Original Article

Histomorphological effects of isoniazid induced hepatotoxicity in male albino mice

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Objective: To observe the histomorphological changes of isoniazid induced hepatotoxicity in male albino mice.

Methodology: This experimental study was carried out at University of Health Sciences, Lahore, Pakistan from January to December 2013. Forty male albino mice selected by simple random technique, were divided into two groups; A-Control, & B-experimental. Group A comprised of 15, while Group B comprised 25 mice. Both the groups were kept under identical conditions and diet. However, experimental group was treated with an additional oral hepatotoxic dose of isoniazid i.e. 100mg/kg bodyweight daily for 30 days. After 30 days, the animals were sacrificed and livers were dissected out. Gross comparison of the organ and stained sections were histologically compared for morphological differences between the groups. Fischer Exact test was used to analyze the qualitative data and a $p<0.05$ was considered significant.

Results: Group A animals showed the normal liver architecture. Whereas, those of Group B showed deranged hepatic histomorphology.

Conclusion: Hepatotoxic dose of Isoniazid caused histomorphological alterations in the liver of male albino mice. (Rawal Med J 201;43:170-174).

Key words: Isoniazid, hepatotoxic, histomorphology.

INTRODUCTION

Isoniazid, also recognized as isonicotinyl hydrazine (INH), is the first choice for prophylaxis and management of tuberculosis. It was discovered in 1912 and later in 1951 it was found to be useful against *Mycobacterium tuberculosis*.[1] When orally administered, it is absorbed readily and produces peak blood levels in 1 to 2 hours. It easily reaches all body fluids, tissues, organs as well as in body waste (saliva, sputum, and feces).[2] The plasma half-life of isoniazid in patients with normal liver and renal function ranges from 1 to 4 hours. It is excreted primarily by kidneys and also by feces. Approximately 50-70% of a dose is excreted in the urine within one day, mostly as its metabolites.[3] Overdose of isoniazid has been associated with uncontrollable seizures, peripheral neuropathy, hepatitis, hematologic abnormalities, hypersensitivity, psychosis, metabolic abnormalities, immunologic and gastrointestinal abnormalities.[4]

Isoniazid hepatotoxicity ranges in severity from asymptomatic elevation of serum transaminases to hepatic failure. It is directly or indirectly metabolized to acetyl hydrazine and hydrazine by N-acetyltransferase and amidohydrolase. Acetyl hydrazine and hydrazine might be oxidized by CYPs to form hepatotoxic metabolites.[5] Human genetic studies have shown that cytochrome P4502E1 (CYP2E1) is involved in antitubercular drug hepatotoxicity. The CYP2E1 c1/c1 genotype is associated with a higher CYP2E1 activity and may lead to a greater production of hepatotoxins. Experimental studies on rats showed that Isoniazid and Hydrazine induce CYP2E1 activity. Isoniazid has an inhibiting effect on CYP1A2, 2A6, 2C19 and 3A4 activity. CYP1A2 is suggested to be involved in hydrazine detoxification. Isoniazid can induce its own toxicity, possibly by the induction or inhibition of this enzymes. In previous studies patients suffering from ATT- induced hepatotoxity have presented with deranged biochemistry of Liver Functions tests[6]. Also, the toxic effects of isoniazid as a combination therapy to various other drugs on liver histology have been previously in rats, rabbits and pigs.[7] However, the histological effects of hepatotoxic dose of Isoniazid alone is still not clear. The aim of this study was to observe the histo-morphological effects of isoniazid induced hepatotoxicity in the adult male mice.

METHODOLOGY

This experimental study was conducted on 40 non-tuberculous healthy male albino mice, which were procured from Veterinary Research Institute (VRI), Lahore. Simple random sampling by balloting method was used for sampling. They were kept in animal house of University of Health Sciences, Lahore, Pakistan. The study was duly approved by the Ethical review committee of University of health Sciences, Lahore Pakistan. Healthy adult male mice, 6-8 weeks of age, weighing 30g±5g were taken. They were divided into two groups A (control) and B (treated). The animals were kept under standard conditions and fed on mouse diet (wheat balls) and water ad libitum. The experiment was started after acclimatization period of 1 week.

Control group A consisted of 15 and treated group B of 25 animals. Group A was given additionally 10 ml/kg of distilled water by mouth, whereas Group B was administered isoniazid orally in a single daily dose of 100mg/kg for 30 days. Dose was based on previous studies in which isoniazid was given at 100 mg/kg/day, orally for 30 days.[8,9]

The animals were sacrificed on the 30th day of experiment. Liver was removed soon and 3-5 mm thick pieces were excised after gross examination of the organ. The specimens were fixed in formalin for 48 hours and processed to prepare paraffin blocks. 4µm thick sections were cut and stained with Haematoxylin and eosin (H & E), Periodic acid Schiff's (PAS) and Periodic acid Schiff's diastase (PASD) stains. The slides were studied microscopically to compare the liver architecture of the two groups. The two groups were statistically compared by SPSS version 18. Fisher's Exact test was applied to compare the qualitative data. p? 0.05 was considered as statistically significant.

RESULTS

On gross examination of the control group, liver was found to be smooth in texture; dark brown in color, possessed four lobes and was surrounded by thin layer of connective tissue capsule. No gross differences were noticed between the control and treated groups.

All slides were observed using 10X, 20X and 40X objectives. The liver of mouse in the control group (Fig 1-A) showed typical hepatolobular architecture. No periportal inflammation was observed.

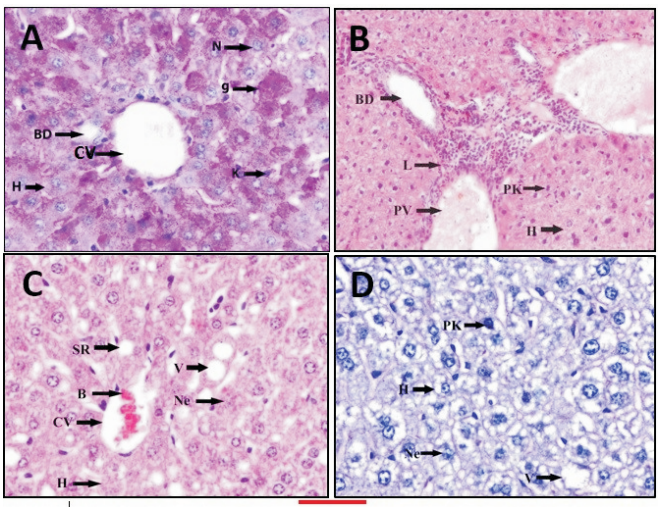
In group B, general architecture was observed to be totally disturbed with loss of radial arrangement of cords of hepatocytes and sinusoids. The hepatocytes were of different sizes. They appeared to be swollen and were empty looking with the loss of their cytoplasmic contents; as their cytoplasm contained large number of micro and macro vacuoles (Fig 1-C&D).

Fig. 1 A. Liver section from group A, showing central vein (CV) and bile duct (BD). Hepatocytes (H), filled with glycogen (g) seen as magenta granules. Nucleus (N) and kupffer cells (K) were visible. PAS stain. (Scale bar = 25 µ approximately).

Fig. 1 B. Photomicrograph of liver section from group B, showing periportal inflammation i.e. abundant lymphocytes (L) was present around bile duct (BD) and portal vein (PV). Vessels were also dilated. Many pyknotic nuclei (PK) were present in hepatocytes (H). H & E stain. (Scale bar = 25 µ, approximately).

Fig. 1 C. Liver section from group B, showing totally disturbed general architecture with deformed hepatocytes (H) having vacuoles (V) in their cytoplasm. Centrilobular necrosis (Ne) was evident by vacuolar degeneration and fatty change around central vein (CV) which was filled with blood (B), showing vascular congestion. Fatty change was indicated by signet ring appearance (SR). H & E stain. (Scale bar = 25 µ, approximately).

Fig 1. D. Liver section from group B, showing hepatocytes (H) with vacuoles (V) in their cytoplasm. Areas of necrosis (Ne) were also evident by fragmented nuclei and pyknotic nuclei (PK). PASD stain. (Scale bar = 150 µ, approximately).



General architecture of the liver was compared for the groups A & B (Table 1). Treated group showed significant architectural changes associated to the isoniazid exposure (p<0.001). In this group, 32% exhibited preserved architecture as compared to 32% manifesting mild and 36% severe architectural loss. In group B, severe vascular congestion was noticed in 40% of the subjects. Also 4% animals exhibited mild, and 10% moderate congestion as compared to Nil in the control group (p<0.001).
In group B, mild inflammation was noticed in 20%, moderate in 48% and severe in 32% of the subjects (p<0.001). Relatively less necrosis was observed in the experimented animals, with 32% of the animals in the treated group not showing any necrosis. However, 20% did show mild, 32% moderate and 16% severe necrosis, as compared to Nil in the control group. The difference was statistically significant with a p<0.001 (Table 2).

Table 1. Qualitative data comparison by Fischer exact test for Group A- Control (n=15) and B - Treated (n=25).

Variable	Parameters studied	A	B	Total	*P* value
General Architecture loss	Preserved	15	8	**23**	< 0.001
	Mildly distorted	0	8	**8**	
	Severely distorted	0	9	**9**	
	TOTAL	**15**	**25**	**40**	
Vascular congestion	Absent	15	1	**16**	< 0.001
	Less than 20% (Mild)	0	4	**4**	
	Between 20- 60% (Moderate)	0	10	**10**	
	More than 20% (Severe)	0	10	**10**	
	TOTAL	**15**	**25**	**40**	
Periportal inflammation	Absent	15	0	**15**	< 0.001
	Less than 20% (Mild)	0	5	**5**	
	Between 20- 60% (Moderate)	0	12	**12**	
	More than 20% (Severe)	0	8	**8**	
	TOTAL	**15**	**25**	**40**	

P<0.05=Significant.

Table 2. Qualitative data comparison by Fischer exact test for Group A- Control (n=15) and B - Treated (n=25).

Variable	Parameters studied	A	B	Total	*P* value
Necrosis	Absent	15	8	23	< 0.001
	Less than 20% (Mild)	0	5	5	
	Between 20- 60% (Moderate)	0	8	8	
	More than 20% (Severe)	0	4	4	
	TOTAL	**15**	**25**	**40**	
Pyknosis	Absent	15	5	20	< 0.001
	Less than 20% (Mild)	0	8	8	
	Between 20- 60% (Moderate)	0	4	4	
	More than 20% (Severe)	0	8	8	
	TOTAL	**15**	**25**	**40**	
Vacuolar degeneration	Absent	15	5	20	< 0.001
	Less than 20% (Mild)	0	8	8	
	Between 20- 60% (Moderate)	0	4	4	
	More than 20% (Severe)	0	8	8	
	TOTAL	**15**	**25**	**40**	
Apoptosis	Absent	15	3	18	< 0.001
	Less than 20% (Mild)	0	4	4	
	Between 20- 60% (Moderate)	0	10	10	
	More than 20% (Severe)	0	8	8	
	TOTAL	**15**	**25**	**40**	

P<0.05=Significant.

Significant pyknosis was observed in the treated group, with 32% severely pyknotic, 16% moderately, and 32% mildly pyknotic. However, 20% animals in the treated and 100% in the control group showed no pyknonis (p<0.001) (Table 2). Regarding the hepatocytes showing vacuolar degeneration, compared to Nil in the control group A, 16% of the treated group B showed mild, 32% moderate and 32% severe level of vacuolar degeneration (p<0.001) (Table 2). The control group did not exhibit apoptosis. Whereas, 16% of the treated group showed mild, 25% moderate and 32% severe apoptosis (p<0.001) (Table 2).

DISCUSSION

Isoniazid hepatotoxity has a wide spectrum, changing from mild transient elevations in aminotransferases to severe hepatitis leading to liver transplantation.[10] Recently, fatal isoniazid-induced hepatotoxicity causing acute liver failure, severe coagulopathy, metabolic acidosis, acute kidney injury and hepatic encephalopathy have been reported.[11,12] Drug-induced liver damage is ultimately a clinical diagnosis of exclusion in which no histologic specimens of the liver are often obtained.[13] In our project, Isoniazid was given in a hepatotoxic dose and actual histology of the organ was studied to observe the induced injury.

Although no marked difference was observed in the gross structure of the two groups, the histological architecture was markedly disrupted in the isoniazide treated group. All the parameters examined were indicative of cellular injury (Table 1 & 2). Scientists have established that apoptosis and necrosis are the most widely recognized forms of hepatocyte cell death and is a pivotal step in most forms of liver injury.[14] In our study, 16% of the animals in the treated group showed mild, 25% moderate and 32% severe apoptosis, while 32% showed moderate and 16% severe levels of necrosis. These histopathological findings confirmed liver injury in the isoniazid treated animals.

However, enhanced hepatocyte apoptosis is tightly coupled with inflammation and fibrosis.[14] Whereas, in our study periportal inflammation was noticed in the treated group and no significant fibrosis was observed. This factor may indicate a potential reversibility of the hepatotoxic effects on discontinuation of the drug. In a recent study, Isoniazid hepatotoxicity was graded as of lower level and generally reversible after cessation of drug.[15]

The pathophysiologic mechanism of drug induced hepatotoxicity has been explained to include both hepatocellular and extracellular insults. Hepatic disruption of the hepatocytes and bile duct injury have earlier been reported as evidence of hepatotoxity.[16] Hepatic disruption was manifested in our study as significant pyknosis in the histological examination with 32% severely pyknotic, 16% moderately, and 32% mildly pyknotic (Table 2). Intense nuclear condensation pyknosis, is the ubiquitous terminus of cells,[17] as reflective in our study.

Earlier bile duct injury has been reported because of amoxicillin treatment, leading to cholesteric hepatitis.[18] The bile duct injury and periportal inflammation was noticed in group B (Fig 1 B) of our study with 20% showing mild, 48% moderate and 32% of the subjects showing severe inflammation.

Drugs have been labelled as a leading cause of liver injury. More than 900 drugs, and other toxins have been reported to cause liver injury, accounting for 20-40% of all instances of fulminant hepatic failure. One of the mechanisms of liver damage is hepatic steatosis (Fatty change). The mechanism of drug induced hepatic steatosis is reported to be their impact on important molecular pathways including increased hepatocytes lipogenesis, decreased secretion of fatty acids, and interruption of mitochondrial β-oxidation as well as altered expression of genes responsible for drug metabolism.

Better understanding of this type of liver injury is important for early identification of drug induced hepatotoxicity and crucial for preventing severe forms of liver injury and cirrhosis.[19] The significant vacuolation of hepatocytes with signet ring appearances in our study support the isoniazid induced liver injury. This form of liver injury is often reversible; nevertheless, over time it may evolve to steatohepatitis and even cirrhosis.[20]

Physicians must identify these injuries because early detection by vigilant monitoring can decrease the severity of damage, if the drug is discontinued.

CONCLUSION

Isoniazid treated hepatoxicity caused histomorphological alterations in liver of male albino mice. Keeping in view the reversible patterns of cell injury noted in our study, it is recommended that combination of Hepatoprotective drugs to isoniazid treatment may reduce or revert the hepatotoxic effects of the drug. Future studies are suggested for detailed histopathological analysis of anti tuberculous drugs and effect of hepatoprotective elements on post or combination administration to them.

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