

## Comparative effects of Silymarin and zinc complex of Betulinic acid on pyrazinamide induced Histopathological changes in Mice liver

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### ABSTRACT

**Introduction:** Liver is an important organ in the body. Due to its role in metabolism of drugs it is also a major site of drug induced liver injury. Oxidative stress plays an important role in DILI therefore use of anti-oxidants has been proposed to combat liver injury.

**Objective of the Study:** To measure and compare Hepatoprotective effects of Zinc complex of Betulinic acid and Silymarin due to their antioxidant effects on Pyrazinamide induced Hepatotoxicity in mice.

**Methodology:** It was an experimental randomized control trial. The research was conducted at the Department of Pharmacology and Therapeutics and Multidisciplinary research laboratory at IIMCT with mutual collaboration of National Institute of Health (NIH) in Islamabad, Pakistan. Research was started after the official approval of synopsis by accredited Ethical Review Committee. Study duration was one year from 1 September 2020 to 31 August 2021. 24 adult Balb-C mice were randomly divided into four groups. Group 1 was Negative Control (NC) and did not receive any intervention. Oral pyrazinamide (500mg/kg) was administered for 28 days to the group 2 (disease control) alone and to group 3 and 4 in combination with Silymarin (100mg/kg) and Zinc complex of Betulinic Acid (1mg/kg) respectively. Mice were dissected after completion of experiment and liver samples were taken for Histopathological analysis. Hepatoprotective function of Silymarin and zinc complex of Betulinic acid was evaluated in group 3 and 4 by Histopathological changes.

**Results:** Significant ( $p < 0.05$ ) changes were seen in parameters (necrosis, inflammatory cell infiltration and vascular congestion) among groups.

**Conclusion:** This study proves protective effects of zinc complex of Betulinic acid and Silymarin on pyrazinamide induced hepatotoxicity.

**Key Words:** Hepatotoxicity, Betulinic Acid, Silymarin, Pyrazinamide, Zinc, Tuberculosis

### INTRODUCTION

Liver is one of the vital organ in the body which performs a variety of important functions such as metabolism of lipids, proteins and carbohydrates, production of coagulation factors, metabolism of drugs, Xenobiotics and nutritional substances.<sup>1</sup> Tuberculosis is a disease which is caused by an infectious agent *Mycobacterium tuberculosis*. It has high incidence in the countries which have low income rate that's why it is also known as disease of poverty. Pakistan is ranked at fifth position among first 22 high burden countries of tuberculosis. WHO report about TB incidence published in 2016 stated that incidence in Pakistan is 270 per 100,000 population.<sup>2</sup> According to a study TB is responsible for causing 1.4 million deaths every year.

<sup>3</sup> First line anti-TB drugs include Isoniazid, rifampicin, pyrazinamide and ethambutol.<sup>4</sup> Among the first line anti-TB drugs first three are hepatotoxic and are known to cause drug induced liver injury. TB patients taking ATT medication has an incidence of about 2-28% of drug induced liver injury.<sup>5</sup> Pyrazinamide has been documented to be more hepatotoxic in comparison with isoniazid and rifampicin.<sup>6</sup> Mechanism of pyrazinamide induced hepatotoxicity involves oxidative stress caused by the changes brought about by pyrazinamide on the anti-oxidants.<sup>7</sup>

Betulinic acid is a lupane-type triterpenoid with a pentacyclic structure which is naturally occurring. It has wide distribution in plant kingdom. Number of plants are

source of BA such as *Dolioscarpus schottianus*, *Ancistrocladus heyneanus*, *Ziziphus spina-christi*.<sup>8</sup> Pharmacological activities associated with BA include anti-tumor activity, anti-oxidant activity, Hepatoprotective activity, cardio protective activity, anti-inflammatory activity as well as nephroprotective effects.<sup>9</sup> Some of the useful effects in hepatic injury are associated with effects of this compound on anti-oxidants such as GSH, superoxide dismutase and the ability of BA to inhibit lipid peroxidation.<sup>10</sup>

Zinc is a commonly occurring trace element in the human body.<sup>11</sup> Antioxidant effects of zinc are attributed to increased production of metallothioneins, activation of catalases and glutathione protection of protein sulfhydryls from oxidation as well as replacement of zinc with copper, iron and other redox active metals on binding sites.<sup>12</sup> Hepatoprotective effect of zinc are due to its ability to inhibit hepatic lipid deposition along with its antioxidant and anti-inflammatory effects.<sup>13</sup>

Silymarin is a combination of flavonolignans which are extracted from the milk thistle. It is standard drug used in various liver ailments due to its anti-oxidant, anti-inflammatory as well as anti-fibrotic properties along with its ability to inhibit free radical generation. Therefore study is designed to observe Hepatoprotective effects of both the Zinc complex of Betulinic acid and *Silymarin* keeping in mind their anti-oxidant properties so that they can be used as adjunct drug therapy.

## MATERIALS AND METHODS

It was an Experimental randomized control study. It was conducted at Pharmacology department, Islamic International Medical College (IIMC) in collaboration with Riphah Institute of Pharmaceutical Sciences (RIPS) and animal house of National Institute of Health (NIH), Islamabad from September 2020 to September 2021. Approval of research proposal was from Institutional Review Committee. 24 healthy male albino Balb/C mice with weight of approximately 30-50g and life of 8 weeks and normal LFT, s was included in study. Mice which have weight less than 30 g, age less than 8 weeks and female mice were not included in the study. Mice were kept under room temperature of  $22 \pm 2$  degree Celsius and 12 hour light dull cycle for 1 week. The mice were randomly divided into four groups each containing 6 mice in each group (n=6). Group 1, control group was given normal diet and tap water. Group 2, drug treated group was given pyrazinamide in dose of 500mg/kg<sup>14</sup> Group 3 was given Silymarin 100mg/kg<sup>15</sup> and group 4 was given zinc complex of Betulinic Acid<sup>10</sup> in 1 % starch jelly .

After completion of 28 days of duration of the trial, mice were anesthetized with chloroform and placed in flat posture with their limbs pinned on dissection table. Abdomen was opened with a longitude cut and liver was identified and exposed. It was detached from neighboring structures and was instantly put into containers having 10% formalin. Dehydration of the tissue is done by ethanol ascending grades. After dehydration liver tissue is cleared by xylene and then its embedding is done by paraffin wax. After that tissue blocks are made which measure 4-5 microns which are then used for histological analysis .Slides were stained by using Eosin and Hematoxylin stain. The slides were then used to analyse the following parameters : necrosis , imflammatory cells infiltration and vascular congestion which are the normal findings found in pyrazinamide induced hepatic injury.

**Data Analysis:** Respective data was entered and analyzed in SPSS version 21. Mean and standard errors were calculated for the quantitative variables. Categorical or qualitative variables were demonstrated by frequency and percentage. Chi square test was applied for comparison of histological qualitative variables.

## RESULTS

Table 1: distribution of necrosis among all groups through Chi-Square test

Group	Absent (%)	Present (%)	P-Value
Group 1	(6) 100%	(0) 0%	0.001*
Group 2	(0) 0%	(6) 100%	
Group 3	(4) 66%	(2) 33%	
Group 4	(4) 66%	(2) 33%	

\*= Significant

Table 2: Chi -Square test showing inflammatory cells infiltration among all Group

Group	Absent (%)	Present (%)	P-Value
Group 1	(6)100%	(0) 0%	0.000*
Group 2	(0) 0%	(6)100%	
Group 3	(4) 66%	(2) 33%	
Group 4	(6) 100%	(0) 0%	

\*= Significan

There was significant ( $p < 0.05$ ) difference in groups and necrosis .Necrosis was present in all mice of group 2, 33% in group 3 and 33 % in group 4.

Distribution of inflammatory cells infiltration showed that there was significant ( $p < 0.05$ ) difference between groups and infiltration. Infiltration of inflammatory cells was present in all animals of group 2, 33% in group 3, 0 % in group 4.

Table 3. Chi-Square test showing distribution of vascular congestion among all groups

Group	Absent (%)	Present (%)	P-Value
Group 1	(6) 100%	(0) 0%	0.002*
Group 2	(0) 0%	(6) 100%	
Group 3	(4) 66%	(2)33%	
Group 4	(5) 84%	(1)16%	

\*= Significant

Distribution of vascular congestion shows significant difference  $p < 0.05$  among groups. Vascular congestion was absent in all animals of group 1 .It was present in all animals of group 2, 33 % in group 3 and 16 % in group 4.

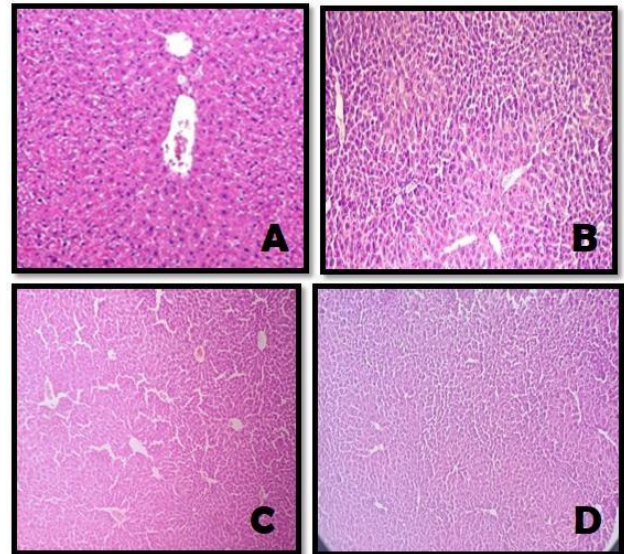


Figure 1 : Histopathological changes in mouse liver (10X) , Group A showing normal hepatic architecture , Group 2 showing necrosis inflammation and vascular congestion , group 3 and 4 showing preservation of normal hepatic architecture.

## DISCUSSION

In the current study Pyrazinamide administration in disease control group caused significant necrosis and inflammatory cell infiltration .These findings were in accordance with the study carries out by V.Kovalenko et al. who used pyrazinamide to study epigenetic changes in rat liver. Use of PZ produced significant histological changes in rat liver .<sup>16</sup>These findings were also supported by S.Taziki et al. who used pyrazinamide to study the Hepatoprotective effects of taurine .In his study administration of PZ produced significant necrosis and inflammation in rats .<sup>17</sup>

In our study there was also significant(  $p < 0.05$ ) improvement in histological parameters including necrosis, vascular congestion and infiltration of inflammatory cells by

the use of Zn and BA in experimental group 4 in comparison with the Disease Control Group. The beneficial effects of BA on liver histology was in accordance with the findings of Abdullah et al.<sup>18</sup> who proved antihepatotoxic activity of Betulinic acid due to its protective effect on antioxidant system and its membrane stabilizing activity. These findings were also in accordance with the study done by A. Ahangarpour<sup>19</sup> who studied the effects of Betulinic Acid on hepatic enzyme levels and histology on streptozotocin –nicotinamide –induced diabetic rats and showed that Betulinic acid pretreatment effectively reduces hepatic necrosis and inflammation. Similar findings regarding zinc were reported by W. Siddique<sup>20</sup> who studied the effect of zinc sulfate on ATT induced hepatotoxicity and showed that zinc protects against liver injury through its antioxidant effect. Findings in the present study were also supported by F.Al-Jawad et al.<sup>21</sup>

Both the biochemical and Histopathological effects of *Silymarin* in our study were supported by E.Sabina et al.<sup>22</sup> and S.Tasduq et al.<sup>23</sup> both of which observed Hepatoprotective effects of *Silymarin* in ATT induced liver damage. Both of these studies demonstrated that use of Silymarin against ATT induced hepatotoxicity results in preservation of normal hepatic architecture with no evidence of necrosis, steatosis and inflammation.

## CONCLUSION

In the present study it is concluded that *Silymarin* and Zinc Complex of Betulinic Acid individually ameliorate the hepatotoxic effect induced by Pyrazinamide and can be used as adjuncts in the treatment DILI caused by oxidative stress.

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