

Combination Therapy of Isoniazid and Hepamerz (L-ornithine, L-aspartate) - Effects on Liver and Kidney Functions of Rabbits

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Background: Isoniazid (INH), first line drug for antitubercular treatment, induces hepatic necrosis in some of human beings in normal doses and in experimental animals in toxic doses. Reactive toxic metabolites of INH are released which disrupt hepatocellular membrane and cause necrosis. So there is a need to find some hepatoprotective and antioxidant agent. Hepamerz is prescribed for liver disease acute hepatitis i.e. viral, non-viral, drug induced, chronic hepatitis, fatty liver with hyperammonaemia, hepatic encephalopathy.

Objective: Purpose of this research was an attempt to observe protective effects of hepamerz on liver and kidney against isoniazid induced toxicity in rabbits.

Methods: Study was conducted on oryctolagus cuniculus rabbits (1-1.5 kg) of either sex for the period of 11 days. Rabbits were divided in five groups at random with 6 animals in each group. In group I normal liver and renal function tests were recorded without any drug. In group II effects of hepamerz (1.5cc/kg/i.p.) were observed. In Group III isoniazid (50 mg/kg/day i.p.) was administered. In group IV (a+b) combined effects of isoniazid (50mg/kg /day i.p) and hepamerz (1.5 cc/kg) intraperitoneally and orally were observed respectively. Liver function tests (bilirubin, ALT and AST) and renal function tests (BUN and creatinine) were performed. Rabbits were sacrificed on 12th day for observing histopathological changes in liver and kidney.

Results: In biochemical analysis, hepamerz (group II) showed hepatoprotective and nephroprotective effects. Liver and kidney architecture remained almost intact in group II. In group III, bilirubin level increased ($P < 0.05$). Histological picture of liver revealed ballooning degeneration, portal inflammation and necrosis of hepatocytes which are signs of acute inflammation. 50% necrosis was observed in kidney. In group IV (a+b) there was decrease ($P < 0.05$) in ALT level, liver architecture manifested increased inflammatory changes as well as apoptosis, rise ($P < 0.05$) in BUN and creatinine levels showing nephrotoxicity. In this group rabbits also manifested central neurological effects (tonic clonic convulsions), peripheral neurological effects (paraplegia). There was no mortality in group I and II while in group III, IVa and IVb there was 33%, 83% and 66% mortality respectively. It can be concluded that INH induced biochemical and histopathological changes are not antagonized by concurrent administration of hepamerz for 11days. Additionally nephrotoxic and neurotoxic effects were observed by this combination therapy.

Key Words: Hepatotoxicity, Nephrotoxicity, INH-induced hepatotoxicity, Hepamerz, Liver Function Tests, Renal Function Tests.

Introduction

Administration of Isoniazid (INH) has been reported to cause hepatic necrosis in some of human beings in normal doses and in experimental animals in toxic doses. Reactive toxic metabolites of INH are released which disrupt hepatocellular membrane and cause necrosis. So there is a need to find some hepatoprotective and antioxidant agent. So there is a need to find hepatoprotective agent against INH-induced toxicity.¹

Hepamerz is a stable combination of two important endogenous amino acids that consists of L-ornithine-L-aspartate. It quickly breaks down into L-ornithine and L-aspartate when administered in body. L-ornithine a substrate of urea cycle, converts toxic ammonia into non-toxic urea which is then eliminated through kidneys. It helps the diseased liver to carry out its normal detoxification. This mechanism lowers the raised level of ammonia in blood that is a com-

mon problem in liver cirrhosis. L-aspartate is a necessary component of citric acid cycle which liberates energy (ATP). It then helps in regeneration of damaged hepatocytes. Hepamerz is generally prescribed for liver disease acute hepatitis like viral, non-viral, drug induced, chronic hepatitis, fatty liver, hepatic encephalopathy. It can also be given as an adjuvant therapy with all hepatotoxic drugs.²⁻⁴

Materials and Methods

Oryctolagus cuniculus rabbits (1 – 1.5 kg) of either sex were kept in the animal house of Department of Pharmacology, King Edward Medical University, Lahore. Liberal amount of water and food was supplied. Before starting the experiment all groups were supplied with normal diet and water for acclimatization. Each experiment started with blood collection to record normal values of all rabbits. Number of animals was kept constant in each group i.e. (n=6).

Animal Study Protocol

Thirty rabbits were divided into five groups with six rabbits in each group. In group I normal hepatic function tests were recorded without any drug. In group II effects of hepamerz (1.5 cc/kg i.p.) were observed. In group III effects of INH (50mg/kg /day i.p.) were observed. In group IVa combined effects of INH (50mg/kg /day i.p.) and hepamerz (1.5 cc/kg/ i.p.) were observed. In group IVb combined effects of INH (50 mg/kg/day i.p.) and hepamerz (1.5 cc/kg p.o.) were observed.

Drugs were given once daily for 11 days according to rabbit's weight. Body weights were recorded at start and

one day after the last dose. Blood samples (at start and on 12th day) were drawn from marginal ear vein and serum samples were stored at 4°C before analysis. Livers and kidneys were preserved for histopathological investigation.

Biochemical Analysis

Liver function tests including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, serum total bilirubin level and renal function tests including blood urea nitrogen (BUN) and serum creatinine were determined by auto-analyzer using kits of Randox laboratories.

Table 1: Effects of different drug treatments on liver and renal function tests of rabbits.

Groups		BIL (mg/dl)	ALT (U/L)	AST (U/L)	BUN (mmol/L)	CREA (mg/dl)
II	C	0.53 ± 0.108	49.67 ± 8.36	43 ± 7.02	36 ± 2.51	0.8 ± 0.06
	T	0.6 ± 0.07	37.5 ± 7.24	52.83 ± 5.21	40.3 ± 3.51	1.03 ± 0.31
	P- value	NS	*	NS	NS	NS
III	C	0.33 ± 0.06	61.6 ± 9.17	34.3 ± 4.59	39.17 ± 7.19	0.983 ± 0.079
	T	0.5 ± 0.03	24.8 ± 2.04	26.6 ± 3.27	40.83 ± 7.35	0.86 ± 0.057
	P- value	*	*	NS	NS	NS
IVb	C	0.43 ± 0.08	46.67 ± 3.14	25.67 ± 4.37	38 ± 5.37	0.85 ± 0.04
	T	0.46 ± 0.05	37.33 ± 4.71	33.5 ± 2.07	47.67 ± 4.318	1.18 ± 0.07
	P- value	NS	*	NS	*	*

BIL = Bilirubin; ALT = Alanine Aminotransferase; AST = Aspartate aminotransferase; BUN = Blood Urea Nitrogen; CREA = Creatinine; NS=not significant; *= significant (P = <0.05); C = Control (before drug treatment on 0th day); T = Test (after drug treatment on 12th day)

Table 2: Histopathological (% age) Changes in Livers and Kidneys of rabbits.

Parameters			
In Liver	Group II	Group III	Group IVb
Fatty Changes	0% (0/6)	0% (0/6)	50% (3/6)
Ballooning Degeneration	16% (1/6)	50% (3/6)	50% (3/6)
Portal Inflammation	33% (2/6)	66% (4/6)	50% (3/6)
Necrosis	0% (0/6)	50% (3/6)	66% (4/6)
Apoptosis	0% (0/6)	0% (0/6)	50% (3/6)
In Kidney	Group II	Group III	Group IVb
Edema	16% (1/6)	50% (3/6)	66% (4/6)
Vascular Congestion	0% (0/6)	33% (2/6)	66% (4/6)
Focal Tubular Necrosis	0% (0/6)	50% (3/6)	83% (5/6)

Histopathological Studies

Small pieces of liver tissues were preserved in 10% formaline and then embedded into paraffin blocks. Thin sections of 5 µM were prepared for microscopic examination. Slides were stained with haematoxylin and eosin (H&E) and observed under light microscope.

Statistical Analysis

The data collected on biochemical parameters was statistically analyzed to test various null hypotheses about the mean values of these parameters. Treatments were estimated at 5% level of significance. Wherever necessary, paired or unpaired 't' tests were also performed.

Results

a) Effect on Food Intake and Weight of Animals

Water and food intake was same in groups I



Fig. 1: Manifestation of paraplegia in group IV(a+b) rabbits.

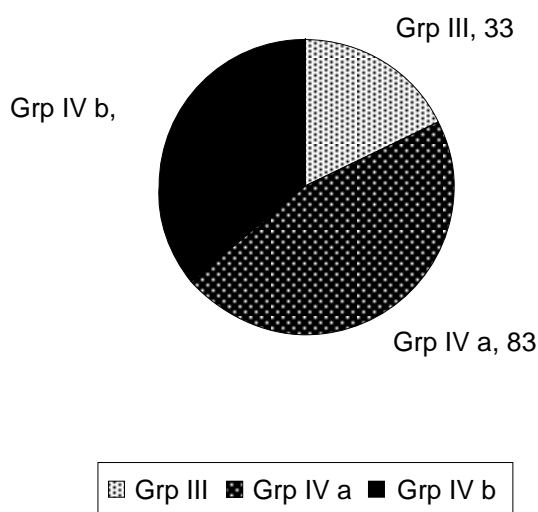


Fig. 2: Mortality (%age) Chart of group III, IVa and IVb.

and II. In group III IVa and IVb there was decreased food intake, decreased motor activity and weight of rabbits reduced.

b) Biochemical and Histopathological Analysis

Group I: In group I no drug was administered. In liver function tests mean value of bilirubin, ALT and AST was 0.38 mg/dL, 48.83U/L and 53.83U/L respectively. In renal function tests mean value of BUN and creatinine was 38.11 mmol/L and 0.98 mg/dL respectively. Liver and kidney biopsies showed normal architecture.

Group II: *Effects on liver:* In liver function tests, hepamerz (1.5 cc/kg; i.p.) caused increase in bilirubin, decrease ($P < 0.05$) in ALT activity and increase in AST activity on 12th day (Table 1). In histological analysis, fatty changes (0%), ballooning degeneration (16%), portal inflammation (33%),

necrosis of hepatocytes (0%) and apoptosis (0%) was observed after postmortem of rabbits (n=6) (Table 2).

Effects on kidney: In renal function tests, hepamerz (1.5 cc/kg; i.p.) alone caused increase in BUN level and creatinine level on 12th day (Table 1). In histological analysis, edema (16%), vascular congestion (0%) and focal tubular necrosis (0%) was observed after postmortem of rabbits (n=6) (Table 2).

Group III: *Effects on liver:* In liver function tests, INH (50mg/kg;i.p) alone caused increase ($P < 0.05$) in bilirubin level, decrease ($P < 0.05$) in ALT and decrease in AST activity on 12th day (Table 1, Figure 1). In histological analysis, fatty changes (0%), ballooning degeneration (50%), portal inflammation (66%), necrosis of hepatocytes (50%) and apoptosis (0%) was observed after postmortem of rabbits (n=6) (Table 2).

Effects on kidney: In renal function tests, INH (50 mg/kg; i.p) alone caused increase in BUN level and decrease in creatinine level on 12th day (Table 1, Figure 2). In histological analysis, edema (50%), vascular congestion (33%) and focal tubular necrosis (50%) was observed after postmortem of rabbits (n=6) (Table 2).

Group IVa and IVb: *Effects on liver:* In both groups same dose of hepamerz was used while mode of administration was different. In group IVa hepamerz was administered intraperitoneally (i.p) but then in group IVb oral route of administration was used. In liver function tests of group IVb, combination of INH (50mg/kg i.p) and hepamerz (1.5 cc/kg p.o) caused increase in bilirubin, decrease ($P < 0.05$) in ALT activity and increase in AST activity on 12th day (Table 1, Figure 1). In histological analysis, fatty changes (50%), ballooning degeneration (50%), portal inflammation (50%) and necrosis of hepatocytes (66%) and apoptosis (50%) was observed after postmortem of rabbits (n=6) (Table 2).

Effects on kidney: In renal function tests, combination of INH (50mg/kg/i.p) and hepamerz (1.5cc/kg/p.o) caused increase ($P < 0.05$) in BUN level and creatinine level on 12th day (Table 1, figure 2). In histological analysis, edema (66%), vascular congestion (66%) and focal tubular necrosis (83%) was observed after postmortem of rabbits (n=6) (Table 2).

c) Neurotoxicity and Mortality

In group III paraplegia (peripheral neurotoxic effect) was observed. There was 33% mortality in this group during 8 to 10 days. In both group IVa and IVb after 3 to 4 days rabbits suffered from limbs weakness and slight blindness. There was totally loss of appetite. Rabbits manifested central and peripheral neurological side effects. Central effects included tonic and clonic convulsions. While peripheral effects included paraplegia (Figure 1) and final fate of these rabbits was death. In group IVa and IVb mortality was 83% up to 6th

day and 66% up to 12th day respectively (Figure 2). Biochemical findings of group IVb are shown (Table 1).

Discussion

Experimental studies on animals suggest that when anti-tubercular drugs are administered in toxic dose there is rise in ALT, AST and ALP in serum, affecting hepatocellular membrane integrity and its organelles.^{5,6}

It is recommended that LFT's should be routinely performed at monthly interval during antituberculosis treatment (ATT). In these subjects, in whom there is disturbance of LFT's, we have to withdraw hepatotoxic AT drugs and restart after an interval of 3-4 weeks. ALT, AST and serum bilirubin are the most sensitive tests for the diagnosis of liver diseases.⁷ Raised levels of serum enzymes indicate cellular leakage and membrane integrity of liver is disturbed.⁸ Hepatotoxin is mainly responsible for increased bile secretion in the serum.⁹ RBC degeneration rate is a measure for binding, conjugating and excretory capacity of hepatocytes. Increased activity of hepatocytes leads to hyperbilirubinaemia which helps to determine integrity of liver.¹⁰

Hepamerz (group II) resulted into fall ($P < 0.05$) in the ALT, though there was non-significant increase in bilirubin and AST. In liver architecture there was lesser incidence of ballooning degeneration, portal inflammation and necrosis compared with other groups. There was increase in urea formation and creatinine, however the incidence of focal tubular necrosis markedly reduced. There was no edema and vascular congestion in renal histology. Hepamerz alone shows hepatoprotective role. In acute liver failure, high blood ammonia levels correlate with mortality and complications. Hepamerz reduces ammonia levels by increasing disposal of hepatic ammonia and peripheral metabolism.¹¹

It has been reported that during sub acute or chronic treatment, INH induced hepatotoxicity occurs in man,¹² rats¹³ and guinea pigs¹⁴ resulting in the rise of serum transaminases and phosphatases activities. But in comparison to this there is also evidence that AST, ALT and ALP activities decrease in serum as well as in liver tissues of rabbits after INH administration.¹⁵

Rise in the levels of activities of ALT, AST and ALP in serum followed by fall in these levels in liver tissue indicates drug and chemical induced hepatotoxicity. As extent of INH induced hepatotoxicity is not clearly shown in the serum so measuring above mentioned levels in serum alone don't reflect the actual position of INH induced liver toxicity during sub acute treatment.¹⁵ We decided to perform bilirubin, ALT, AST and liver biopsy in order to verify the disturbed functions and actual necrotic and inflammatory changes in the liver. So in our experiments histological parameters were added to support the biochemical findings. Isoniazid-induced hepatitis is associated with ballooning degeneration and focal hepatocyte necrosis.¹⁶ In our study same changes were observed so proving the validity of our animal model. As there is excessive production of ammonia in case of jaundice, urea cycle becomes activated and most

of ammonia is converted into urea, which is the end product of protein metabolism. We have tried to find out the correlation of increased urea formation with renal parenchymal changes.

Histopathological appearance with INH induced toxicity resembles with viral hepatitis. Direct toxicity of the drug or its metabolite is mainly responsible for hepatocyte death.¹⁷ Acetylhydrazine is a drug metabolite implicated in causing INH hepatitis.¹⁸

In group III, there was hyperbilirubinemia ($P < 0.05$), fall in ALT ($P < 0.05$). It is supported by past literature in which there was a fall in the activities of ALT not only in serum but also in liver tissue of rabbits treated with INH (50 mg/kg i.p).¹⁵ Histological picture of liver showed that there was portal inflammation and hepatic necrosis also. Blood urea nitrogen level raised and in the renal biopsy there was tubular necrosis along with edema and vascular congestion. INH also caused neurotoxic manifestations i.e. convulsions having tonic and clonic convulsions. Animal studies have shown that isoniazid has neurotoxic properties.^{19, 20} Thirty three % mortality was probably due to hepatic, renal and neurotoxic manifestations.

In combination therapy group IVa and IVb, ALT decreased ($P < 0.05$). Liver histology revealed ballooning degeneration, necrosis inflammation as well as patchy apoptosis. Urea and creatinine levels increased ($P < 0.05$). Histopathological changes showed massive tubular necrosis. In group IVa, increased mortality was also observed as compared to group IVb in which route of administration was changed. Hepamerz administration both intraperitoneally (IVa) and orally (IVb) along with INH caused neurotoxicity in which rabbits suffered from convulsions (tonic and clonic) and hyperextension of body.

Conclusion

Biochemical and histopathological studies showed that Hepamerz alone (group II) has hepatic and renal protective action while INH (group III) caused hepatic and renal toxicity. Combination of INH and Hepamerz increased ($P < 0.05$) the level of BUN and creatinine causing nephrotoxicity leading to increased mortality. Nephrotoxicity and neurotoxicity is a new finding in combination therapy of INH and Hepamerz.

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