ISONiazid INDUCED LIVER DAMAGE;
HEALTHY EFFECTS OF SilyMARIN ON LIVER HISTOPATHOLOGY AGAINST
ISONIAZID INDUCED LIVER DAMAGE IN RABBITS

Sarwat Jahan¹, Sana Imran², Naveed Ahsan²

ABSTRACT… Objectives: To observe healthy effects of silymarin on liver histopathology against liver damage, caused by isoniazid in rabbits. Study Design: Interventional study. Setting: Animal House of Jinnah Postgraduate Medical Centre, Karachi. Period: April to September 2013. Methods: Total 28 rabbits of weight 1-1.5kg of either sex were used in this study. Which were divided randomly into four equal groups: Group I was control group. In group II silymarin (50mg/kg/day orally) was administered, in group III isoniazid (50mg/kg/day orally) was given; and in group IV, effects of combination therapy of isoniazid and silymarin were observed. Before starting the drug therapy, at day 0 and one day after the end of study period i.e., at day 19, body weight of each animal was recorded. Rabbits were sacrificed on 19th day and the required liver sample was taken for histopathological examination. The data feeding and analysis at the end of study was done on computer package SPSS (Statistical packages of social science) version 16. Results: No mortality was recorded in any group. In group II (silymarin treated) animals in this group exhibited no any histological changes in the hepatic lobule except few inflammatory cells 28.5% were seen in the portal tract. The liver microscopic examination in group III (isoniazid treated), animals showed the disturbed architecture of the lobule. There were no fatty changes, whereas ballooning degeneration was 42.9%, hepatocytes necrosis was 71% and portal inflammation was 71.4% which was very severe. Animals in group IV, given combination of silymarin and isoniazid showed the intact architecture of the hepatic lobule, in which 14.29% ballooning degeneration, whereas necrosis of hepatocytes and portal inflammation was mild in nature which may be due to hepatoprotective role of silymarin. Conclusion: Silymarin has hepatoprotective effects when given in combination with isoniazid.

ABSTRACT... Objectives: 

Introduction: 
Liver is the first place where various types of oral therapeutic drugs and other xenobiotic come in contact from intestinal absorption and thus causing this organ more prone to chemical-induced injury.¹ Hepatotoxicity is one of the most important adverse drug reaction associated with anti tuberculous drugs that may limit their use. It may involve metabolism of drugs to toxic, reactive intermediates and covalent binding with cellular components, interference, with membrane transport or with cellular biochemistry such as protein synthesis, or immunological mechanisms.² One of the isoniazid metabolite named hydrazine cause hepatotoxicity. It is an important product of cytochrome P450 enzyme metabolizing activity which mainly causes oxidatdivestress.³ One pathway of oxidative stress is lipid peroxidation, as established in animal models treated with anti-tuberculosis drugs such as isoniazidor hydrazine.⁴ Further more hydrazine inhibits mitochondrial complex II and reduce the function of electron transport chain and ATP production in hepatocytes. In case of combination therapy of hydrazine and a complex I inhibitor, hepatocyte death can occur.⁵ According to recent studies, isoniazid bind to liver proteins and cause immune-mediated hepatotoxicity.⁶⁷ Silymarin is a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin. The antioxidant property and cell regenerating functions due to increased protein
isoniazid induced liver damage

MATERIAL AND METHODS
This study was conducted in the Department of Pharmacology and Therapeutics, BMSI, JPMC, Karachi in collaboration with Department of Pathology, Biochemistry and BMSI Animal House from April 2013 to September 2013. This animal study protocol was approved by JPMC’s Ethical Committee. In this interventional study, 28 locally bred sexually mature oryctolaguscuniculus rabbits of both sexes were taken. Their body weight was ranging from 1-1.5 kg. The animals were kept in the BMSI animal House. General examination was done to evaluate their general health condition. All the animals were kept on standard laboratory diet (containing wheat, floor, vitamin etc.) and water. The rabbits were given identification by labeling them with permanent marker on ear of each rabbit and housed in labeled stainless steel cages with holed steel top in well-ventilated room under the acceptable 12 hours day/night light-cycle of the animal house. The animals were kept a week for attaining acclimatization to the environment before starting the treatment.

These animals were divided randomly into four equal groups: Group I was control group without any drug; in group II, silymarin (50mg/kg/day orally) was given. In group III, Isoniazid (INH) (50mg/kg/day orally) was administered; and in group IV combination of isoniazid and silymarin were given. The animals were sacrificed on 19th day that is one day after the end of treatment period after recording their final weight. A midline incision extending from manubrium sterni to the lower abdomen was made with the help of a scalpel. The skin flaps and muscles of abdomen retracted by pair of sharp scissor and blunt forcep and liver was expose by incising the diaphragm. The liver was removed from abdomen by dissecting the omental bursa, blood vessels and ligaments attached to it. The required liver sample was taken for histopathological examination. All the sections were studied under light microscope at low and high magnification and oil immersion objective. The architecture of the liver lobule and hepatocytes were studied.

Sample size was calculated according to the previous study as a reference by using computer program open Epi version 16. Data was analyzed using SPSS 16. In all statistical analysis, only p<0.05 was considered significant.

RESULTS
Present study was aimed to observe healthy effects of silymarin on liver histopathology against liver damage, caused by isoniazid in rabbits. No mortality was recorded in any group. Animals of group-I (control group) showed normal histological appearance of hepatic lobules when seen under the light microscope (Table-I, Figure-1). In group II (silymarin treated) there was non-significant histopathological findings in this group when compared to control group except few inflammatory cells 28.5% (n=2) were seen in the portal tract as depicted in Table-I, Figure-2. The liver microscopic examination in group III (Isoniazid treated) animals showed the disturbed architecture of the lobule. The hepatic lobules showed degenerative changes and focal necrosis. There was no fatty changes 0% (n=0), whereas ballooning degeneration was 42.9% (n=3), hepatocytes necrosis was 71% (n=5) and portal inflammation was 71.4% (n=5), as depicted in Table-I and Figure-3. In Group IV (Silymarin + Isoniazid), combination of silymarin and isoniazid exhibited the intact architecture of the hepatic lobule. Other histological findings were 14.29% (n=1) ballooning degeneration, necrosis of hepatocytes and portal inflammation as depicted in Table-I and Figures-4 (A,B).

DISCUSSION
According to the recent data from the Drug-Induced Liver Injury Network (DILI), the occurrence of Isoniazid-induced liver injury is high in the United States and it is the second most frequent drug that causes liver injury. Hydrazine one of the important metabolite is responsible for hepatic necrosis, macrovesicular degeneration, and steatosis in mice and rabbits.
### Table-I. Histopathological changes in liver tissue among different groups of rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control) (n=7)</th>
<th>Group II (Silymarin) (n=7)</th>
<th>Group III (Isoniazid) (n=7)</th>
<th>Group IV (Combination) (n=7)</th>
</tr>
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<tbody>
<tr>
<td>Fatty changes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ballooning degeneration</td>
<td>No</td>
<td>No</td>
<td>3/7 (42.9%)</td>
<td>1/7 (14.29%)</td>
</tr>
<tr>
<td>Necrosis</td>
<td>No</td>
<td>No</td>
<td>5/7 (71.42%)</td>
<td>1/7 (14.29%)</td>
</tr>
<tr>
<td>Portal inflammation</td>
<td>No</td>
<td>2/7 (28.5%)</td>
<td>5/7 (71.42%)</td>
<td>1/7 (14.29%)</td>
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**Figure-I.** Control group 10x hepatic parenchyma showing normal portal tract with no ballooning degeneration

**Figure-II.** Effect of silymarin 10x hepatic parenchyma showing near normal morphology with focal mild portal inflammatory infiltrate

**Figure-III.** Effect of isoniazid 40x hepatic parenchyma showing portal inflammation and ballooning degeneration

**Figure-IV (A) Effect of combination of drugs 10x hepatic parenchyma showing mild portal inflammations**
Isoniazid has an inhibiting effect on CYP1A2, 2A6, 2C19 and 3A4 activity, which is involved in hydrazine detoxification. Isoniazid is also found to bind itself with liver proteins and cause immune-mediated hepatotoxicity. The use of natural herbs like silymarin may cause protective effects on the liver and thus can prevent from such adverse drug reactions due to their dual pharmacological properties including hepatoprotective and antioxidant activity. Silymarin has also been found to have immunomodulatory, anti-fibrotic, anti-proliferative, and antiviral properties.

Animal studies have proven that isoniazid (INH) toxicity results in the induction of liver apoptosis with associated disruption of mitochondrial membrane potential and DNA strand breaks. In this study Isoniazid induced liver toxicity was manifested by changes in the liver histopathological architecture. Thus, we also used histological markers in study. Animals in group II (silymarin treated animals), showed minimal reversible changes in the hepatic lobule and only 28.5% (n=2) portal inflammation was observed.

When we compared this group with the control group, there was no significant difference observed in histopathological findings except few inflammatory cells in the portal tract. These effects of silymarin indicate the protective effects on liver tissue. Group-III (isoniazid treated animals showed 42.9% (n=3) ballooning degeneration and 71.4% (n=5) portal inflammation and hepatic necrosis. According to the previous study, isoniazid treated animals showed multifocal and centrilobular mononuclear cell infiltration in hepatocytes, the same histopathological findings observed in isoniazid treated group in this study. In group IV, combination of isoniazid and silymarin was administered, 14.3% (n=1) ballooning degeneration, necrosis and portal inflammation was observed. Our findings match with the results of previous studies who reported that the effect of combination therapy of isoniazid and silymarin results in decreased incidence and severity of liver histopathological changes.

CONCLUSION
Silymarin has healthy effects on liver, when given in combination with the drug causing hepatotoxicity like isoniazid. It prevents isoniazid induced liver damage which was proven by histopathological evidence.

Acknowledgements
We are grateful to the departments of Pharmacology, Biochemistry and Pathology and animal house BMSI, JPMC, Karachi, for providing manpower, laboratory facilities, high technological equipment and chemicals.

REFERENCES
ISONIAZID INDUCED LIVER DAMAGE


AUTHORSHIP AND CONTRIBUTION DECLARATION

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“He who opens a school door, closes a prison.”

– Victor Hugo –