ORIGINAL ARTICLE

AZATHIOPURINE INDUCED HISTOLOGICAL CHANGES IN LIVER IN RABBIT MODEL

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Objective: To evaluate azathioprine (AZA) induced liver injury and protective effects of Zinc sulphate (Zn) and L-Ascorbic acid (AA) in rabbit model. This experimental-analytical study was carried out at animal house of Isra University, Hyderabad from July 2013 to November 2014. **Methods:** One hundred rabbits were selected through inclusion and exclusion criteria. Group 1 (Controls, n=20) were given 0.9% isotonic saline orally, Group 2 (n=20) AZA, Group 3 (n=20) AZA+Zn, Group 4 (n=20) AZA+AA and Group 5 (n=20) AZA+Zn+AA orally for 4 weeks. At end of 4th week, the rabbits were sacrificed. Abdomen was incised in midline and liver was removed, fixed in 4% formaldehyde and was paraffin embedded. 5 micron thick liver tissue sections were stained with Haematoxylin & Eosin. Data was completed using Statistix 8.1 and Chi-square test, and $p \leq 0.05$ was taken as significant. **Results:** The liver histological findings showed significant differences among the controls, AZA, AZA+Zn, AZA+AA and AZA+Zn+AA (p=0.001). AZA induced severe liver injury. Zn and AA treated rabbits showed improvement in liver histology. AZA induced changes includes hepatocytes necrosis parenchyma regenerative nodular hyperplasia, peri-sinusoidal and sinusoidal dilation, cholestasis and peliosis hepatis. Disarray of hepatocytes cords was observed. Hydropic changes with central venule congestion and sinusoid congestion was visible. **Conclusion:** Azathioprine produces severe liver injury. Liver histology was improved with concomitant administration of zinc and ascorbic acid.

Keywords: Azathioprine, Liver injury, Zinc sulphate, Ascorbic acid

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INTRODUCTION

Azathioprine (AZA) is one of the immunosuppresor agent, widely used drug in clinical practice. AZA is used for various autoimmune disorders such as the rheumatoid arthritis and systemic lupus erythematosus. It is also used against rejection of kidney transplants.[1]

AZA is also used for inflammatory bowel disease, lymphomas, multiple sclerosis and myasthenia gravis. AZA therapy is notorious for a number of serious adverse drug reactions. Bone marrow and liver are the seriously affected organs by AZA.[2] Recent hospital based studies reported sided effects of AZA therapy which included fatigue, hepatomegaly, splenomegaly, icterus and abdominal distension due to ascites. Blood counts showed pancytopenia. Liver function testing showed elevated serum bilirubin and transaminases.[2,3]

Zn is one of the trace elements. Its essentiality lies in its participation with enzyme catalysis. Zn is needed by metallo enzymes such as superoxide dismutase (SOD). SOD scavenges the free radicals in body cells including hepatocytes. The hepatocytes are reported zinc deficient patients suffering from liver cirrhosis.[4] Zn is very effective in acute liver injury induced by ethanol toxicity.[5] Being part of biological enzyme activity, the Zn is essential for redox reactions which reduce the cellular injury. Zn plays essential role against oxidative injury in living cells. Zn reduces post ischemic reperfusion induced injury in various tissues and organs through unknown mechanisms. Once suggested mechanism is through its antagonism of copper reactivity.[6] A previous study reported zinc was effective against Chlorpyrifos induced liver toxicity. Zinc protected the liver histology in Chlorpyrifos treated animals.[7]

L-Ascorbic acid (AA) is a water soluble vitamin which exerts antioxidant activity. It helps in reducing reactive oxygen species (ROS) which are biochemically reactive and damage body cells and tissues. ROS are produced during metabolism, phagocytosis, exposure to toxins and carcinogens.[8–10] AA is very potent hydrophilic anti oxidant agent. Hydroxyl radicals, superoxide, sulphydryl radicals, oxidised LDL, and hypochlorite radicals are neutralised by AA. A growing body of evidence suggests AA protects against oxidative stress, thus may be used in various chronic diseases in which primary underlying mechanism is free radical formation.[9–12]

This study was conducted on rabbit model for evaluating toxic effects of azathioprine on liver. Zinc and ascorbic acid were evaluated for their hepatoprotective effects against azathioprine induced liver injury in rabbit model.

MATERIAL AND METHODS

One hundred rabbits were selected for study of Azathioprine induced histological changes of liver at Animal House of Isra University Hyderabad, from July 2013 to November 2014. Male rabbits weighing

1,000−1,500 grams were included. The animals were kept at an optimal room temperature with 55−60% humidity and exposed to 12 hour light-dark cycles. Fresh alfalfa and clean water were provided freely.

Group 1 (n=20) was given 0.9% isotonic saline orally on alternate day for 4 weeks and served as control group. Group 2 (n=20) was administered Azathioprine 15 mg/Kg orally for 4 weeks; Group 3 (n=20) was given Azathioprine 15 mg/Kg+Zinc sulphate 5 mg/Kg orally for 4 weeks; Group 4 (n=20) was fed Azathioprine 15 mg/Kg+L-Ascorbic acid 200 mg/Kg orally for 4 weeks; and Group 5 (n=20) received Azathioprine 15 mg/Kg+Zinc sulphate 5 mg/Kg+L-Ascorbic acid 200 mg/Kg orally for 4 weeks.

Azathioprine, Ascorbic acid, and Zinc sulphate were obtained from Pharmacy of Isra University Hospital. The animals were sacrificed by over-dose of Ketamine and Xylazil[13] and liver was removed promptly for histological study.

Each sample of liver was washed in normal saline and tissues were fixed in previously marked containers containing 10% formaldehyde. The tissues were embedded in paraffin, cut into 5 µm thick sections and stained with Haematoxylin-Eosin (H&E), Masson's trichrome, and Methanamine. The histological criteria included vacuolar degeneration, inflammatory cell infiltrate, congestion and necrosis. The histological parameters were graded as 0 (no abnormal findings), + (mild injury), ++ (moderate injury), and +++ (severe injury).[14]

Data were analysed on Statistix 8.1 using Chi-square test and $p \leq 0.05$ was taken statistically significant.

RESULTS

The liver sections from control group showed intact central portal venules and compact hepatocytes arrangement. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm were seen in control group (Figure-1−3).

The liver histological findings showed significant differences among the controls, AZA, AZA+Zinc sulphate, AZA+Ascorbic acid, and AZA+Zinc sulphate+Ascorbic acid (p=0.001). Liver histology was significantly deranged in AZA group compared to controls and other groups (p=0.01). The AZA+Zinc sulphate+Ascorbic acid group showed less derangement in liver histology when compared to AZA group (p=0.01). The AZA group showed nodular regenerative hyperplasia, veno-occlusinve disease; peliosis hepatis, sinusoidal dilatation, cholestasis, hepatocyte necrosis, and perisinusoidal fibrosis. Derangement of hepatocyte cords, hydropic changes with congestion of central venules and sinusoids were observed. (Figure-4−8). Minimal changes in Zinc sulphate+Ascorbic acid group were observed.

Table-1: Liver injury of controls, Azathioprine, Zinc sulphate, and L-Ascorbic acid groups

	Sinusoidal dilation and Periportal inflammation	Steatoheaptitis	Fibrosis	Peliosis Hepatis	Nodular regenerative hyperplasia	Veno-occlusive Disease
Azathioprine	++++	+++	++++	++++	++++	+++
Azathioprine+Zinc sulphate	++++	++	+++	+++	+++	+++
Azathioprine+Ascorbic acid	+++	++	++	+++	+++	++
Azathioprine+Zinc Sulphate+Ascorbic acid	++	++	+	+	++	+

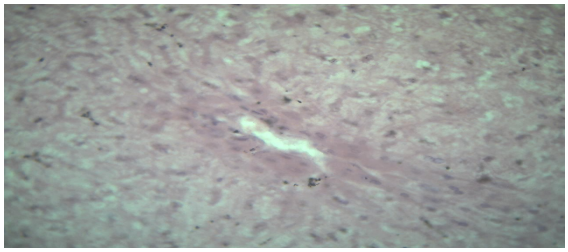

Figure-1: Liver slide of control group showing normal hepatocyte cords. Sinusoids with central venules are visible (H&E, ×40)

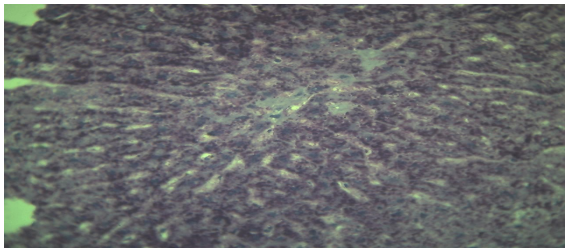

Figure-2: Liver tissue of control group showing normal glycogen content on PAS staining. (×40)

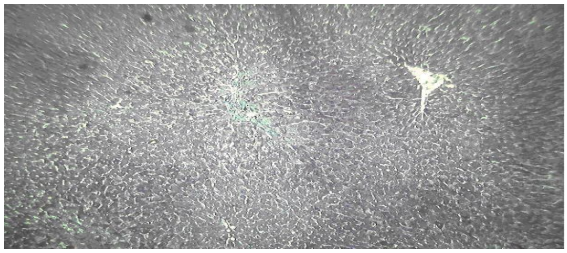

Figure-3: Liver tissue showing no fibrosis on methanemine staining, normal glycogen content on PAS staining. (×40)

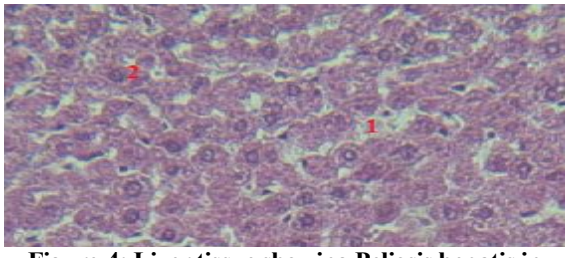

Figure-4: Liver tissue showing Peliosis hepatis in azathioprine group (H&E, ×40)

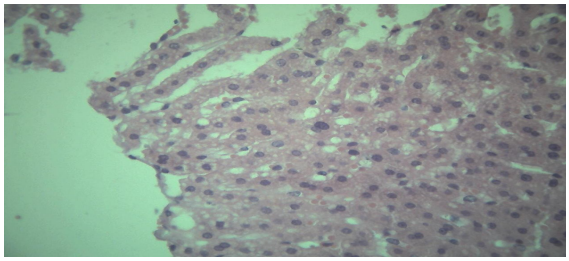

Figure-5: Liver tissue showing near liver histology in Azathioprine+Zinc sulphate group (H&E, ×40)

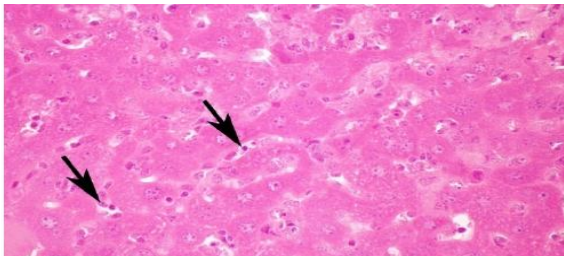

Figure-6: Liver tissue showing liver in Azathioprine +ascorbic acid group; areas of cellular injury are visible (H&E, ×40)

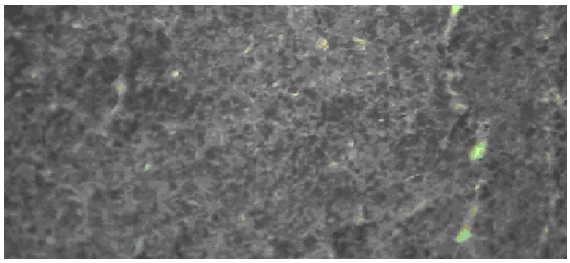

Figure-7: Liver tissue of Azathioprine+Ascorbic acid group showing normal glycogen content. (H&E, ×40)

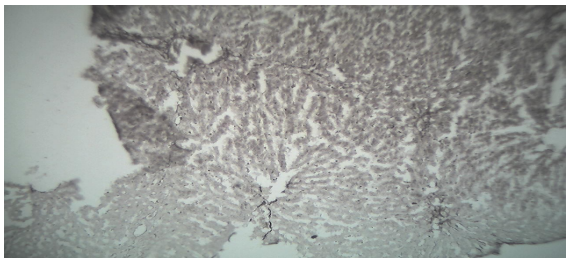

Figure-8: Slide from Azathioprine+Zinc sulphate+Ascorbic acid group showing no fibrosis on methanemine silver staining. (H&E, ×40)

DISCUSSION

AZA treated liver showed a variety of histological findings. Severely altered liver histology was noted in the AZA treated rabbits (p=0.01). An improvement in liver histology was observed in rabbits treated with Zn and AA (p=0.01) (Figure 4–8). Zn and AA treated rabbits showed improvement in liver histology. AZA induced changes includes hepatocytes necrosis parenchyma regenerative nodular hyperplasia, peri-sinusoidal and sinusoidal dilation, cholestasis and peliosis hepatis. Disarray of hepatocytes cords was observed. Hydropic changes with central venule congestion and sinusoid congestion was visible. The findings of present study are consistent to previous studies.[15–17]

Venous congestion, cell vacoulation, and cytoplasm showed degeneration in AZA treated rabbits. Previous studies[15–17] reported that the purine synthesis is inhibited by the AZA which are essential nucleotides for DNA synthesis. Zn produced good effects and findings are really astonishing. Zn 5 mg/Kg body weight showed protective effect against AZA induced liver injury. It is reported that the Zn is a good anti oxidant activity as it is co factor for the SOD enzymes. The SOD catalyses the reaction in which superoxide radical is removed. Zn inhibits the NADPH oxidase, a cell membrane enzyme, which produces the superoxide O^{-} radical from O_2. Zn also inhibits the release of various cytokines such as IL-8, IL-1β and TNF-α. These cytokines are involved in cellular injury. Zn induces the cysteine-rich metallothionein which is an excellent scavenger of OH^{-} ions.[18,19] Zn provides protection against toxins induced liver injury. Zn is used in hepatic encephalopathy refractory to standard treatment.[20,21] Our findings of zinc as cell protective agent is supported by above cited studies. It is reported that the Zn reduces blood ammonia in hepatic encephalopathy and blood ammonia levels.[20]

Zn supplements to chronic Hepatitis C patients reduced gut symptoms, reduces weight loss, inhibits hair loss and prevents anemia.[20–23] The present study reports a protective effect of Zn against oxidative load induced by toxic drugs such as AZA. Our findings are highly consistent with previous studies.[22–27]

Ascorbic acid (AA) has shown astonishing results against AZA induced liver injury in present study. AA 200 mg/Kg improved liver histology, this shows protective role of AA against AZA induced liver injury. The present study reports that the zinc sulphate and ascorbic acid protect against AZA induced liver injury.

CONCLUSION

Azathioprine produces severe liver injury. Azathioprine produced hepatocytes necrosis, parenchyma regenerative nodular hyperplasia, peri-sinusoidal and sinusoidal dilation, cholestasis and peliosis hepatis. Liver histology was improved with concomitant administration of Zinc and ascorbic acid. The present study concludes ascorbic acid and zinc sulphate may be used to minimize the azathioprine induced liver injury.

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