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# AZATHIOPRINE EFFECTS ON KIDNEY AND LIVER IN FRUCTOSE-INDUCED DIABETIC RAT (WISTAR)

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## ABSTRACT

**Background** : Azathioprine is an immunosuppressive drug that widely administered in the clinic for multiple uses (autoimmune diseases, post-transplant immunosuppression and cancer ).Using this drug cause complications like toxicity in some organs. Method: 56 Wistar rat weighing 200±20 gr 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 interaperitoneal at 98th day. Groups 5 and 6 (adiabatic) were injected (15 and 21) mg/kg/b.wt azathioprine interaperitoneal. 24 hours after, blood samples were taken from all groups and their serum separated for biochemical analyzes. Data were analyzed by ANOVA and Duncan test ($P<0.01$).

**Results**: Obtained finding showed that serumic amounts of blood glucose, Triglyceride, Total and direct bilirubin, total protein and ALP increased significantly and in all experimental groups and AST, increased in groups 1-4, comparing control group ($p<0.01$). In the other hand, urea nitrogen, creatinine and cholesterol (HDL) were increased in diabetic groups. **Conclusion**: Azotioprin is dose- dependent and had synergistic effects with diabetes. Although the effect of this drug on kidney is more than liver but seems diabetes is effective more in this result. Therefore, diabetics are taking limits for consuming Azathiprin.

**Keywords:** Azatioprin, Tissue damage, Liver enzyme.

## INTRODUCTION

Azathioprine (AZA) is an immune suppressive drug that treat diseases such as leukemia; acute lymphoblastic, inflammatory bowel disease and rheumatoid arthritis. Azathioprine with

corticosteroids is the best option to prevent organ rejection. Despite the widespread use of this drug, AZA has been observed that had other effect such as suppressing the patient's lymphocytes, causing toxicity in the bone marrow, gastrointestinal tract and liver (Amouoghli Tabrizi 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). 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 AZA increase liver enzymes level as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Increased malondialdehyde and reduced glutathione levels, due to its effect on the creation of reactive oxygen radicals cause changes in tissues, tissue necrosis, enlarged mitochondria and the rough endoplasmic reticulum (Amin and Hamza, 2003).

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 function 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. In this study, parameters including alanin aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, malondialdehyde and fasting blood sugar (FBS) measured.

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 ($p< 0.01$ ). All the results were expressed as mean + S.D.

## RESULTS

Obtained results are showed in below figures. All the parameters changed in diabetic groups but some of them didn't change in nondiabetic groups. The level of concentration of kidney marker (Fig. 1, 2, 3) increased significantly. The other parameter (Fig. 4- 10) spatially liver enzyme (Fig. 11, 12, 13) is good marker for liver function.

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

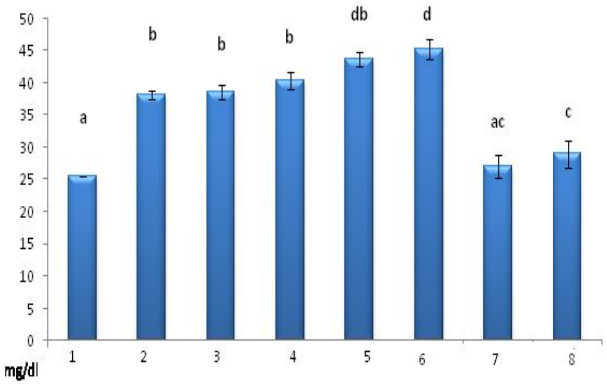


**Fig. 1: BUN level in serum**

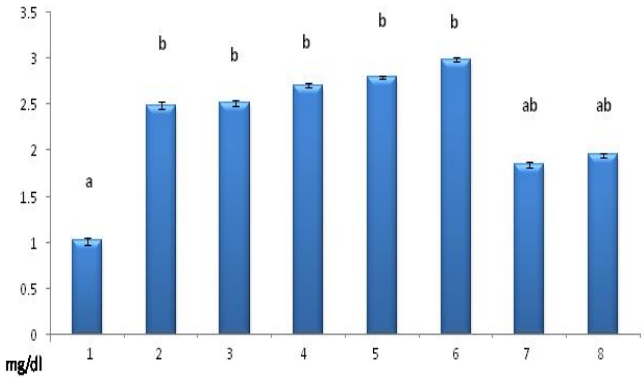


**Fig. 2: Creatinine level in serum**

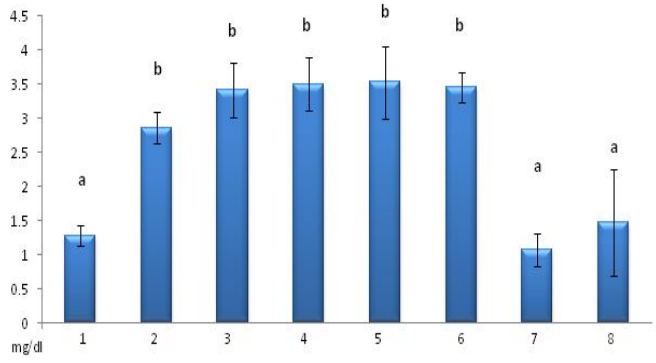


**Fig. 3: Uricacid level in serum**

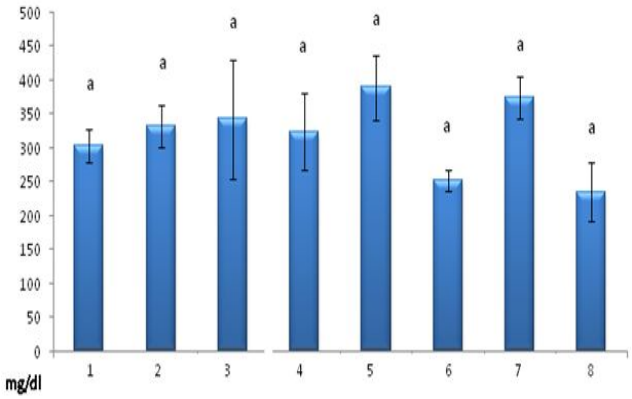


**Fig. 4: Malondialdehyde level in tissue**

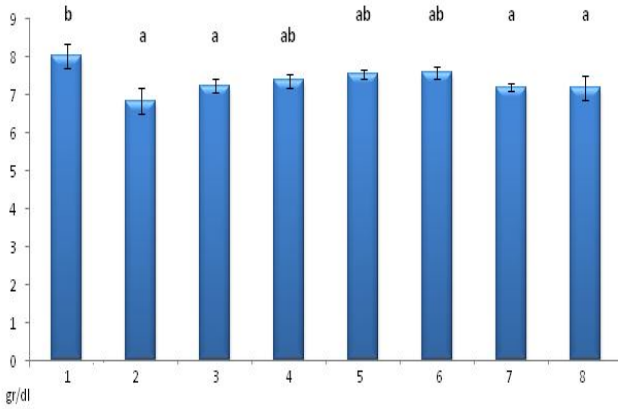


**Fig. 5: Totel protein levein serum**

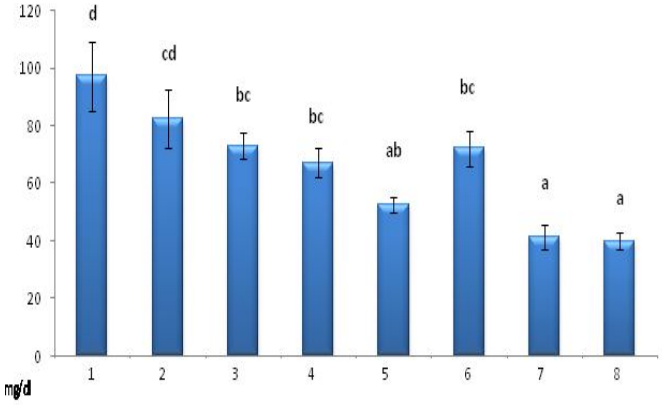


**Fig. 6: Triglycerid level in serum**

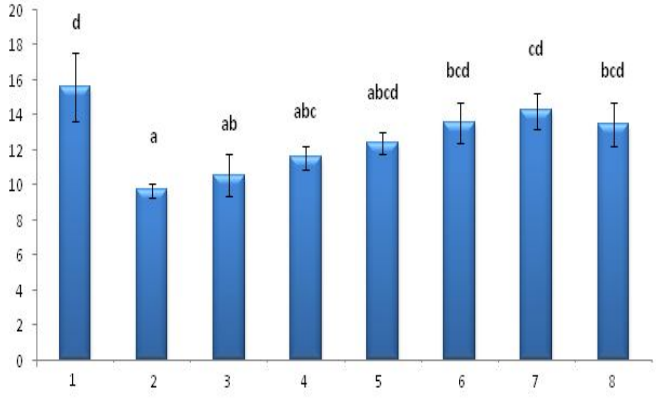


**Fig. 7: HDL Colesterol level in serum**

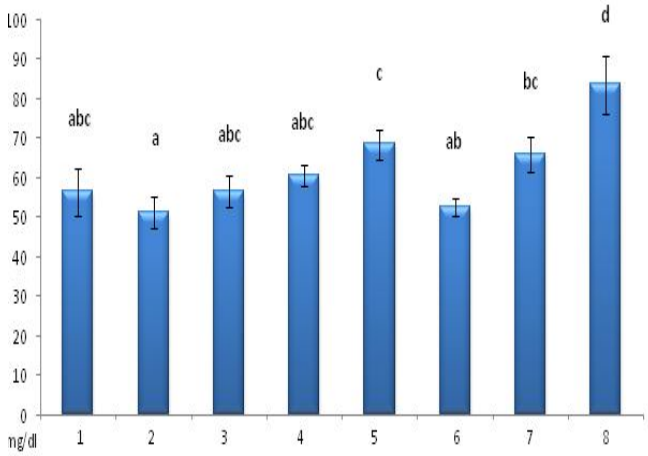


**Fig. 8: LDL Colestrol level in serum**

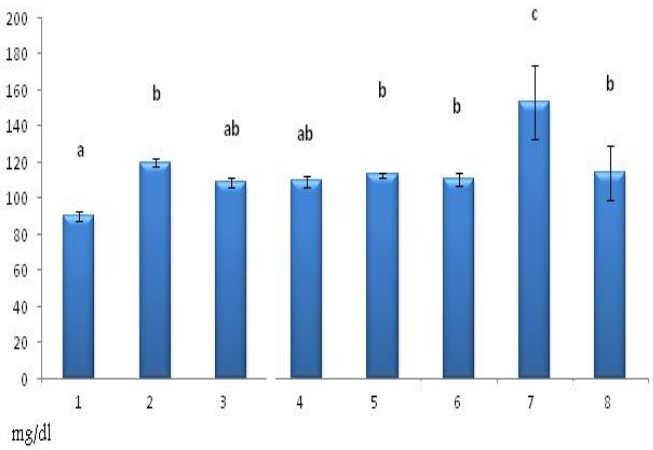


**Fig. 9: FBS level in serum**

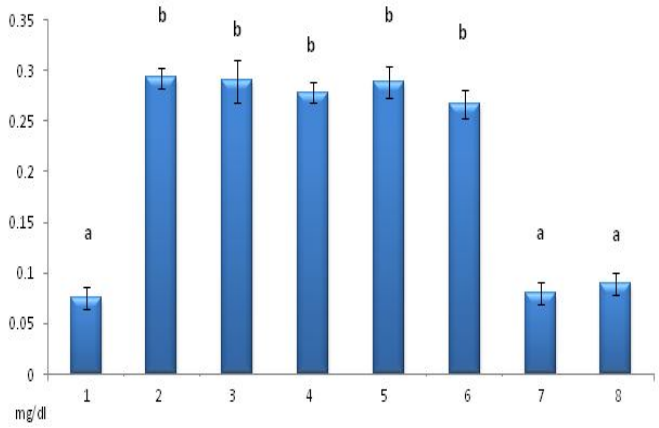


**Fig. 10: Bilirubin level in serum**

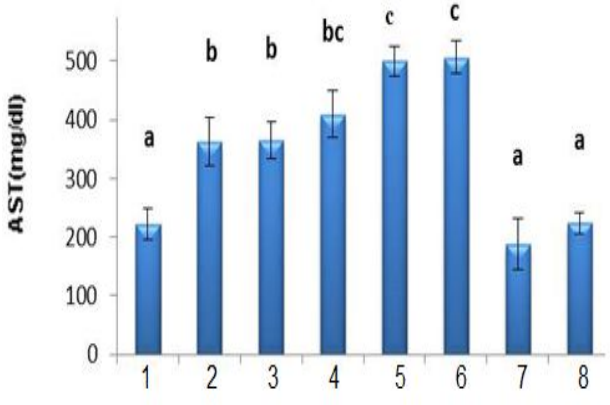


**Fig. 11: AST level in serum**

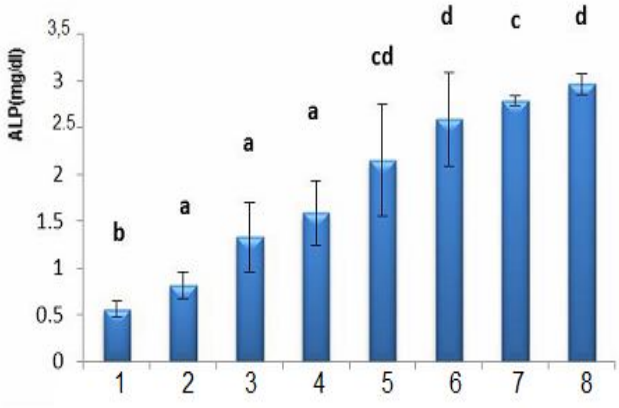


**Fig. 12: ALP level in serum**

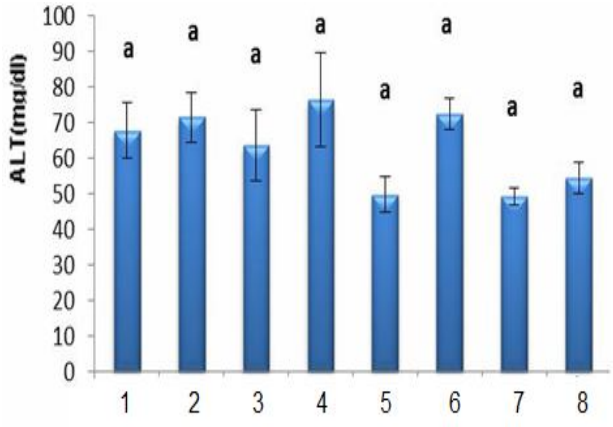


**Fig. 13: ALT level in serum**

## DISCUSSION

In hyperglycemia, most cells cannot use glucose for nutrition (Memisogullari et al., 2003). Evidence indicates that hyperglycemia is an important factor for kidney damage. Increased levels of uric acid, BUN,creatinin (Fig 1-3), indicating kidney damage in diabetic into the tubular secretion of creatinine and glomerular filtration on urine (Memisogullari et al., 2003). Some drugs can despite normal kidney function, decreased tubular secretion may increase serum creatinine. In addition to drugs, other substances such as glucose, uric acid, ketones and bilirubin levels can also cause a sharp rise in serum creatinine are false changes (Raza et al., 2003). 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. Increased urea and uric acid in the control group (diabetic) can be related to the metabolism of the drug in the liver. The impact of drug-induced damage to the liver may be a natural cycle and its effects on some materials just does not happen all come together. If amino acids are produced in the liver converts ammonia to urea in the blood that cannot be moved. Ammonia is highly toxic and can cause damage to the kidney tissue (Raza et al., 2003).

An other hand, based on the results this study are is consistent with the research (Noemí et al., 2008; Erejuwa et al., 2011), the effect of the drug which can't be ignored also increasing serum Alkaline phosphatase (ALP) in the treatment groups (Fig. 12) who received high doses of the drug, especially in diabetics rats comparing to control group showed the synergistic effect diabetes with drug (César et al., 2004). The drug appears to be dose-related acts but on the other hand no significant change in Alanine amino transferase (ALT) (Fig. 13) and malondialdehyde (MDA) in this study were consistent with the expected results (Vozarova et al., 2002; Tohidi et al., 2008) at least with these doses. Alanine aminotransferase is the best marker for liver damage because level of this enzyme in the cytoplasm of hepatic cells is several times higher than the serum's level of that and when the membrane was damaged, the enzyme was removed from the cells and its serumic concentration increases.

The increastion of Aspartate aminotransferase in groups treated diabetes refers to diabetes complications though higher doses have been shown that the optimizer can work together and the sum effects of both drugs and diabetes on various organs in the body is the main cause of this result can be considered (Fig. 11). When tissue damaged, these 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).

According to studies, blood glucose and lipid peroxidation, glycosylation due to changes in the structure of proteins and lipids, changes in cell membrane structure and permeability changes, it leak out of the cell and into the cytoplasm of some enzymes in the serum occurs (Raza et al., 2008).

Changing some parameters, such as increased Bilirubin (Fig. 10), cholesterol. LDL(Fig. 8) in the treatment of diabetic control, consistent with the findings of others, mostly due to complications from diabetes due to insulin resistance, cholesterol metabolism and lipid is disrupted (Wu et al., 2006).

Previous reports (César et al., 2004) showed that AZA selectively inhibit synthesis of purine nucleotides, which are required for DNA synthesis. It has been suggested that, in rat hepatocytes treated with AZA, 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.

No significant differences in the levels of MDA (Fig.4) as the most products of lipid peroxidation in the groups studied but according to some studies, after taking some toxins (Ceyhun et al., 2010), or drugs such as AZA (Amouoghli Tabrizi et al, 2009), MDA increased in blood or tissue, and antioxidants significantly reduced (Park & Lee, 2008). Probably short duration of the study, opportunity for complications due to oxidative stress were not enough, especially in diabetes (type II) that the effect is long lasting.

Increase in fasting glucose in diabetes treatment (Fig. 9) due to resistance in skeletal muscle, liver and adipose tissue to insulin leading to reduced glucose uptake (increased glucose), increased hepatic glucose production is lipogenesis and the effects of drugs on the synthesis of insulin receptors in cells can increase fasting blood sugar (FBS) (Aninat et al., 2006).Therefore, it should be more cautiously in diabetic patients.

## CONCLUSIONS

results obtained showed azathioprine have less toxicity in rat hepatocytes and no induction stress oxidative but in diabetic rats, due to synergistic effects of AZA with diabetes medications can cause severe damage. Therefore, in such cases the drug has limitations.

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