Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2013 Vol. 3 (4) October-December, pp.177-183/Farangis et al.
Research Article

THE EFFECTS OF AZATHIOPRINE ON LIVER AND KIDNEY TISSUE IN DIABETIC RATS

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ABSTRACT
Azathioprine belongs to the chemical class of purine analogues and it has been widely used as an immunosuppressant. Using this drug causes complications like toxicity in some organs. Liver and kidney are important in metabolism of drugs, so in present study, the effects of AZA on these organs were evaluated. 56 Wistar rat weighing 200±20 g were classified in 8 groups. Control (without treatment) and sham (140cc fructose 10% as a daily feeding). Groups 1 to 4 (diabetic) in addition froctose 10 % feeding, were injected (3.75, 7.5, 15 and 21) mg/kg/b.wt azathioprine at 98th day and Groups 5 and 6 were injected (15 and 21) mg/kg/b.wt azathioprine interaperitoneal. 24 hours after, blood samples were taken from all groups for biochemical analyzes. Then liver and kidneys were dissected, fixed in formalin 10% and sections were prepared by tissue prossesing for histological study. Data were analyzed by ANOVA and groups were compared by Duncan test. The histological damages in liver and kidney of diabetics treated groups spatially in high dose of drug were observed. Lymphocyte invasion, hydropic changes in portal area, cytoplasm granularity and necrosis of hepatic parenchyma. Also significant decrease in the diameter of the renal cortex and medulla in diabetic and non-diabetic treatment groups and a significant increase in proximal and distal convouluted, hanle's loop and glomerules were observed. Azotioprin had synergistic effects with diabetes is dose- dependent and degenerate changes in kidney tissue are more than liver. Therefore 1t is suggested that the diabetic patients are limited to consumption.

***Key Words**: Azatioprin, Tissue Damage, Liver, Kidney*

INTRODUCTION
Azathioprine (AZA) is an immune suppressive drug that treats diseases such as leukemia; acute lymphoblastic, inflammatory bowel disease and rheumatoid arthritis. This drug acts as a prodrug for mercaptopurine, inhibiting an enzyme that is required for the synthesis of DNA. Thus it most strongly affects proliferating cells, such as the T cells and B cells of the immune system (Aninat *et al.*, 2006). So it prevent rejection following organ transplantation, and to treat an array of autoimmune diseases, including rheumatoid arthritis, pemphigus, systemic lupus erythematosus, Behçet's disease, autoimmune hepatitis and atopic dermatitis, restrictive lung disease, and others (Amouoghli *et al.*, 2009).
Toxic effects of the drug are the cause of production free radical in organs, tissues and oxidative injury (Sweetman *et al.*, 2002; César *et al.*, 2004). It act by selectively inhibiting the synthesis of purine nucleotides (adenine) and reducing DNA synthesis of a variety of immunologic and other specialized cells, including hepatocytes due to oral administration of azatioprin increase liver enzymes level as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Diabetes (Type 2) or non-insulin dependent diabetes is the world's largest hormone disorder (Tripathi and Srivastava, 2004). In this type of diabetes, skeletal muscle, liver and adipose tissue will resistance to insulin which can lead to decreased glucose uptake, increased hepatic glucose and lipid. Insulin resistance is associated with many disorders including high blood pressure, elevated blood lipids and renal disorders (Park and Lee, 2005). Diabetes can change metabolism and excretion of drugs and toxins (Maritim *et al.*, 2000). Furthermore, the role of the liver is in detoxification and metabolism of some drugs, such as carbon tetrachloride, thioacetamide (Wang *et al.*, 2000), and aspirin (Doi and Ishida, 2009), has been studied in rat. In the current study, effects of AZA on liver and kidney tissue in rats that resistant to insulin were evaluated.

MATERIALS AND METHODS

In this study, 56 male Wistar rats weighing 20 ± 200 g in standard conditions (12 h dark, 12 h light and temperature 22 ±2c) were kept and fed food and water intensive. LD50 was determined by taking a dose of medication for induction of diabetes, along with drinking water for 98 days, daily fructose solvent cc 140 (10%) were used. By shedding a drop of blood taken from the tail of mice after 12 hours of starvation on specific kits, blood glucose levels were measured. The rats were divided in 8 sub-groups as follows:
Control group: no treatment, sham group: 140cc fructose 10% as a daily feeding. Groups 1 to 4 (diabetic) in addition intake fructose 10 %, were injected (3.75, 7.5, 15 and 21) mg/kg/b.wt AZA interaperitoneal at 98th day. Groups 5 and 6 (non-diabatic) were injected (15 and 21) mg/kg/b.wt AZA interaperitoneal at 98th day. 24 hours after drug injection, blood samples were taken from all groups, centrifuge with 2500 rpm for 10 minutes and their serum separated for biochemical analyzes. Then liver and kidney was disected and sections prepared for histological study as follow:
Specimen were fixed in 10% formalin, were placed in tissue processor and dehydrated in graded series of ethanol (70%- 100%), then impregnated with paraffin and serial 4µ thickness sections were obtained and subjected to Haematoxylin and Eosin (H&E) stains. Sections were mounted with binocular light microscope (x40, x100) and photomicrograph was prepared. In this research, liver enzymatic, diameter of renal tubules and change of liver and kidney tissue were studied. Data were were statistically evaluated with Statistical Package for Social Sciences (SPSS), Version 17. Hypothesis testing methods included one-way analysis of variance (ANOVA) and all groups compared by Duncan test ($p< 0.01$).

RESULTS AND DISCUSSION

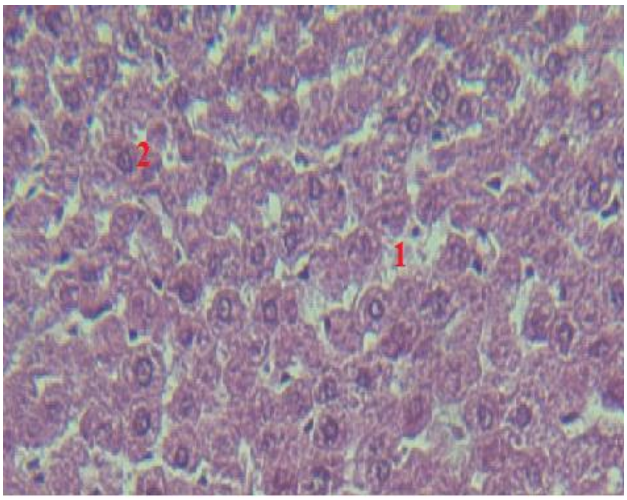
Results

The histological changes including lymphocyte.invasion, hydropic changes in portal areas in diabetics treated groups with minimum does of drug and infiltration, cytoplasm granularity and necrosis of peri-portal area with the maximum dose was observed (Figure 1: C-F). In the non-diabetic treatment groups (Figure 1: G, H), slight changes were seen.
Also significant decrease in the diameter of the renal cortex and medulla in diabetic and non-diabetic treatment groups (Figure 2: A, B) and a significant increase in proximal and distal convoluted, handle's loop and glomerules were observed (Figure 2: C-F).

Discussion

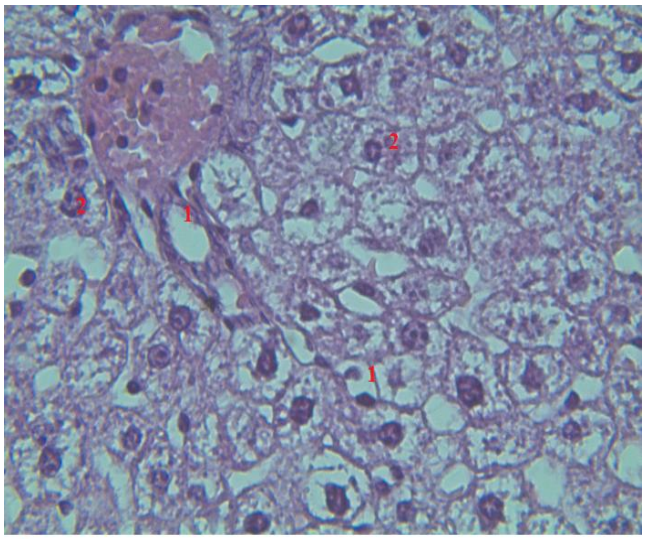
The toxicity of the drugs depends on the chemical nature of the drug and patient's factors. Some diseases affect the absorption, distribution, metabolism and excretion of drugs affecting modern society, nowadays lifestyle-related diseases such as obesity, diabetes, hypertension and hyperlipidemia is increasing (Vozarova *et al.,* 2002).
Diabetes is the most common endocrine diseases and metabolic disorders can be applied to this disease. In hyperglycemia, most cells cannot use glucose for nutrition. Evidence indicates that hyperglycemia is an important factor for kidney damage (Memisogullari *et al.,* 2003).
According to obtained results Azatioprine in nondiabetic groups has little effect on these organs spetialy liver, whereas the most tissue changes were observed in diabetic groups. Given the dose-dependent and time-consuming toxins, can cause changes in tissue, in addition to the physiological state of the target tissue dose and time of exposure to the drug involved the effect of the drug (Erbey *et al.,* 2000).
Based on result of this study is to some extended justified. When tissue damaged, liver enzymes, from liver and other tissues as heart muscle leaking to serum and drug effects on other organs cannot be ignored (Vozarova *et al.,* 2002; Tohidi *et al.,* 2008). Lymphocytic invasion around the portal triad indicate the presence of smaller vessels in the fibrous mass that cannot be observed with an optical microscope.

The previous studies showed that changes in cell membrane structure and permeability changes couse to leaking out of the cell and into the cytoplasm of some liver enzymes as alanin transfrease and aspartat transfrase in the serum occurs (Raza *et al.*, 2008).



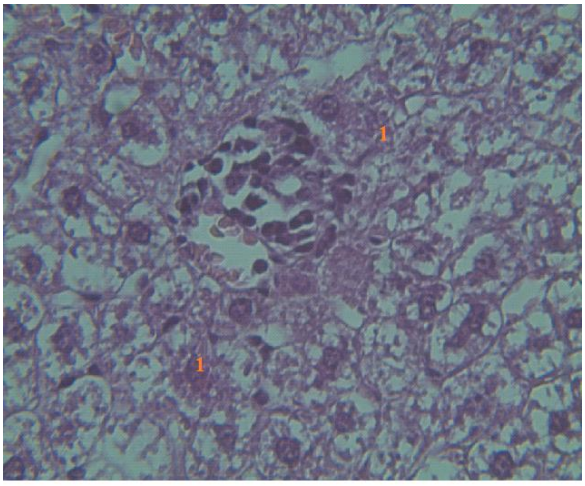
A

Figure 1A: Light micrographs of liver in control group blood sinusoids (1) and hepatocytes (2) are normal



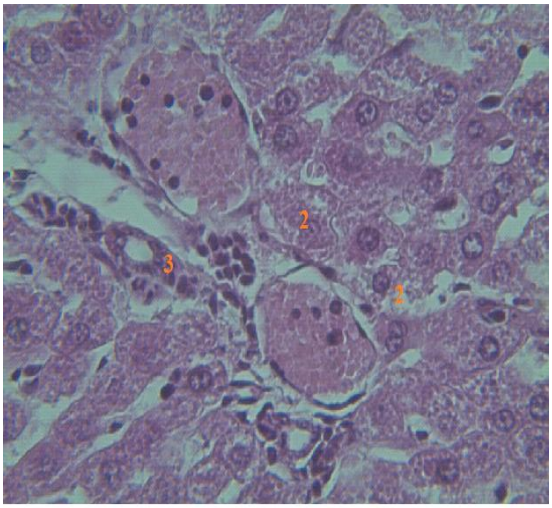
B

Figure 1B: Light micrographs of liver in sham group blood sinosoids (1) are dilated and congested with blood and hepatocytes are grnulated (3)



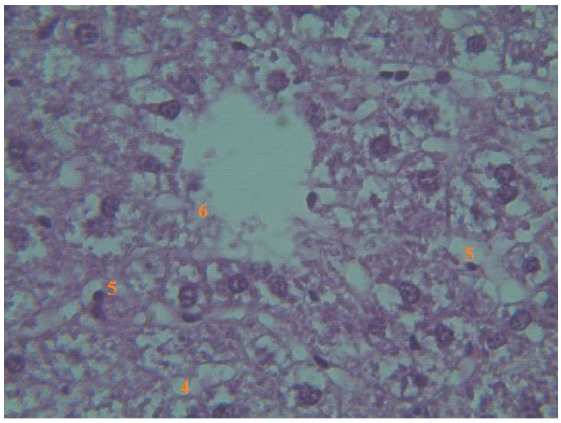
C

Figure 1C: Light micrographs of liver in group 3 Granularity in hepatocyte (1)



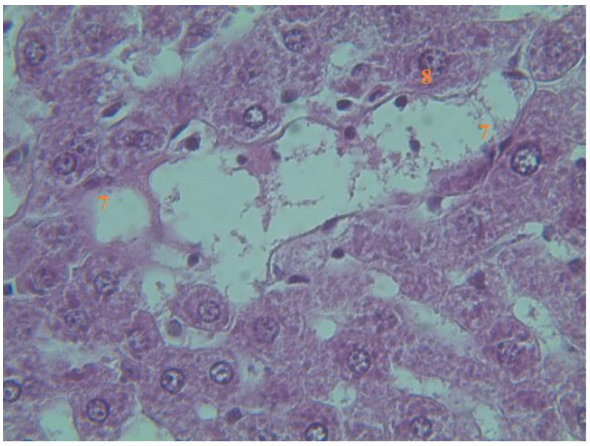
D

Figure 1D: Light micrographs of liver in group 4 Degeneration of hepatocyte (2) and moderate infiltration of inflammatory cells around portal triad (3)



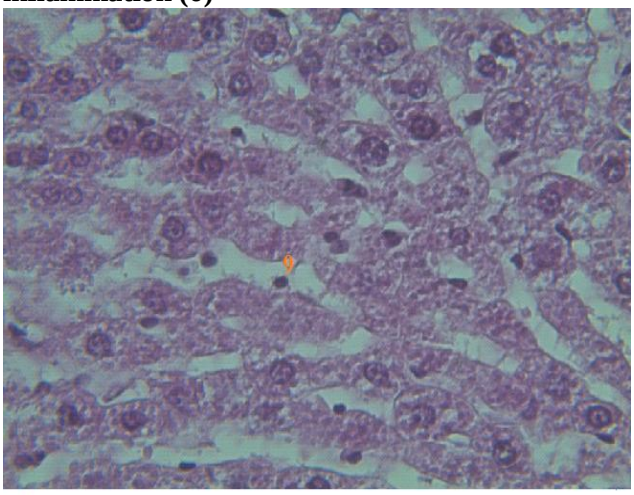
E

Figure 1E: Light micrographs of liver in group 5 Vein is widely dilated (4) and kupfer cells are in sinusoid (5), Eosinophilic cytoplasm and inflammation (6)



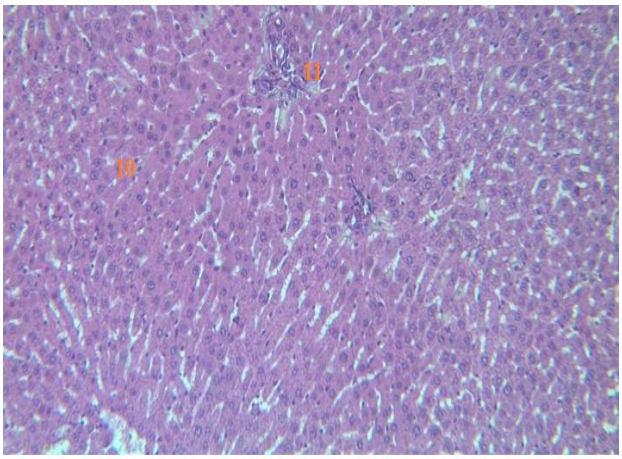
F

Figure 1F: Light micrographs of liver in group 6 Hydropic degenerating (7) and Inflammation cells (8)



G

Figure 1G: Light micrographs of liver in group 7 Lymphocytes influx in the sinusoid (9)



H

Figure 1H: Light micrographs of liver in group 8 Hepatocytes are more and less Normal (10) and granularity around portal triad (11)

Figure 1: The change of liver tissue in experimental groups (40x,100x), staining: H&E

1: Control	**3:** AZA(3.75)+ diabet	**5:** AZA(15)+ diabet	**7:** AZA(15)
2: Sham	**4:** AZA(7.5)+ diabet	**6:** AZA(21)+ diabet	**8:** AZA(21)

It has been suggested that, in rat hepatocytes treated with Azatioprin, ROS production could damage membranes and macromolecules at this level (Farrell, 2004), although there are no convincing results supporting this hypothesis.

Another potential source of ROS that could initiate oxidative stress may be related to production some metabolytes as 6-mercaptopurine that is toxin and ROS may be formed during their metabolism.

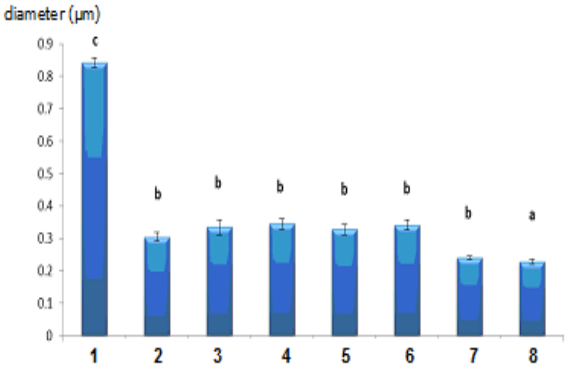


Figure 2A: Graph of the diameter change in Cortex

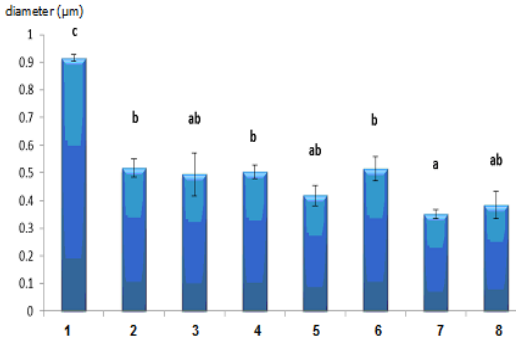


Figure 2B: Graph of the diameter change in Medula

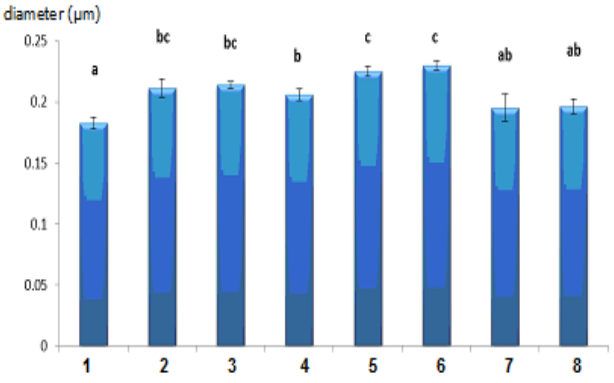


Figure 2C: Graph of the diameter change in Proximal convoluted

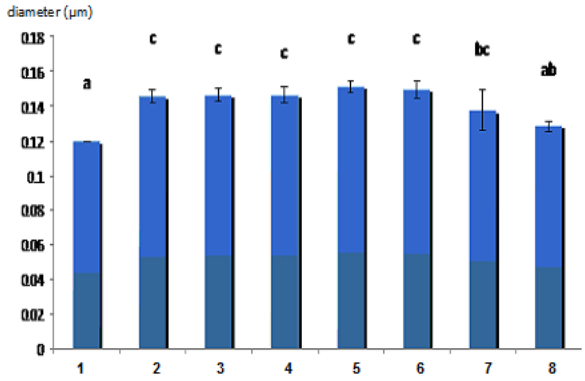


Figure 2D: Graph of the diameter change in Distal convoluted

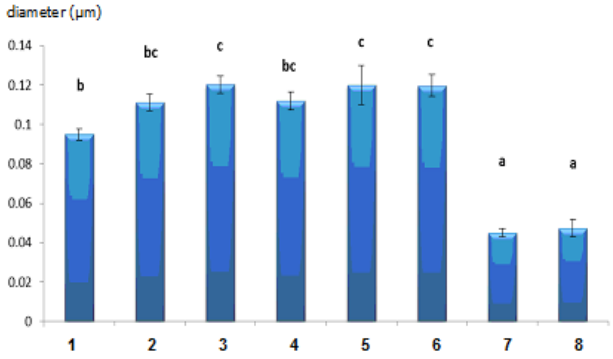


Figure 2E: Graph of the diameter change in Bowman's capsule

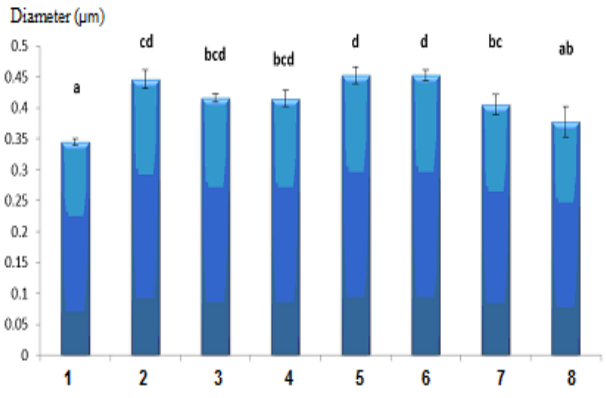


Figure 2F: Graph of the diameter change in Glomerul

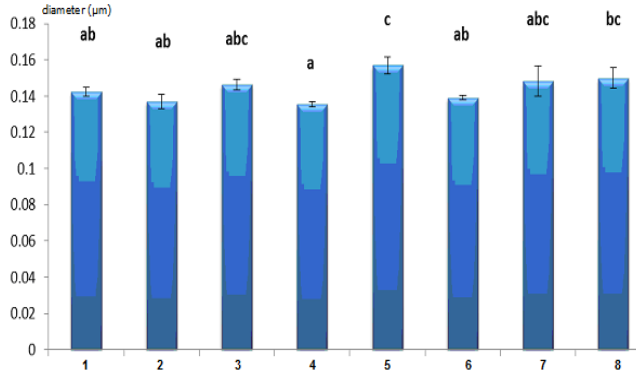


Figure 2G: Graph of the diameter change in Collecting tube

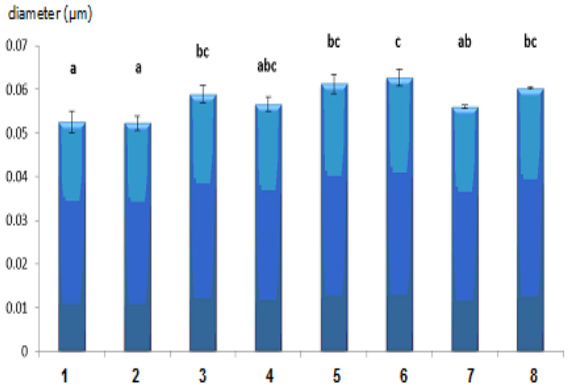


Figure 2H: Graph of the diameter change in Loop of henle

Figure 2: The change of renal tube diameter in experimental groups

A few damage spatially in liver, probably due to short duration of the study, opportunity for complications due to oxidative stress were not enough. Moreover in diabetes (type II) that the effect is long lasting. Approximately 2% of Azathioprine and its metabolite '6 - mercaptopurine' intake in the urine and most of the secondary metabolites of the drug are excreted by the kidneys (Amouoghli *et al.*, 2009). For this reason it is expected that this drug is used today to treat some illnesses on all side effects must be But according to the AZA metabolism, purine antagonist and perhaps product free radicals, which inhibit the synthesis of nucleic acids, proteins, and lipids (Aninat *et al.*, 2006). On the other, probably urea and uric acid that are produced in the diabetic groups increased the damage of tissue. Ammonia from the amino acid is converted to urea in the liver by a cyclic mechanism and inner medullary nephron reabsorption in the urine collecting tubes. The impact of drug-induced damage to the liver or kidney may be a natural but its effects on some materials just does not normal (Amin and Hamza, 2003).

Diabetes is an indicator of kidney damage. Ammonia is highly toxic and can cause damage to the kidney tissue. Azathioprine metabolism purine antagonist and perhaps create free radicals, which inhibit the synthesis of nucleic acids and proteins, and lipids (Aninat *et al.*, 2006).

In present study, significant decrease in the diameter of the renal cortex and medulla in diabetic and non-diabetic treatment groups and increase in proximal and distal convouluted, hanle's loop and glomerules were seen. Glomerular diameter decrease due to diabetes and disorder filter in the blood filteration. Azathioprine proliferate endothelial cell of glomeruls and increased its thickness, since reduced the dimeter of this tubul (Rainer and Schleicher, 2000).

May be when the drug entry of into the kidney tissue, neutrophils begin to swell and also tremendous number of lysosomal occurs in the cytoplasm and granularity is happen. Invasion of neutrophils into the inflamed area of the inner surface of the capillary endothelium changes and creating pores in the capillary walls into the interstitial space. Neutrophils and macrophages move to the site and inflammatory reactions via releasing of inflammatory mediators such as quinine, prostaglandins, leukotrienes and cytokines (interleukin 1) lead to tissue necrosis (Sundby *et al.*, 1979).

It is noteworthy that the low toxic effects Azatioprin on liver and kidney in this study, justify widespread use in treatment comparing corticosteroids and cyclophosphamide. Although the physiological conditions of the exposure and the disease should be considered.

Conclusion

Obtained results showed Azathioprine have less toxicity in rat hepatocytes and renal but in diabetic rats, due to synergistic effects of this drug with diabetes medications can cause severe damage. Therefore, it should be more cautiously in diabetic patients.

ACKNOWLEDGMENT
We gratitude manager of Islamic Azad University Jahrom branch and all those who helped us in doing this research.

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