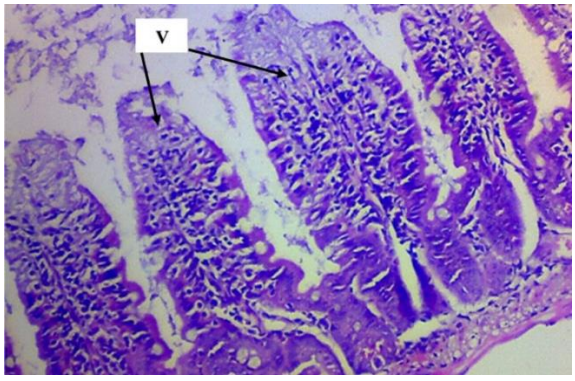

Figure 14: Crosse section in the normal small intestine mice architecture appearance normal villi (V) with epithelial cells of the intestinal mucosa (H&E, X10).

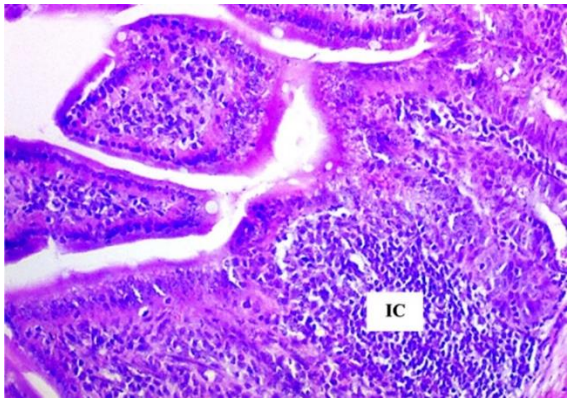

Figure 16: Cross section in the small intestine of the treated mice shows inflammatory cells infiltration in villi (IC) (H &E, X10).

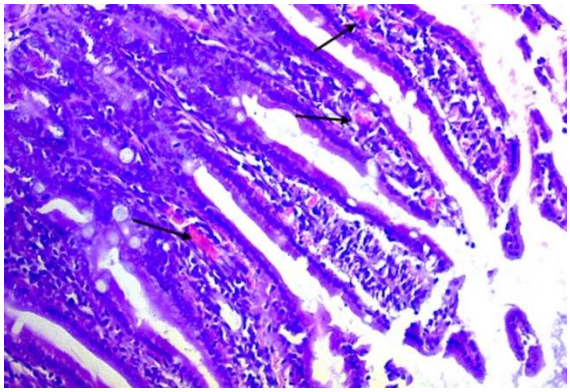

Figure 17: Cross section in the small intestine of the treated mice shows haemorrhage in villi (arrows) (H &E, X10).

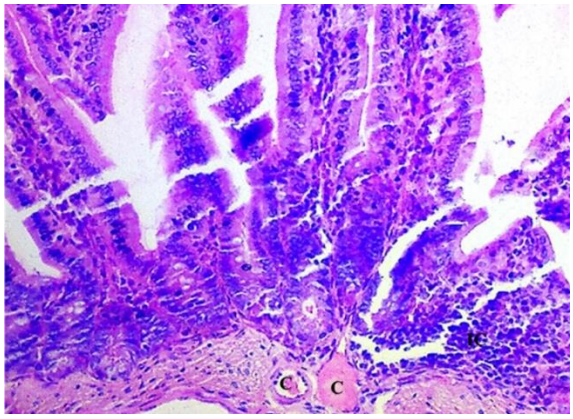

Figure 18: Cross section in the small intestine of the treated mice shows congestion of blood vessels (C) with inflammatory cells infiltration (IC) (H &E, X10).

Discussion

After examining the current study results of the liver tissue, there was an increase in histological changes in mice treated with cyclophosphamide drug more than those treated with prednisolone. Jiang *et al.* (17) illustrate that malondialdehyde is a sensitive indicator of the quantity of free radical metabolism since it is the end product of various lipid peroxides created by lipid peroxidation. Furthermore, acrolein (a toxic cyclophosphamide metabolism) is combined with glutathione. It might increase reactive oxygen species and cause lipid peroxidation.

Additionally, the antioxidant system is a critical defense against cyclophosphamide-induced oxidative damage. It is a condition that causes a breakdown in the body's natural antioxidative defense mechanism, allowing ROS to accumulate unabated and attacks on tissue antioxidant enzymes to occur (18,19).

Cyclophosphamide generates acrolein and phosphoramide mustard. Acrolein is responsible for the toxicity that affects the tissues, as acrolein produces high types of ROS in liver cells and therefore, it interacts with protein and causes changes in the structure and function of enzymes, and affects the defense mechanism antioxidants in tissues. Also, the toxicity caused by cyclophosphamide results from mitochondrial dysfunction, which leads to a decrease in ATP due to oxidative stress (20,21).

In the administration of cyclophosphamide, Oyagbemi *et al.* (22) found that injection the rats with 200 mg/kg body weight causes hepatic tissue periportal inflammation, hemorrhage, and congestion in the liver. Intraperitoneal injection of cyclophosphamide showed loss of hepatocytes architecture, blood sinusoids congestion, vacuolar degeneration, inflammatory cellular infiltration and in hepatic sinusoid, formation of pyknotic nuclei and hepatocellular necrosis, which is a prevalent finding in chronic and acute liver diseases, and with the firmness of the underlying cause, it is pursued by gradual fibrosis.

While the prednisolone caused a more negligible effect on liver tissue, the potential mechanism of these changes may result from the glucocorticoids causing a change in lipid metabolism and visceral adiposity or producing oxidative stress. It has been declared a significant cause of glucocorticoid-induced liver damage and the extrema production of free radicals (23,24).

Alzubaidy *et al.* (25) found that prednisolone administration was found to improve many symptoms and develop many histologic and biochemical abnormal changes in many kinds of liver diseases, for example, cases of liver transplantation, autoimmune hepatitis, liver cirrhosis, and septic shock patients and that the prednisolone has no effect on the histology of the kidney and liver in these organs in rabbits. In addition, a previous study by Kumar *et al.* (26) confirms that the treated rats with dexamethasone at different doses induced increasing liver necrosis, fibrosis.

Finally, all the histological changes in the liver tissue were more severe when the mice were dosed with cyclophosphamide than the changes observed in the liver tissue of the mice dosed with prednisolone, which may be due to the high toxicity of the first drug.

The microscopic examination of the kidney tissue showed some histological changes that were more severe in the tissues of animals dosed with cyclophosphamide compared to the prednisolone drug. According to the previous studies, the number of reactive oxygen species can be raised with drug delivery, causing oxidative injury in renal tissue. Sharef *et al.* (27) found that treating the male rats with dexamethasone caused significant necrosis of the tubule, hyaline deposition of glomerular, increase of fibrosis, and edema in the proximal convoluted tubule.

On the other hand, the toxic cyclophosphamide effect in kidney tissues can be observed in previous research by El-Shabrawy *et al.* (28) and Sayed-Ahmed (29) that causes severe histopathological lesions in kidney tissues. Histopathological analysis of kidneys of animals treated with a single dose of cyclophosphamide intraperitoneally demonstrated minor congestion, prominent enlargement of the capsule cavities, glomerular atrophy, and fragmentation injury to the parietal layer of the visceral layer and renal capsule. It also showed cloudiness in the structure of epithelial cells vacuolization in several inflammatory epithelial cells and cell fragments in the tubules (30). Lin *et al.* (31) illustrates that cyclophosphamide accumulates inside a cell. It disables the cell's antioxidant defense mechanisms and increases the formation of reactive oxygen species that can oxidize polyunsaturated fatty acids, resulting in lipid peroxidation and the generation of malondialdehyde.

Also, our study focused on the effects of cyclophosphamide and prednisolone on the small intestine. Yamamoto *et al.* (32) revealed prednisone was able to decrease the villus height in the duodenum which is associated with a change in intestinal absorption. In addition, as expected, there was a decrease in mucosal leukocytes after prednisone treatments (33). Ruiz-Irastorza *et al.* (34) showed the low doses of prednisone which treatment can be considered safe. However, Sciascia *et al.* (35) and Zahr *et al.* (36) illustrated even low doses can be caused organ damage.

The administration of cyclophosphamide may have affected the mucosa layer and increased intestinal permeability through its effect on intercellular connections in the intestinal epithelium (37-39).

A previous study by Lima *et al.* (40) found that the treated rats orally with prednisolone decreased villus height, whereas crypt depth, longitudinal and circular muscles were not affected. These findings prove a reduction of intestinal absorption, which may be connected with symptoms and diverse gastrointestinal dysfunctions.

Another study by Sheeja and Kuttan (41) found extensive damage to the intestinal villi in mice treated with cyclophosphamide, with villi lengths significantly shortened

and crypt architecture largely disrupted. Most of its cells degenerated, while vertical villi were sticking together and compressed and short with no brush border. They also missed the gaps between them due to the necrosis and degeneration of their cells.

Conclusions

After all of the above, our study found that rats dosed with cyclophosphamide causes histological changes in (liver, kidney, small intestine) more severe than what was revealed in rats dosed with prednisolone drug to the same organs, and this is due to the high cytotoxicity of the first drug compared with the second.

Acknowledgments

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Conflicts of interest

The authors certify that the content of this work does not contain any conflicts of interest for them.

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تقييم التغيرات النسجية المستحثة بالبريدنيزولون والسيكلوفوسفاميد في بعض أعضاء ذكور الفئران البيضاء

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الخلاصة

بريدنيزولون هو كورتيكوستيرويد مصنع يستخدم لعلاج أمراض مختلفة، المعروف أنه يستخدم لعلاج العديد من الحالات مثل أمراض المناعة الذاتية والربو. أما السيكلوفوسفاميد هو نوع من العلاج بخردل النيتروجين الذي يحدث تأثيره عن طريق ألكلة الحمض النووي، يتم استخدامه كمثبط للمناعة في التهاب المفاصل الرثوي وعلاج العديد من السرطانات أيضا. نظرا إلى الاستعمال الواسع لهذين العقارين هدفت الدراسة إلى تقييم التغيرات النسجية في كبد وكلى والأمعاء الدقيقة لذكور الفئران. تم استخدام خمسة وسبعين فأرا بالغا بعمر من ٨-١٢ أسبوعا والتي قسمت إلى ثلاث مجموعات، المجموعة الأولى جرعت فمويا بـ ٠,١ ملغم / كغم من البريدنيزولون، المجموعة الثانية جرعت فمويا بـ ٠,١ ملغم / كغم من السيكلوفوسفاميد، والمجموعة الثالثة تلقى الماء المقطر فمويا لمدة ٣٠ يوما يوميا. بعد ٢٤ ساعة من العلاج الأخير، تم التضحية بالحيوانات وإخراج الأعضاء (الكبد، الكلى، الأمعاء الدقيقة) ووضعها في محلول الفورمالين بنسبة ١٠٪ إلى حين إجراء التقنيات النسجية عليها. أظهرت نتائج الدراسة زيادة بالفروق المعنوية عند أ<٠,٠٥ بالتغيرات النسجية في الأعضاء المدروسة المتمثل بالنخر والتليف وتنكس الخلايا واحتقان ونزيف الأوعية الدموية والخلايا الالتهابية عند مقارنتها مع مجموعة السيطرة، وأن اعلى فرقا معنويا لهذه التغيرات كانت عند درجة ١ و ٢. تؤكد دراستنا أن هذه الأدوية تسبب تغيرات نسجية اختلفت شدتها بين الأعضاء وكذلك ضمن العضو الواحد عند مقارنتها بمجموعة السيطرة وأن السيكلوفوسفاميد يسبب تغيرات نسجية أكثر من بريدنيزولون.