Effects of Jetepar (glucometamine, glucodiamine and nicotinamide ascorbate) on Isoniazid Induced Hepatotoxicity in 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. Jetepar, combination of glucometamine, glucodiamine and nictonamide ascorbate is non-toxic to liver and used for viral hepatitis. Purpose of our research was to observe the effects of jetepar against INH-induced hepatotoxicity in rabbit model.

Methods: Study was conducted on oryctolagus cuniculus rabbits (1-1.5 kg) of either sex for the period of 11 days. Animals were divided in five groups at random with 6 animals in each group. In group I normal liver function tests were recorded without any drug. In group II isoniazid (50mg/kg /day i.p) was administered. In Group III effects of jetepar (3cc/kg /day i.m) were observed. In group IV and V combined effects of isoniazid and jetepar were observed with two different doses. In liver function tests serum bilirubin, ALT and AST were performed on 12^{th} day. Animals were sacrificed on 12^{th} day for observing histopathological changes in liver.

Results: In group II, bilirubin level increased significantly. Histological picture revealed ballooning degeneration, portal inflammation and necrosis of hepatocytes which are signs of acute inflammation. In group III, rabbits gained weight and liver architecture remained intact. In group IV and V, there was significant improvement in liver architecture when compared with group II. So it can be concluded that INH induced biochemical and histopathological changes are significantly antagonized by concurrent administration of jetepar for 11days.

Key Words: INH-induced hepatotoxicity, liver function tests**,** liver histology.

Introduction

Liver is exposed to metabolites that may cause direct toxicity or there may be chance of immunological reaction either by drug itself or its active metabolite.[1] It has been reported that 62% of withdrawn drugs produce toxic metabolites when administered.[2]

During anti TB treatment hepatotoxicity is common side effect and it may range from rise in levels of transaminases up to acute liver failure (ALF).[3] Ten to 20% of patients receiving isoniazid manifest liver injury and some may develop hepatitis. In human beings isoniazid is metabolized at variable rates and this rate is controlled genetically.[4]

Isoniazid (INH, isonicotinic acid hydrazide) is a synthetic antibiotic introduced in 1952 which is bactericidal against replicating Mycobacterium tuberculosis. The drug acts by inhibiting the oxygen-dependent steps in the synthesis of mycolic acid, a component of the mycobacterial cell wall. 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.[5] INH toxicity can be reduced prophylactically with *pyridoxine*. For treatment it is strongly recommended that drug should be administered concurrently with another agent.

Jetepar is a drug with specific anti-toxic activity. It contains glucometamine (betaine glucuronate), glucodiamine (diethanolamine glucuronate), nicotinamide ascorbate. Its components have the following therapeutic activity. (a) Betaine glucuronate has a remarkable antitoxic and glycogenetic action due to glucuronic acid which is one of the main substances employed by liver to eliminate endogenous or exogenous toxins. (b) Diethanolamine glucuronate adds to the detoxicating and liver protective action of glucuronic acid, the advantage of diethanolamine is that it remarkably reduces hepatic lipids of both aortic and serum cholesterol. (c) Nicotinamide as ascorbate preserves the antitoxic, eutrophic and anti-asthenic activity of ascorbic acid and the hypocholesterolemic action of nicotinamide.

After a few days of treatment, jetepar determines a fast elimination of endogenous toxins as glucuronjugates and a fall of the hyperlipemic values. A clear improvement of the liver functions is thus rapidly achieved. Jetepar is used for intoxications either endogenous or exogenous, liver diseases, cirrhosis of liver, fatty liver and alcoholism.

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). INH was obtained from IRZA Pharmaceuticals Lahore, Pakistan. Following injections, chemicals, kits and equipment and apparatus was used during study. Jetepar injections 10 ml vials, normal saline 1000 ml I/V soln, lignocain injections vials (local anesthetic), xylene, 10% formaldehyde, alcohol, hemotoxicilin, 1% acid alcohol (HCl), 0.1% ammonia, 1% eosine. Kits for serum bilirubin, ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase). Automated clinical chemical analyzer, automated processing chamber routery microtome, weighing balance (for small animals), electronic weighing balance (Shimadzu AY220), UV Spectrophotometer, refrigerator, centrifuge machine (SANYO MSE), operation table (For small animals), Light microscope, operation light, EDTA containing tubes, disposable syringes 5cc 23G, disposable needles 23G, cotton bags, butterfly needles (22G,23G), scissors, measuring cylinder 100 ml, glass pipette, glass rod, dissection box, filter paper, sharp disposable blades, glass slides, hot plate of 56°C.

A. **Grouping of the Animals**

The rabbits (n = 30) were divided into five groups with six rabbits in each group. Body weights were recorded at start and one day after last dose. In group I normal liver function tests were recorded without any drug. In group II effects of isoniazid (50 mg/kg /day i.p) were observed. In group III effects of jetepar (3 cc/kg /day i.m) were observed. One cc of jetepar contains betaine glucuronate, diethanolamine glucuronate and nictonamide ascorbate. In group IV combined effects of isoniazid (50 mg/kg/day i.p) and jetepar (3 cc/kg /day i.m) were observed. In group V combined effects of isoniazid (50 mg/kg /day i.p) and jetepar (6 cc/kg /day i.m) were observed.

B. **Drug Injection Protocol:**

The dosing schedule for INH[3] was 50 mg/kg intraperitoneally for a period of 11 days. Same duration was applied for other groups with different dose according to rabbit's body weight.

C. **Blood Sampling**

Blood (4 ml) was drawn from marginal ear vein with the help of a fine 22G needle and by putting EDTA containing vial under this for blood collection. Xylene was applied on target surface to dilate vein. Lignocain swab (local anesthetic) was applied on the target to protect from pain. Blood samples obtained from ear vein (before drug treatment) were centrifuged and serum was stored at -20°C till analyzed. A baseline specimens was drawn on 0th day (before administration of any drug) and on 12th day (the day after last day of drug administration).

D. **Biochemical Analysis**

Extracted serum was subjected for the hepatic function tests. Hepatic profile included estimations of bilirubin, AST, ALT. All these parameters were recorded by auto-analyzer using kits.

E. **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.

F. **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

1) **Effect on Food Intake, Weight of Animals and Mortality**

In group II, IV and V there was decreased food intake and decreased motor activity. While in group III due to increased food intake rise ($P<0.05$) in weight was observed (Table 1). In this group no mortality was observed as compared to group IV and V in which 10% mortality occurred.

Table 1: *Effect on Body Weight of Rabbits.*

Groups		Weight (Kg)	
		C	T
III	Mean	0.9 ± 0.1125	1.075 ± 0.089
	"P" value	*	
IV	Mean	1.083 ± 0.0833	1.117 ± 0.079
	"P" value	NS	
V	Mean	1.133 ± 0.152	0.9917 ± 0.1165
	"P" value	*	

C = Control (before drug treatment on 0th day)
T = Test (after drug treatment on 12th day)
NS = not significant; *=significant (P=<0.05)
Group III = Jetepar (3cc/kg /day i.m)
Group IV = INH (50mg/kg /day i.p) + Jetepar (3cc/kg /day i.m)
Group V = INH (50mg/kg /day i.p) + Jetepar (6cc/kg /day i.m)

2) **Biochemical and Histopathological Analysis**

(a) In liver function tests bilirubin, AST and ALT were performed.

(b) In histopathological analysis ballooning degeneration, fatty changes, portal inflammation and necrosis of hepatocytes were examined in liver sections with the help of light microscope.

Group I:

(a) In this group 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.

(b) Normal liver parenchyma was also observed (Figure 1).

Group II:

(a) In this group there was increase (P<0.05) in bilirubin level, decrease (P<0.05) in ALT and decrease in AST activity on 12th day (Table 2).

(b) Fatty changes 0% (n = 0), ballooning degeneration 50% (n = 3), necrosis of hepatocytes 66% (n = 4) and portal inflammation 50% (n = 3) were observed after postmortem of rabbits (n = 6) (Figure 2, 3).

Table 2: *Effects of different drug treatments on Liver function tests of rabbits.*

Groups		Bilirubin (mg/dl)	ALT (U/L)	AST (U/L)
II	C	0.33 ± 0.0614	61.67± 9.175	34.33 ± 4.598
	T	0.5 ± 0.0365	24.83 ± 2.04	26.67± 3.273
	"p" value	*	*	NS
III	C	0.45 ±0.0846	70.17 ± 4.269	47.5 ± 6.371
	T	0.35 ± 0.0619	60.17 ± 8.002	70.67 ± 7.99
	"p" value	NS	NS	*
IV	C	0.35 ± 0.0848	65.17 ± 8.154	26.67 ± 2.216
	T	0.466 ± 0.071	34 ± 1.291	38.83 ± 6.107
	"p" value	NS	*	NS
V	C	0.38 ± 0.0846	62.67 0.016	29.83 ± 8.109
	T	0.48 ± 0.0763	43.17 ± 11.81	41.83 ± 10.41
	"p" value	NS	*	*

ALT = Alanine Aminotransferase; AST = Aspartate aminotransferase
NS = not significant; * = significant (P = <0.05), C = Control (before drug treatment on 0th day) T = Test (after drug treatment on 12th day)
Group II = INH (50 mg/kg /day i.p)
Group III = Jetepar (3 cc/kg /day i.m)
Group IV = INH (50 mg/kg /day i.p) + Jetepar (3 cc/kg /day i.m)
Group V = INH (50 mg/kg /day i.p) + Jetepar (6 cc/kg /day i.m)

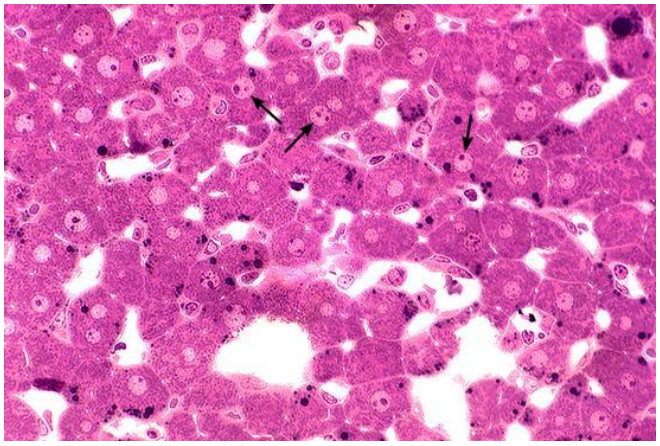

Fig. 1: *Normal Liver Parenchyma of Rabbits (Group I) (H&E, 20x).*
Group I = (Normal without any drug)

Group III:

(a) In this group there was no change in bilirubin level, decrease in ALT activity and increase (P<0.05) in AST activity on 12th day (Table 2).

(b) Fatty changes 0% (n = 0), ballooning degeneration 0% (n = 0), necrosis of hepatocytes 0% (n = 0) and portal inflammation 50% (n = 3) were observed after postmortem of rabbits (n = 6) (Figure 2, 4).

Group IV:

(a) In this group there was increase in bilirubin level, decrease (P<0.05) in ALT activity and increase in AST activity on 12th day (Table 2).

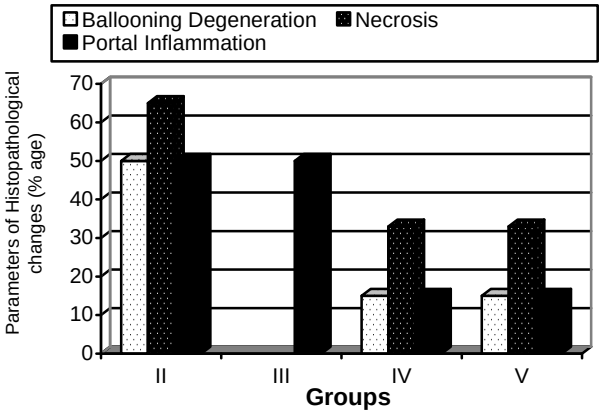

Fig. 2: *Parameters of Histopathological Changes in % age observed in all Groups.*
Group II = INH (50 mg/kg /day i.p)
Group III = Jetepar (3 cc/kg /day i.m)
Group IV = INH (50 mg/kg /day i.p) + Jetepar (3cc/kg/ day i.m)
Group V = INH (50 mg/kg /day i.p) + Jetepar (6cc/ kg / day i.m)

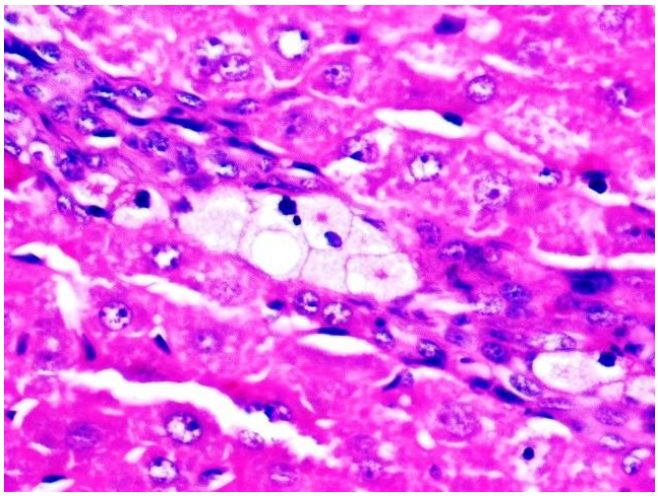

Fig. 3: *Liver Parenchyma of Rabbits (Group II) showing Portal triad inflammation, ballooning degeneration, necrosis (H&E, 40x).*
Group II = INH (50mg/kg /day i.p)

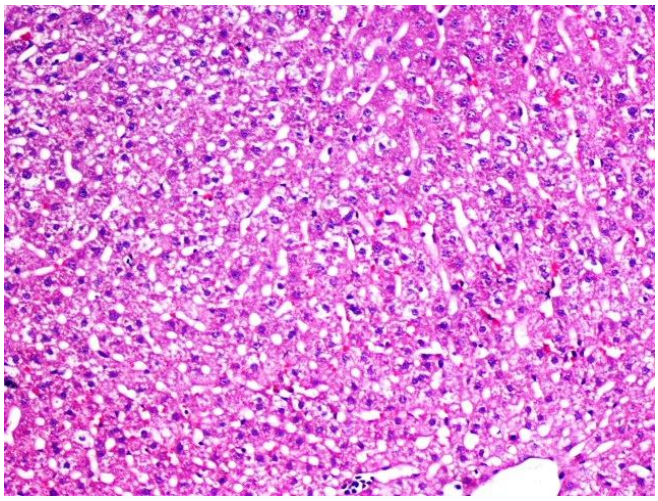

Fig. 4: *Liver Parenchyma of Rabbits (Group III) representing normal hepatocytes(H&E, 10x).*
Group III = Jetepar (3cc/kg /day i.m)

(b) fatty changes 0% (n = 0), ballooning degeneration 16% (n = 1), necrosis of hepatocytes 33% (n = 2) and portal inflammation 16% (n = 1) were observed after postmortem of rabbits (n = 6) (Figure 2,5).

Group V:

(a) In this group there was increase in bilirubin level, decrease ($P < 0.05$) in ALT activity and increase ($P < 0.05$) in AST activity on 12th day (Table 2).

(b) Fatty changes 0% (n = 0), ballooning degeneration 16% (n = 1), necrosis of hepatocytes 33% (n = 2) and portal inflammation 16% (n = 1) were observed after postmortem of rabbits (n = 6) analysis (Figure 2, 5).

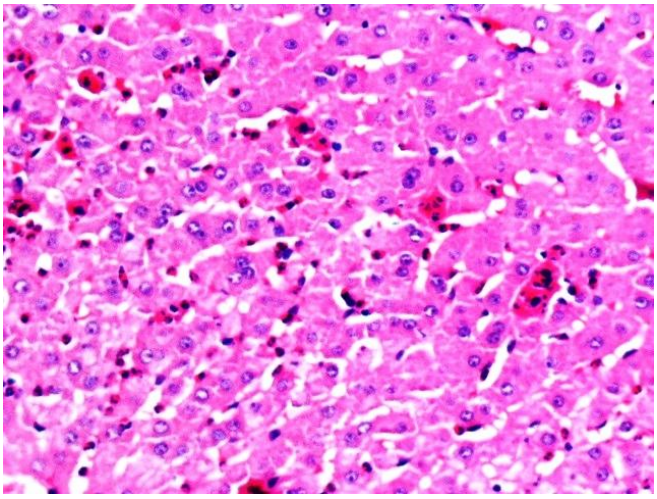

Fig. 5: *Liver Parenchyma of Rabbits (Group IV and V) representing mild ballooning degeneration and necrosis in liver parenchyma (H&E, 20x).*
Group IV = INH (50mg/kg /day i.p) + Jetepar (3cc/kg /day i.m)
Group V = INH (50 mg/kg /day i.p) + Jetepar (6cc/kg /day i.m)

Discussion

Isoniazid (INH), first-line drug in the treatment of tuberculosis, causes mild changes in serum transaminases levels resulting in hepatotoxicity. It is preferred to measure these levels during INH treatment so that the extent of toxicity can be estimated. INH is only 1 to 2% associated with a risk of severe hepatotoxicity. It has been found that hydrazine, INH metabolite, is involved in toxicity.[6]

Hepatic profile mainly includes estimating bilirubin, ALT, AST, GGT, ALP, albumin and PT test. It is more preferred to evaluate every month during antitubercular treatment (ATT). In these subjects, in whom there is disturbance of LFT's, hepatotoxic antitubercular (AT) drugs are withdrawn and restarted after an interval of 3-4 weeks. Levels of ALT, AST and bilirubin are helpful in diagnosing hepatic diseases.[7] Raised levels of serum enzymes indicate disturbances in hepatocellular integrity.[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] The mechanism of liver damage due to INH is incompletely understood. The histopathologic appearance closely resembles viral hepatitis, although direct toxicity of the drug or a metabolite are probably responsible for hepatocyte death.[11] Acetylhydrazine is the drug metabolite that has been most consistently implicated in the pathogenesis of INH hepatitis.[12]

Experimental studies on animals suggest that when antitubercular drugs are administered in toxic dose, ALT,

AST and ALP are released in serum, affecting hepatocellular membrane integrity and its organelles.[13,14]

It has been reported that during sub acute or chronic treatment, INH induced hepatotoxicity occurs in man,[15] rats[16] and guinea pigs[17] 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. 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.[18] 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. Histopathological analysis in rabbits with INH induced hepatotoxicity shows inflammation and necrosis at focal and centrilobular regions.[19] In our study same changes were observed so proving the validity of our animal model.

In group II bilirubin level raised ($P < 0.05$) and ALT activity decreased ($P<0.05$). As this finding correlates with literature that when rabbits are treated with INH (50 mg/kg) there is fall in the levels of ALT, AST and ALP in serum as well as in liver tissue.[18] Histological picture of liver showed that there was 50% ballooning degeneration, 50% portal inflammation and also 66% hepatic necrosis (Figure 2, 3).

In group III AST level increased ($P < 0.05$). Liver parenchyma showed reversible changes i.e. only 50% portal inflammation (Figure 2, 4). So this effect of jetepar manifests that it may have hepatoprotective effects when combined with INH. In this group animals showed increased appetite and weight gain. Jetepar is non-toxic to liver. As regards the serum ALT and AST levels and clotting time there was no change observed between drug and control groups, indicating the non-toxic reaction of the jetepar on liver tissue.[20]

In combination group IV, ALT activity decreased ($P < 0.05$). Liver histological changes showed 16% ballooning degeneration, 33% necrosis and 16% portal inflammation (Figure 2, 5).

In combination group V, ALT activity decreased ($P < 0.05$) and AST activity increased ($P < 0.05$). By doubling the dose of Jetepar, there was no significant effect on histopathological picture of liver. Liver parenchyma presented exactly same picture (in same percentage) as in group IV (Figure 2, 5). However significant increase in AST manifests that liver parenchyma has tendency to be improved more (comparing with group II) if duration of dose is enhanced. By observing significant improvement (P-value <0.05 i.e. 0.0124) in histopathological parameter of groups IV and V (Figure 2), it can be concluded that INH induced biochemical and histopathological changes are significantly antagonized by concurrent administration of jetepar for 11 days. But scope for further evaluation is there by prolonging the experimental time period.

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References

1. Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis 2002; 22: 137–44.
2. Temple RJ, Himmel MH. Safety of newly approved drugs: implications for prescribing. JAMA 2002; 287: 2273 - 2275.
3. Zaleskis, R. The side-effects of TB therapy. *Breathe*., 2005: 2 (1).
4. Mitchell, J.R., Zimmerman, H.J., Ishak, K.G., Thorgiersson, U.P., Timbrell, J.A., Snodgrass, W.R., *et al.* Isoniazid liver injury: clinical spectrum, pathology and probable pathogenesis. *Ann Intern Med.*, 1976; 84: 181-92.
5. Priya, S., Victor, Mohan, C.R., Pradeep, K., Vijayalakshmi, P. and Karthikeyan, S. Membrane stabilizing and antioxidant properties of silymarin against isoniazid induced hepatotoxicity in rats. *Adis Data Information BV.* 2004.
6. Sarich, T.C., Adams, S.P., Petricca, G and Wright, J.M. *Pharmacology and Experimental Therapeutics* 1999; 289 (2): 695-702.
7. Harper, H.A. The functions and tests of the liver, In: Review of physiological chemistry. Los Altos, California: Lange Medical Publishers, 2005: 271-283.
8. Drotman, R.B. and Lawhorn, G.T. Serum enzymes as indicators of chemical induced liver damage. *Drug Chem. Toxicol.*, 1978; 1: 163-171.
9. Rao, R.R. Mechanism of drug induced hepatotoxicity. *Ind. J. Pharmacol.*, 1973; 5: 313-318.
10. Singh, B., Saxena, A.K., Chandan, B.K., Anand, K.K., Suri, O.P., Suri, K.A. and Satti, N.K. Hepatoprotective activity of verbenalin on experimental liver damage in rodents. *Fitoterapia.*, 1998; 69: 135-140.
11. Black, M., Mitchell, J.R., Zimmerman, H.J., Ishak, K.G. and Epler, G.R. Liver physiology and disease: Isoniazid-associated hepatitis in 114 patients. *Gastroenterology.*, 1975; 69: 289.

12. Nelson, S.D., Mitchell, J.R., Timbrell, J.A., *et al.* Isoniazid and iproniazid: Activation of metabolites to toxic intermediates in man and rat. *Science*., 1976; 193: 901.
13. Parthasarathy, R., Sarma, G.R., Janardhanam, B., Ramachandran, P., Santha, T. and Sivasubramanian, S. Hepatic toxicity in South Indian patients during treatment of tuberculosis with short-course regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle.*, 1986; 67: 99-108.
14. Shakun, N.P. and Tabachuk, O.P. The comparative action of isoniazide, rifampicin and ethambutol on liver function. *Eksp. Klin. Farmakol.*, 1992; 55: 45.
15. Mitchell, J.R., Long, M.W., Thorgeirsson, U.P. and Jollow, D.J. Acetylation rates and monthly liver function tests during one year of isoniazid preventive therapy. *Chest*., 1974; 68: 181-190.
16. Snodgrass, W., Potter, W.Z, Timbrell, J.A., Jollow, D.J. and Mitchell, J.R. Possible mechanism of isoniazid related hepatic injury. *Clin Res.*, 1974; 22: 323.
17. Heisey, G.B., Hughes, H.C., Long, C.M. and Rozmiarke, H. The guinea pig as a model for isoniazid-induced reactions. *Lab Anim Sci.*, 1980; 30: 42-50.
18. Karthikeyan, S. Hepatotoxicity of isoniazid: A study on the activity of marker enzymes of liver toxicity in serum and liver tissue of rabbits. *Indian J Pharmacol.*, 2004; 36 (4): 247-249.
19. Sarich, T.C., Zhou, T., Adams, S.P., Bain, A.I., Wall, R.A and Wright, J.M. A model of isoniazid-induced hepatotoxicity in rabbits. *J Pharmacol Toxicol Methods* 1995; 34: 109-116.
20. Qadri, N.M., Qureshi, S., Zakir-Ur-Rehman. and Yaqeen, Z. Studies on beneficial role and subchronic toxicity of livergen: A polyherbal hepatoprotective drug. *Hamdard-Medicus (Pakistan).*, 2003; 46 (2): 127-154.