ORIGINAL ARTICLE

Comparison of Hepatoprotective effect of Silymarin and Zinc Sulfate against Hepatotoxicity induced by Isoniazid and Rifampicin in albino rats

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ABSTRACT

Liver the most vital organ performing functions like protein synthesis, metabolic processes, clearance of xenobiotic (drug) and various toxins. Anti-tuberculosis drugs induced liver injury is a main cause of acute liver failure (ALF). Current treatments available for liver protection are not ineffective enough. Development of regenerative medicines such as (Zinc sulfate or Silymarine) are in great demand. Supplemental use is effective for prevention of liver damage. Both have antioxidant properties. Hepatic protection of Zinc Sulfate and Silymarin was evaluated and compared against 1st line anti TB (Isoniazid 50mg/kg/day and Rifampicin 100 mg/kg/day). 28 rats were divided into 4 groups G1, G2, G3 and G4. Liver injury was induced by Isoniazid and Rifampicin in G2, G3 and G4. Hepatoprotection of Silymarin (200mg /kg/day) and Zinc sulfate (7mg /kg/day) was evaluated in G3 and G4 respectively by serum ALT, AL P, AST, total bilirubin, total protein, albumin, globulin, A/G ratio (LFTs). There was significant elevation of LFTs in G2, showing induction of hepatotoxicity. The levels of ALT, ALK PO4, AST and Total bilirubin were significantly reduced at day 13 p-value ***< 0.0001 in G3, G4. While values of Total Protein, Albumin, Globulin and A/G ratio significant at day 6. Comparing G3, G4 values of ALT and ALT shows significant difference while ALP, Total Bilirubin, Total Protein, Albumin, Globulin and A/G ratio shows no significant difference.

Keywords: hepatotoxicity, hepatoprotaction, Isonazid, Rifampin, Zinc sulfate, Albion rats, LFTs, Silymarin.

INTRODUCTION

Liver is center for drugs and xenobiotic metabolism[1]. Drug induced liver injury is a leading clinical problem[2]. Liver is main site for transformation of exogenous substances (medicines)[3]. Tuberculosis is a global pandemic affecting millions of people. (4) Isoniazid and Rifampicin are first line anti-TB drugs[5]. These two are associated with risk of liver damage[6]. According to Meta-analysis hepatotoxicity by Isoniazid alone is 1.6% and Rifampicin is 1.1%, and with combination it is 2.6%[7]. At present there is no promising therapy available to treat patient with hepatotoxicity[8]. Silymarin antioxidant isolated from plant *Silybum marianum* (milk thistle), widely used in treatment of the liver diseases. (9) Silymarin a C25 flavonoid mixture is a gold standard liver tonic, used to treat hepatotoxicity[10]. Its administration increases glutathione S-transferase (GST) and Quinone reductase (QR), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) are involved in clearance of free radicals[10]. Zn a trace element with growth-promoting and antioxidant activities[11]. It is vital micronutrient for >1000 transcription factors, >300 enzymes and gene expression. (12) Zinc protects liver by metallothionine MT induction and antioxidant action[13].

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MATERIAL AND METHODS

28 albino male rats (each group containing 7), weighing 150-200g were divided in 4 groups under hygienic conditions , at 23±2 ºC, under natural cycles provided with rodent chow and water ad libitum. Identification marks were given to animals. Hepatotoxicity was induced by of Isoniazid 50mg/kg/day (14) and Rifampicin 100 mg/kg/day (14) co-administration as single morning dose per oral daily for 14 days. Silymarin was given orally daily 200 mg /kg/day (15) for 14 days in **G3.** Zinc sulfate was through oral route daily for 14 days 7mg /kg/day[16].

The dose was adjusted on weekly basis by measuring body weights of rats**.** 1 ml blood was taken by cardiac puncture after ether anesthesia (17) on day 0, 6 and 13. Blood was allowed to clot at room temperature and centrifuged for 15 minutes at 3000 rpm. Serum was stored at -20 ºC. (18, 19) By using commercially available kits following lab tests were performed: ALP, ALT, AST, Total bilirubin, Total protein, albumin/globulin ratio Albumin, Globulin. (17) All the tests were performed on All drugs given by oral route for 14 Sya (Isoniazid 50mg/kg/day, Rifampicin 100 mg/kg/day, Silymarin 200 mg /kg/day

Chemistry Analyzer Sphera. Methods were followed as provided by manufacturer. All data was entered on graph pad prism version 5. One way analysis of variance and turkey multiple comparison test were used for analysis of data. P-value ≤ 0.05was considered significant. Zinc sulfate14 days 7mg /kg/day)

Groups	Inducing agents	Protective agents

G 1	0.3ml Distilled Water	0.3ml Distilled Water
G 2	Isoniazid + Rifampicin	0.3ml Distilled Water
G 3	Isoniazid + Rifampicin	Silymarin
G 4	Isoniazid + Rifampicin	Zinc Sulfate

RESULTS AND DISCUSSION

ALT levels of G1 was 37±7.3, 38±5.5 and 39±4.8 U/L at day 0, 6 and 13 respectively. ALT levels of G2 increased from 37.9±8.6 to 78±5.4 and 104±4.1 U/L at day 6 and 13 respectively. ALT levels of G3 was increased from 43.6±1.8 to 50±3 and 56.7±2.9 U/L at day 6 and 13 respectively. ALT levels of G4 was increased from 37±5.9 to 59.7±3.3and 63.3±2.9 U/L at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.2120, ***< 0.0001 and ***< 0.0001 respectively.

AST levels of G1 was 133±17.3, 125±10.1 and 125±9.6 U/L at day 0, 6 and 13 respectively. AST levels of G2 increased from 113.6±21.9 to 358.3±67.6 and 728.3±37.5 U/L at day 6 and 13 respectively. AST levels of G3 was increased from 133.7±7.04 to219.3±45.97 and 276.4±12.3U/L at day 6 and 13 respectively. AST levels of G4 was increased from 136.7±20.6 to 207.6±27.4 and 330 ±17.7 U/L at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.0820, ***< 0.0001 and ***< 0.0001 respectively.

ALP levels of G1 was 117±23.6, 143.7±32.8 and 145±32.2 U/L at day 0, 6 and 13 respectively. ALK PO4 levels of G2 increased from 157±42.75 to 885.7±131.5 and 1210±108.9 U/L at day 6 and 13 respectively. ALK PO4 levels of G3 was increased from 170±53.7 to 186±74.7 and 339.7±21.5 U/L at day 6 and 13 respectively. ALK PO4 levels of G4 was increased from 160.9±39.3 to221.6±65.7 and 391.9±12.5 U/L at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.1057, ***< 0.0001 and ***< 0.0001 respectively.

Total Bilirubin levels of G1 was 0.0186±0.0491, 0.0614± 0.0584 and 0.0429± 0.0535 mg/dl at day 0, 6 and 13 respectively. Total Bilirubin levels of G2 increased from 0.0143± 0.0378 to 1.214± 0.434 and 0.529± 0.17 mg/dl at day 6 and 13 respectively. Total Bilirubin levels of G3 was increased from 0.0343± 0.0597to 0.643± 0.3599 and 0.343± 0.127 mg/dl at day 6 and 13 respectively. Total Bilirubin levels of G4 was increased from 0.0143± 0.038to 0.857± 0.127and 0.443± 0.237 mg/dl at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.8348, ***< 0.0001 and ***< 0.0001 respectively.

Albumin levels of G1 was 4.51±0.324, 4.49±0.308 and 4.43±0.298 g/dl at day 0, 6 and 13 respectively. Albumin levels of G2 increased from 4.1±0.554 to 4.3±0.16 and 4.3±0.195 g/dl at day 6 and 13 respectively. Albumin levels of G3 was increased from 4.43±0.269 to 4.16±0.127 and 4.39±0.217 g/dl at day 6 and 13 respectively. Albumin levels of G4 was increased from 4.39±0.344 to 4.2±0.116 and 4.21±0.19 g/dl at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.2399, *0.0199 and 0.3349 respectively.

Globulin levels of G1 was 3.1±0.337, 3.27±0.415 and 3.31±0.402 g/dl at day 0, 6 and 13 respectively. Globulin levels of G2 increased from 3.24±0.55 to 4.23±0.45 and 2.99±0.56 g/dl at day 6 and 13 respectively. Globulin levels of G3 was increased from 3.49±0.297 to 3.9±0.26 and 3.1±0.49 g/dl at day 6 and 13 respectively. Globulin levels of G4 was increased from 3.43±0.33 to 3.96±0.28 and 2.93±0.399 g/dl at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.2842, *** 0.0004 and 0.4437 respectively.

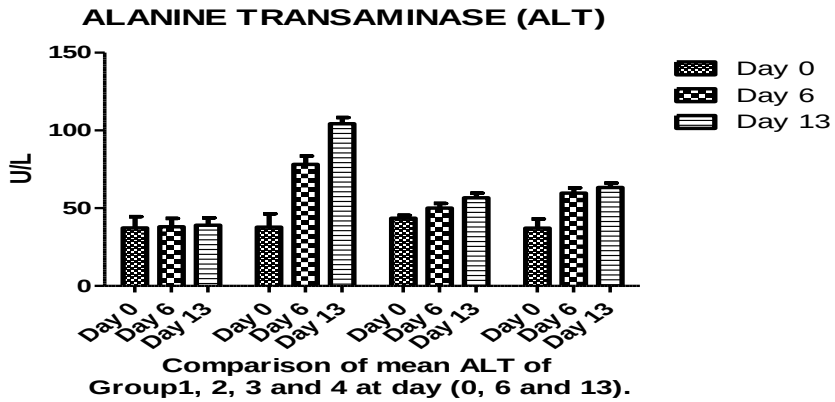
Albumin/ Globulin levels of G1 was 1.45±0.09 to 1.38±0.12 and 1.34±0.12at day 0, 6 and 13 respectively. Albumin/ Globulin levels of G2 increased from 1.28±0.17 to1.02±0.13 and 1.55±0.073 at day 6 and 13 respectively. Albumin/ Globulin levels of G3 was Increased from 1.29±0.11 to 1.06±0.085 and 1.44±0.225 at day 6 and 13 respectively. Albumin/ Globulin levels of G4 was increased from 1.28±0.0597 to 1.06±0.0929 and 1.45±0.18 at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.0219, ***< 0.0001 and 0.1472 respectively.

Total protein levels of G1 was 7.63±0.64, 7.79±0.71 and 7.74±0.64 g/dl at day 0, 6 and 13 respectively. Total protein levels of G2 increased from 7.34±1.008 to 8.53±0.415 and 7.29±0.609 g/dl at day 6 and 13 respectively. Total protein levels of G3 was increased from 7.9±0.414 to 8.07±0.256 and 7.49±0.518 g/dl at day 6 and 13 respectively. Total protein levels of G4 was increased from 7.84±0.719 to 8.16±0.223 and 7.14±0.443 g/dl at day 6 and 13 respectively. Significant difference was observed among the groups at day 6 and 13 with p-value of 0.4693, 0.0370 and 0.2349 respectively.

Mean ALT values (G1, G2, G3 and G4) Comparison.

ALT On Day	G1	G2	G3	G4	P-value
0	37±7.3	37.9±8.6	43.6±1.8	37±5.9	0.2120
6	38±5.5	78±5.4	50±3	59.7±3.3	***< 0.0001
13	39±4.8	104±4.1	56.7±2.9	63.3±2.9	***< 0.0001

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05

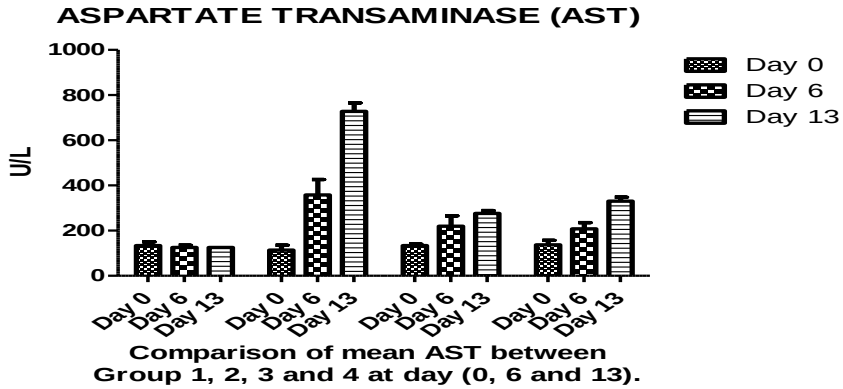


G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).

Mean AST values (G1, G2, G3 and G4) Comparison.

AST On Day	G1	G2	G3	G4	P-value
0	133±17.3	113.6±21.9	133.7±7.04	136.7±20.6	0.0820
6	125±10.1	358.3±67.6	219.3±45.97	207.6±27.4	***< 0.0001
13	125±9.6	728.3±37.5	276.4±12.3	330±17.7	***< 0.0001

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05

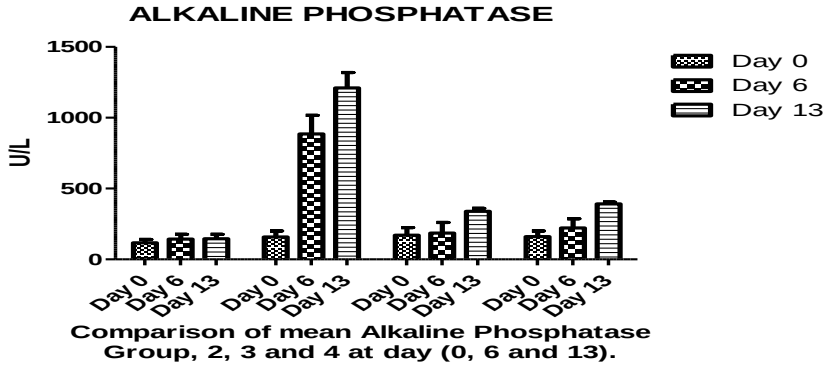


G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).

Mean ALP values (G1, G2, G3 and G4) comparison

ALP On Day	G1	G2	G3	G4	P-value
0	117±23.6	157±42.75	170±53.7	160.9±39.3	0.1057
6	143.7±32.8	885.7±131.5	186±74.7	221.6±65.7	***< 0.0001
13	145±32.2	1210±108.9	339.7±21.5	391.9±12.5	***< 0.0001

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05

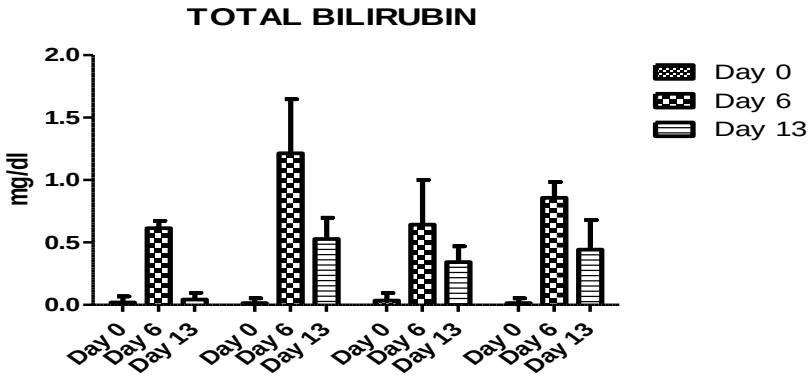


G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).

Mean Total Bilirubin values (G1, G2, G3 and G4) comparison

Total Bilirubin On Day	G1	G2	G3	G4	P-value
0	0.0186±0.0491	0.0143±0.0378	0.0343±0.0597	0.0143±0.038	0.8348
6	0.0614±0.0584	1.214±0.434	0.643±0.3599	0.857±0.127	***< 0.0001
13	0.0429±0.0535	0.529±0.17	0.343±0.127	0.443±0.237	***< 0.0001

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05



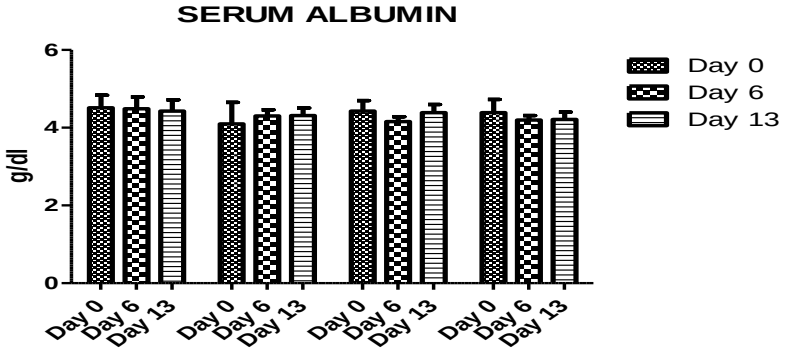
Comparison of mean Total Bilirubin between Group1, 2, 3 and 4 at day (0, 6 and 13).

G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).

Mean albumin values (G1, G2, G3 and G4) comparison.

Albumin On Day	G1	G2	G3	G4	P-value
0	4.51±0.324	4.1±0.554	4.43±0.269	4.39±0.344	0.2399
6	4.49±0.308	4.3±0.16	4.16±0.127	4.2±0.116	*0.0199
13	4.43±0.298	4.3±0.195	4.39±0.217	4.21±0.19	0.3349

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05



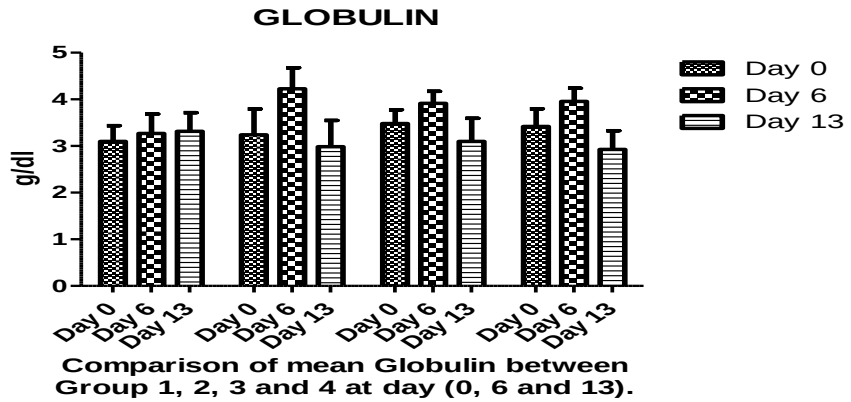
Comparison of mean Serum Albumin between Group1, 2, 3 and 4 at day (0, 6 and 130.

G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).

Mean globulin values (G1, G2, G3 and G4) comparison.

Globulin On Day	G1	G2	G3	G4	P-VALUE
0	3.1±0.337	3.24±0.55	3.49±0.297	3.43±0.33	0.2842
6	3.27±0.415	4.23±0.45	3.9±0.26	3.96±0.28	***0.0004
13	3.31±0.402	2.99±0.56	3.1±0.49	2.93±0.399	0.4437

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05

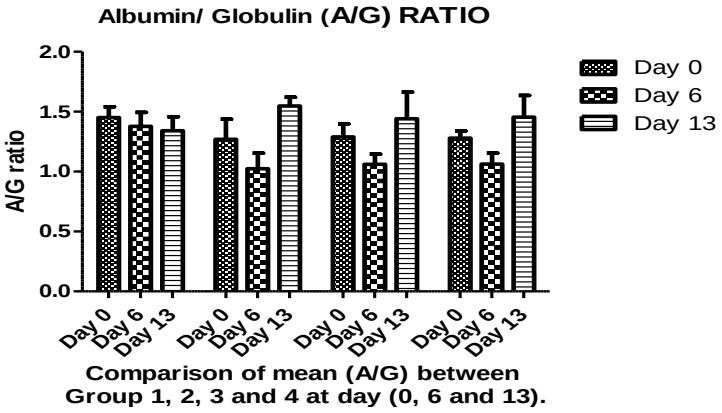


G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).

Mean Albumin/ Globulin values (G1, G2, G3 and G4) comparison

Albumin/ Globulin On Day	G1	G2	G3	G4	P-VALUE
0	1.45±0.09	1.28±0.17	1.29±0.11	1.28±0.0597	*0.0219
6	1.38±0.12	1.02±0.13	1.06±0.085	1.06±0.0929	***< 0.0001
13	1.34±0.12	1.55±0.073	1.44±0.225	1.45±0.18	0.1472

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05

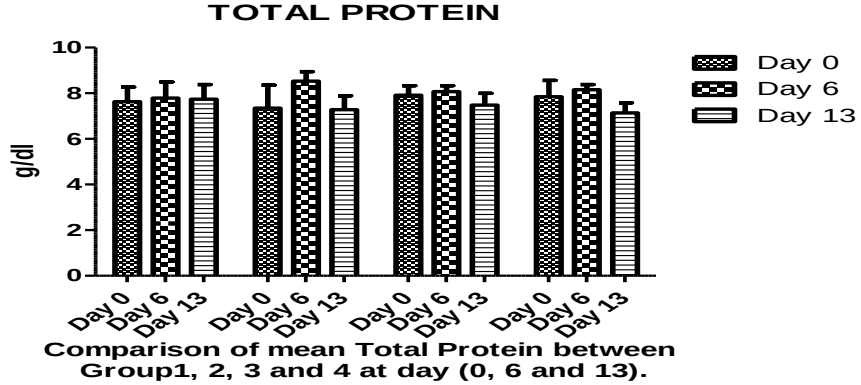


G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).

Mean Total protein values (G1, G2, G3 and G4) comparison.

Total protein On Day	G1	G2	G3	G4	P-VALUE
0	7.63±0.64	7.34±1.008	7.9±0.414	7.84±0.719	0.4693
6	7.79±0.71	8.53±0.415	8.07±0.256	8.16±0.223	*0.0370
13	7.74±0.64	7.29±0.609	7.49±0.518	7.14±0.443	0.2349

***p-value ≤ 0.0001, ** p-value ≤ 0.001, * p-value ≤ 0.05



G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Silymarin) **G4** = (Isoniazid+ Rifampicin+ Zinc sulfate).
Post hoc Tukey's test

Multiple comparisons of means for LFTs parameters between groups.

Comparison of groups	ALT	AST	ALP	Total Bilirubin	Albumin	Globulin	Albumin/Globulin ratio	Total Protein
G1 VS G2	***	***	***	***	ns	ns	ns	ns
G1 VS G3	***	***	***	***	ns	ns	ns	ns
G1 VS G4	***	***	***	***	ns	ns	ns	ns
G2 VS G3	***	***	***	ns	ns	ns	ns	ns
G2 VS G4	***	***	***	ns	ns	ns	ns	ns
G3 VS G4	*	***	ns	ns	ns	ns	ns	ns

Significant (p-value ***p-value ≤ 0.001, ** p-value ≤ 0.01, * p-value < 0.05), Non-significant p-value (ns)

ALT is biomarker for protein catabolism. (20) Increased levels of enzyme ALT indicate cell membrane dysfunction. (21) Serum ALT level was significantly raised in G2 with p-value ***p-value ≤ 0.001. ALT levels are reduced in G3 as compared to G2 ***p-value ≤ 0.001. These results were same with previous study by Chote Luangchosiri. This reduction in ALT is because of anti-inflammatory, antifibrotic activity and liver regeneration capacity. (22) According to Alciona Sasu Silymarin protects against Epirubicin induced liver injury by production of anti-ROS proteins, prevention of mitochondrial DNA damage, stimulation of replication, inhibition of membrane lipases and protecting electron transport chain for ATP synthesis. (23) G4 as compared to positive control G2 showed significant reduction in ALT ***p-value ≤ 0.001. These results were similar to the results of study conducted by Rasha H.G. Hasan against CCl_4 induced hepatotoxicity. Zinc sulfate by production of various metallothioneins and antioxidant action by enzyme zinc superoxide dismutase (Zn, SOD)[17] Metallothioneins protect against Isoniazid induced hepatotoxicity. (24) Significant difference * p-value < 0.05 was observed in ALT level with between G3 and G4 due to antioxidant, anti-inflammation, antifibrotic activity and liver regeneration capacity. (124)**.** Serum ALP is not specific to liver only but may be found elevated in other disease like bone disorders[25]. Treatment with Isoniazid and Rifampicin in G2 caused significant increase in level of serum AST and ALP as compared to G1 ***p-value ≤ 0.001. These results were same as study conducted by Chao Wang and Mohd Mujahid Respectively[26,27]. G3 showed significant reduction in serum AST as compared to G2 with ***p-value ≤ 0.001. These results were in consistence with previous study conducted by Chote Luangchosiri decrease in serum level by Silymarin was due to oxygen free radical scavenging action, inhibition of proinflammatory cytokines, enhancing immuno-modulatory effect, antifibrotic activity, stimulation of protein synthesis and liver regeneration[28]. Serum ALP level was significantly reduced in G3 as compared to G2 ***p-value ≤ 0.001 due to antioxidant, anti-inflammatory, immuno-modulatory, antifibrotic activity, protein synthesis stimulation and liver regeneration.(22) G4 showed significant reduction in serum AST and ALP as compared to G2 with ***p-value ≤ 0.001. These results were similar to previous study conducted by Chote Luangchosiri and Mohd Mujahid respectively. (22) (27) AST decrease by Zinc Sulfate may be due to antioxidant action by enzyme zinc superoxide dismutase (Zn, SOD) and various metallothioneins production. (17) Metallothioneins induction protect against hepatotoxicity. (24) Decrease in ALP was due to antioxidant, anti-inflammatory, immuno-modulatory, antifibrotic activity, stimulation of protein synthesis and liver regeneration capacity. (22) G4 as compared to G2 showed significant reduction in ALP ***p-value ≤ 0.001. These results were in accordance with study conducted by Vijayta Dani Chadha. This effect may be due to antiperoxidative property of Zinc. Zinc also inhibits lipid peroxidase formation and increase in glutathione. (28) Significant difference with ***p-value ≤ 0.001 was observed in ALT level between G3 and G4 but only mathematical decrease in ALP levels were observed statistically results were non-significant (22)**.** Raised bilirubin is indicator for cholestasis and bile flow.(20) it is an insensitive marker raised only when hepatic injury is very extensive.(25) Treatment with Isoniazid and Rifampicin in G2 caused significant increase in level of serum total bilirubin as compared to G1 ***p-value ≤ 0.001. These results were similar with study conducted by Adnan Jehangir. (29) G3 and G4 as compared to G2 shows no significant decrease in total bilirubin. As experimental duration was 2 weeks the results are similar with Eunyoung Heo study results up till 2 weeks, longer duration study is needed to confirm the preventive effect of Zinc Sulfate and Silymarin against Isoniazid, Rifampicin induced hepatotoxicity, as mathematical reduction in bilirubin level was obvious in 2 weeks study duration. Similarly only mathematical decrease was observed in albumin, globulin, albumin/globulin ratio and total protein in G2, while mathematical increase was evident from values of G3 and G4, longer duration study is required to find out the preventive effect in G3 and G4.

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