The Role of Glutathione on Azathioprine-Induced Cytotoxicity in Rats: Histological Study

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ABSTRACT

Objective: the current work aims to find out whether glutathione has a protective role against hepatic and renal tissue toxicity induced by the use of azathioprine.

Methods: A total of twenty-eight Wistar rats were assigned to 4 equal groups, the control group (I) was given distilled water. Azathioprine in a dose of 5mg/kg body weight was administered to group II. Group III received azathioprine 5mg/kg body weight with glutathione 100 mg/ kg body weight, the last group (IV) received glutathione alone 100 mg/ kg body weight. The treated groups were given the treatment by oral plastic gavage daily for 6 weeks. The liver and kidney of the animals were dissected and prepared for histological examination.

Results: The obtained results indicated that in group II Liver sections showed steatohepatitis with marked Ballooning degeneration of the hepatocytes, Coagulative necrosis, focal inflammatory cells infiltration in the Portal triads with hyperplasia and hypertrophy of Kupffer cells. Renal sections of group II revealed vacuolar degeneration in the epithelial lining cells of renal tubules with focal lymphocytic inflammatory cells aggregation in renal parenchyma. dilatation of the Bowman's space with formation of renal cysts and necrosis of some glomeruli. In group III the histology of rats' liver and kidney sections showed improvement of the histological lesions. In liver section, some necrotic hepatocytes with central veins congestion and slight inflammatory cells infiltration. The renal tissue slides revealed tubular necrotic epithelium with marked inflammatory cells infiltration.

Conclusion: The current study concluded that azathioprine in a dose of 5mg/kg for 6 weeks can produce hepatic and renal cellular damage. Glutathione in a dose of 100mg/kg for 6 weeks can reduce this tissue damage.

Keywords: glutathione , Azathioprine , liver , kidney , rats .

دور الجلوتاثايون في السمية الخلوية التي يسببها الازوثيوبرين في الفئران : دراسة نسيجية

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الخلاصة

الهدف : يهدف العمل الحالي إلى معرفة ما إذا كان للجلوتاثيون دور وقائي ضد سمية الأنسجة الكبدية والكلوية الناتجة عن استخدام الآزوثيوبرين.

الطريقة : تم تخصيص ما مجموعه ثمانية وعشرين فأر من Wistar إلى ٤ مجموعات متساوية ، المجموعة الضابطة (I) أعطيت الماء المقطر. تم إعطاء الآزوثيوبرين بجرعة ٥ مجم / كجم من وزن الجسم للمجموعة الثانية. تلقت المجموعة الثالثة الآزاثيوبرين ٥ مجم / كجم من وزن الجسم مع الجلوتاثيون ١٠٠ مجم / كجم من وزن الجسم ، أما المجموعة الأخيرة (IV) فقد تلقت الجلوتاثيون بمفرده ١٠٠ مجم / كجم من وزن الجسم. أعطيت المجموعات المعالجة بالتزقيم الفموي يومياً لمدة ٦ أسابيع. تم تشريح كبد وكلى الحيوانات وتجهيزها للفحص النسيجي.

النتائج : أشارت النتائج التي تم الحصول عليها إلى أن أقسام الكبد في المجموعة الثانية أظهرت التهاب الكبد الدهني مع تنكس البالون الملحوظ للخلايا الكبدية ، ونخر تخثر الدم ، وتسلل الخلايا الالتهابية البؤرية في ثلاثية البوابة مع تضخم وتضخم خلايا كوبفر. كشفت أقسام الكلى من المجموعة الثانية عن تنكس فجوي في خلايا البطانة الظهارية للأنابيب الكلوية مع تجمع الخلايا الالتهابية اللمفاوية البؤرية في الحمة الكلوية. توسع مساحة بومان مع تكوين أكياس كلوية ونخر في بعض الكبيبات. في المجموعة الثالثة أظهرت أنسجة أقسام الكبد والكلى لدى الفئران تحسنًا في الآفات النسيجية. في قسم الكبد ، بعض خلايا الكبد النخرية مع احتقان الأوردة المركزية وتسلل الخلايا الالتهابية الطفيفة. كشفت شرائح الأنسجة الكلوية عن ظهارة نخرية أنبوبي مع تسلل ملحوظ للخلايا الالتهابية.

خاتمة : خلصت الدراسة الحالية إلى أن جرعة من الآزوثيوبرين ٥ مجم / كجم لمدة ٦ أسابيع يمكن أن تسبب تلف الخلايا الكبدية والكلوية. يمكن أن يقلل الجلوتاثيون بجرعة ١٠٠ مجم / كجم لمدة ٦ أسابيع من تلف الأنسجة.

الكلمات المفتاحية : كلوتاثايون ، ازوثيوبرين ، الكبد ، الكلية ، فئران .

INTRODUCTION

Azathioprine is a well-known immunosuppressive compound that is a purine analog that induces cellular death [1]. It is absorbed very well by GIT and about half percent of the drug is excreted in urine [2]. The remaining amount of the drug will be metabolized to the main principal metabolite that is 6-Mercaptopurine by thiopurine methyltransferase which will be changed to 6-thioinisine 5-monophosphate that will inhibit DNA replication and purine synthesis [3].

It may even inhibit different genes in T -cell that are included in different processes like intestinal inflammation [4].

It is commonly recommended in the management of many diseases including rheumatoid arthritis [5], many types of inflammatory intestinal disorders especially in long time remission [6], lupus nephritis [7], systemic lupus erythematosus [8], dermatitis [9], acute lymphatic leukemia [10], psoriasis[11], renal transplant to prevent graft rejection [12].

Glutathione is an endogenous tripeptide it is involved in different defense biological pathways and immune system like detoxification of xenobiotics and internal compounds, protein folding, controlling the action of mitochondria and cellular progression and apoptosis, and it enhance the action of vitamin C and E which considered as antioxidants agents.[13, 14]

It protects the cellular core from reactive oxygen and nitrogen species as it acts straightly with the sources of oxidative stress like heavy metals and biological pollutants. It conjugates with these compounds to be easily excreted by the body and this is the main role of glutathione as antioxidant compound. In addition, it deactivates hydroxyl and superoxide radicals. [15]

MATERIALS AND METHODS

Chemicals

Azathioprine, 50 mg tablet, aspen Pharma Trading Limited, Ireland. Glutathione, 500mg capsules, NOW FOODS, 395 S, USA.

Ethical Committee Approval

The article was approved by the medical research ethical committee of Mosul University, Mosul, Iraq.

Animals

The treated animals were taken from the laboratory animal section in the Veterinary College/University of Mosul, Iraq. They were kept in groups in plastic cages at room temperature of 25 ± 2ºC and were received diet ad libitum and normal tape water.

Experimental Design

A total of twenty-eight Wistar rats were assigned to 4 equal groups, the control group (I) was given distilled water. Azathioprine in therapeutic dose of 5mg/kg body weight was administered to group II. Group III received azathioprine 5mg/kg body weight with glutathione 100 mg/ kg body weight, and glutathione in a dose of 100 mg/ kg was administered to group (IV). The treated groups were given the treatment by oral plastic gavage daily for 6 weeks.

The liver and kidney of the treated groups were dissected, fixed in 10% neutral buffered formaldehyde solution, the dehydration was maintained by alcohol, the clearing process was continued by xylol, then they were embedded in paraffin wax blocks. Lastly, tissue slides stained with hematoxylin and eosin for subsequent histological examination were prepared.

OBSERVATION AND RESULTS

Histological Criteria

The Microscopic finding of hepatic and renal slide tissues revealed different histological lesions: In Glutathione treated rats there are no histological changes in the liver and kidney parenchyma.

In groups treated with Azathioprine , the Liver section showed steatohepatitis which marked by ballooning degeneration of the hepatocytes characterized by rounded hepatocytes and clear cytoplasm as well as present of Mallory Denk bodies in some hepatocytes , other hepatocytes showed coagulative necrosis. Also formation of thrombus in veins of portal area are noticed. In the portal triads area, there are focal inflammatory cells infiltration mainly lymphocytes and neutrophils. The histology of rats liver in group 3 (treated with Azathioprine and Glutathione) there is an improvement of the histological lesions. In liver section, there are mild necrotic hepatocytes with central veins congestion and slight inflammatory cells infiltration. (Figure 1).

The kidney slide sections revealed present of vacuolar degeneration and coagulative necrosis in the epithelia cells lining the renal tubules with focal lymphocytic inflammatory cells aggregation in renal parenchyma. Renal Glomeruli showed dilatation of the Bowman's space with formation of renal cysts due to necrosis of some glomeruli, as well as, there is congestion and thickening of the blood vessels wall.

The histology of rats' kidney in group III (treated with Azathioprine and Glutathione) there is an improvement of the histological lesions. , the renal tissue slides, revealed mild necrotic tubular necrotic epithelium with marked inflammatory cells infiltration. (Figure 2).

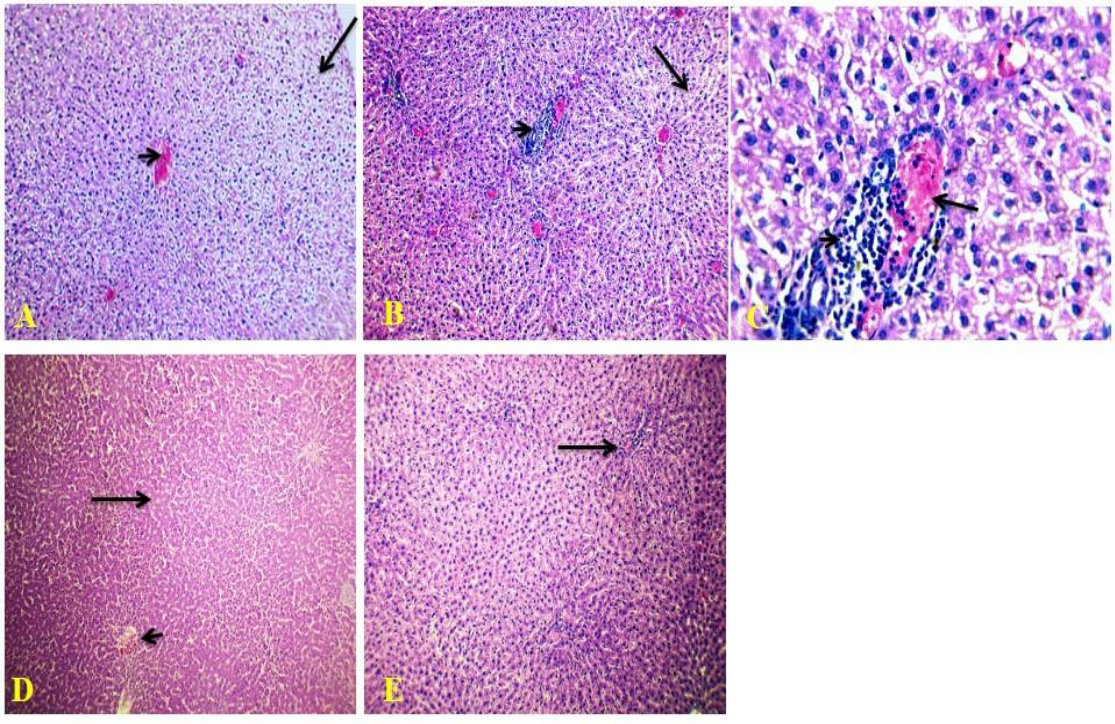

Figure 1: Micrograph of Rat liver treated with Azathioprine. (A) steatohepatitis (black arrow) and congestion of central vein (Arrowhead) ,(H&E 100X). (B) Hepatocytes coagulative necrosis (Black Arrow) with focal inflammatory cells infiltration in Portal triads are (Arrow head) (H&E 100X). (C) Infiltration of mononuclear inflammatory cells in Portal triads (Arrow head) with thrombus in the portal vein (Black Arrow) (H&E 40٠X). (D) & (E) Rat liver treated with Azathioprine and Glutathione there is an improvement of the histological lesions, mild necrotic hepatocytes with central veins congestion and slight inflammatory cells infiltration. (H&E 100X).

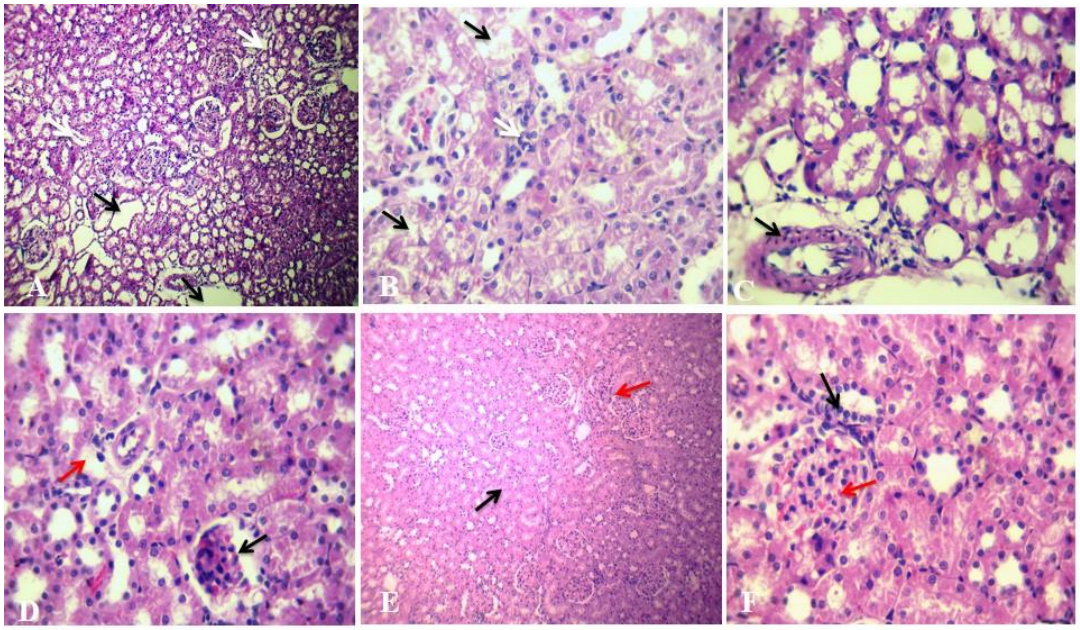

Figure 2: Micrograph of Rat kidney, treated with Azathioprine (A) formation of renal cysts due to necrosis of some glomeruli (Black Arrow), Necrosis of renal tubules (White Arrow) (H&E 100X). (B) Necrosis of renal tubules (Black Arrow) and focal aggregation of inflammatory cells (White Arrow) (H&E 400X). (C) Thickening in the wall of blood vessels (Arrow) (H&E 400X). (D) Nephrosclerosis of the glomerular tuff and expansion of Bowman's space (Black Arrow) with necrosis of the renal tubules (red arrow) (H&E 400X). (E) & (F) Rat renal parenchyma, treated with Azathioprine and Glutathione there is an improvement of the histological lesions, revealed mild necrotic glomerular and tubular necrotic epithelium with marked inflammatory cells infiltration (H&E 100 & 400X).

DISCUSSION

In group II (rats treated with Azathioprine) liver tissue sections showed marked Ballooning degeneration with steatohepatitis, coagulative necrosis and marked focal inflammatory cells infiltration. While in group III (rats treated with Azathioprine and glutathione) showed an improvement of the histological lesions.

Kidney slide sections of group II revealed vacuolar degeneration and coagulative necrosis in the epithelia cells lining the renal tubules with focal inflammatory cells aggregations, dilatation of the Bowman's space with formation of renal cysts. While in group III renal sections showed an improvement in the histological lesions.

The effect of azathioprine on hepatocytes is due to stimulation of protein kinases enzymes which are directly associated with mitochondrial destruction and lactate dehydrogenase secretion ending with ATP and glutathione lack [16], in addition to higher levels of ALT, AST and ALP [17]

These changes were reported because azathioprine cause depletion of natural antioxidants including glutathione and superoxide dismutase, so the drug increases both oxidative stress and DNA damage. [18]

Al Maruf *et al* [19] studied toxic changes of rat hepatocyte when exposed to azathioprine in culture and they found that this tissue damage is due to liberation reactive oxygen species and increase oxidative stress.

Tapner *et al*. [20], demonstrated that azathioprine can cause liver cell damage in rats due to direct effect on mitochondria leading to necrosis and ATP depletion and excess liberation of reactive oxygen species.

Lee and Farrell [21] studied the mechanism of azathioprine in inducing liver cell damage in rats and they found that this process is started with glutathione depletion with subsequent hypoxia of mitochondria leading to ATP depletion and disseminated necrosis of hepatic cells.

The results of this article were in agreement with Tabrizi *et al* [22] results, they found that azathioprine in a dose of 15mg/kg daily for seven days can induce liver tissue damage including severe degenerative necrosis of hepatic parenchyma with inflammatory cell infiltration and portal vein destruction.

These results were in coincides with Matsuo *et al* [23] study, they found that administration of 100,200, 300 mg/kg of azathioprine in mice will lead to liver tissue necrosis and damage due to increase levels of reactive oxygen species.

The results in the current work were in agreement with Ismail *et al*. [24] study. They administer azathioprine 10 and 20mg/kg for 21 days in rats and they reported hepatotoxicity liver cell necrosis with infiltration with chronic inflammatory cells and vascular congestion.

Also, the results were in acceptance with Abd Elfatah *et al* [25] study which reported that 10 mg/kg of azathioprine for 7 days was enough to cause liver tissue damage including cellular necrosis with nuclear karyolysis and hyperplasia of Kupffer cells.

The results in this study were in agreement with Godarzian and Hosseini study [26] They found that 50 mg/kg of azathioprine when given for 21 days in rats can lead to renal tissue damage including tubular epithelial cells necrosis with focal inflammatory cellular infiltration in renal parenchyma.

These results were in agreement with Akinlolu *et al*. [27] study. They found that 5mg/kg of azathioprine when administered to rats for 35 consecutive days was enough to create renal tissue damage like widening of Bowmans space, glomerular atrophy, with necrotic foci in the renal tissue parenchyma. High levels of malondialdehyde were detected in the renal cells.

It has been stated that azathioprine induces direct renal and hepatic tissue damage through its effect on mitochondria and enhancing lipid peroxidation production, severe reduction in the natural antioxidant enzymes. [28]

CONCLUSION

The current study concluded that azathioprine in a dose of 5mg/kg for 6 weeks can produce hepatic and renal cellular damage. Glutathione in a dose of 100mg/kg for 6 weeks can reduce this tissue damage.

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