ORIGINAL ARTICLE

Evaluation of Hepatoprotective effect of Zinc Sulfate against Isoniazid and Rifampicin (first line Anti- tuberculous drugs) induced Hepatotoxicity in Albino rats

ZUJAJA ZAHEER[1], WARDAH SIDDIQUE[2], NAZIA RASHID[3], *NEELOFAR YOUSAF WARRAICH[4], AYELA EMAN ZIA[5], AMNA ASGHAR[6], AJAZ FATIMA[7]*

[1]Professor Head of the department Pharmacology KEMU,
[2]Assistant Prof. Pharmacology LMDC.
[3]Assistant Prof. Pharmacology FJMU,
[4]Assistant Prof. Pharmacology AMDC Lahore.
[5]Lecturer Pharmacology UCMD, the University of Lahore.
[6]Senior Demonstrator Pharmacology FMH Lahore.
[7]Professor Pharmacology LMDC.
Correspondence to Dr. Wardah Siddique, Email: dr4humanities@gmail.com, Cell: 0336-4138407

ABSTRACT

Background: Liver diseases are becoming significant health problem which can lead to liver failure. Currently available therapies for liver failure, are ineffective with the exception of organ transplantation.
Aim: Development of alternative methods for treatment of liver pathology are in great demand so regenerative medicines offer newer approaches for prevention of liver disorders.
Methods: Zinc sulfate is promising agent available to treat patient with hepatotoxicity. It has antioxidant properties. Hepatoprotective effect of Zinc Sulfate was evaluated against Isoniazid 50mg/kg/day and Rifampicin 100 mg/kg/day combination induced hepatotoxicity, in this study.
Results: 21 rats were divided into 3 groups (containing 7 rats in each group). Liver injury was induced by Isoniazid and Rifampicin combination in G2, 3. Hepatoprotective effect of Zinc sulfate 7mg /kg/day was evaluated in G3 by serum ALT, ALK PO4, AST, total bilirubin, total protein, albumin, globulin, A/G ratio (LFTs) analysis. There was significant elevation of serum ALT, ALK PO4, AST, total bilirubin indicating development of hepatotoxicity while total protein, albumin, globulin, A/G ratio were not significantly altered. The levels of ALK PO4, AST and Total bilirubin were significantly reduced (p value ***< 0.0001) in group G3 treated with Zinc Sulfate.
Conclusion: This study proved hepatoprotection of Zinc sulfate by improvement in deranged LFTs
Keywords: Hepatotoxicity, hepatoprotaction, Isonazid, Rifampin, Zinc sulfate, albion rats, LFTs.

INTRODUCTION

Hepatotoxicity is defined as liver injury associated with impaired liver function[1]. Drug-induced liver injury is a major cause of liver failure[2]. It is linked to thousands of marketed drugs[3]. Like Anti-TB drugs induced hepatotoxicity is a serious health problem[4]. Organ transplantation is the only effective available therapy for liver failure[5]. It is an efficient method, but practically its application is limited due to supply of donor organ, immunological reactions, and economic reasons. Development of less expensive, alternative methods for treatment of liver pathology, are in great demand. So regenerative medicines offer an ecnomical approach for prevention of liver disorders[5]. At present there is no promising therapy available to treat patient with hepatotoxicity[6]. Zinc is qualified to be the first essential trace element used in prophylaxis and management of liver diseases[7]. More than 300 enzymes and more than 1000 transcription factors require Zinc for their normal functioning[8]. Zinc carry out biological functions as part of numerous enzymes such as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.(9) Zinc has antioxidant properties at physiological level[10]. Its antioxidant activity is through induction of glutathione, catalase, sulfhydryls and a cysteine rich metallothionine protein (involved in Zinc homeostasis and electrophilic scavenging). It is also essential for regulation of anti-inflammatory activities, signaling pathways, cellular metabolic processes like structural, regulatory or catalytic processes and modulation of immune responses[11].

Received on 42-06-2019
Accepted on 16-11-2019

MATERIAL AND METHODS

Twenty one male albino rats weighing 150-200g were divided in 3 groups G1, G2 and G3 (7 rats in each group) and kept in animal house in hygienic conditions , at 23±2 degree centigrade ,under natural light and dark cycles, provided with rodent chow and water ad libitum. Animals were marked for identification. Hepatotoxicity was induced by co-administration of Isoniazid 50mg/kg/day[12] and Rifampicin 100 mg/kg/day (12) solution, prepared in distilled water and given orally daily as single morning dose for 14 days (0 day to 13th day). Zinc sulfate 7mg /kg/day[13] solution was prepared by dissolving in distilled water and given orally daily for 14 days in G3**.**

Body weight of rats were measured weekly to adjust the dose**.** Blood sample (1ml) was drawn after anesthetizing rat with ether through cardiac puncture[14] using 5ml disposable syringes on day 0, 6 and 13. Blood sample was allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes. Resulting serum was stored at -20 degree centigrade and assayed for biochemical tests[15,16]. Following lab tests were performed

by using commercially available kits: Alkaline phosphatase (ALK PO4), Alanine transaminase (ALT), Aspartate transaminase (AST), Total bilirubin, Total protein, Albumin, Globulin, Albumin/Globulin ratio. (14) All the tests were performed on Chemistry Analyzer Sphera. Methods were followed as provided by kits manufacturers. **Data analysis:** All data was entered on graph pad prism version 5 for statistical analysis. The data was evaluated by one way analysis of variance followed by tukeys multiple comparison test. P value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Serum Alanine Transaminase levels in all groups at day 0 was similar. Serum ALT levels of G1 was 37.29±7.251 U/L, 38.00±5.477 U/L and 39.00±4.830 U/L at day 0, 6 and 13 respectively. Serum ALT levels of G2 increased from 37.86±8.611 U/L to 78.14±5.460 U/L and 104.3±4.071 U/L at day 6 and 13 respectively. Serum ALT levels of G3 was increased from 37.14±5.928 U/L to 59.71±3.302 U/L and 63.29±2.870 U/L at day 6 and 13 respectively. There was mathematical improvement in Serum ALT levels in G3 but no significant difference was observed among the groups at day 0, 6 and 13 with p-value of 0.6826, 0.4388 and 0.4833 respectively..

Serum Aspartate Transaminase levels of G1 were 133.4±17.34 U/L, 125.1±10.11U/L and 125.4±9.624U/L at day 0, 6 and 13 respectively. Serum AST levels of G2 increased from 113.6±21.88 U/L to 358.3±67.56U/L and 728.3±37.52U/L at day 6 and 13 respectively. Serum AST levels of G3 was increased from 136.7±20.64U/L to 207.6±27.43 U/L and 330.0±17.74U/L at day 6 and 13 respectively. No significant difference was observed among the groups at day 0 with p-value of 0.0921. There was significant difference among the groups on day 6 and 13 with p-value ***<0.0001.

Serum Alkaline Phosphatase levels of G1were117.0± 23.59, 143.7± 32.77 and 145.0± 32.24 at day 0, 6 and 13 respectively. Serum ALK PO4 levels of G2 increased from 157.9**±**42.81 U/L to 885.7±131.5 U/L and 1210±108.9U/L at day 6 and 13 respectively. Serum ALK PO4 levels of G3 were increased from 160.9**±**39.34U/L to 221.6±65.66 U/L and 347.6±19.65 U/L at day 6 and 13 respectively. No significant difference was observed among the groups at day 0 with p-value of 0.0658. There was significant difference among the groups on day 6 and 13 with p-value ***<0.0001.

Serum Total Bilirubin levels of G1 was 0.01857±0.04914 mg/dl, 0.06143±0.05843 mg/dl and 0.04286±0.05345 mg/dl at day 0, 6 and 13 respectively. Serum total bilirubin levels of G2 increased from 0.01429±0.03780 mg/dl to 1.214±0.4337 mg/dl and 0.5286±0.1704 mg/dl at day 0, 6 and 13 respectively. In G3 serum total bilirubin level was increased from 0.01429±0.03780 mg/dl to 0.8571±0.1272 mg/dl and 0.4429±0.2370 mg/dl at day 0, 6 and 13 respectively. No significant difference was observed among the groups at day 0 with p-value of 0.9759. There was significant difference among the groups on day 6 and 13 with p-value ***<0.0001.

Serum Albumin levels of G1 was 4.514±0.3237 g/dl, 4.486±0.3078 g/dl and 4.429±0.2984 g/dl at day 0, 6 and 13 respectively. Serum albumin levels of G2 increased from 4.100±0.5538 g/dl to 4.300±0.1633 g/dl and 4.314±0.1952 g/dl at day 0, 6 and 13 respectively. In G3 serum total bilirubin level was increased from 4.386±0.3436 g/dl to 4.200±0.1155 g/dl and 4.214±0.1864 g/dl at day 0, 6 and 13 respectively. Only mathematical difference exists between G2, G3 but no significant difference was observed among the groups at day 0, 6 and 13 with p-value of 0.1965, 0.0612 and 0.2516 respectively.

AST and ALT are biomarkers for protein catabolism[17]. Raised levels of enzymes ALT and AST indicate defective cell membrane function.(18) Serum ALP is not liver-specific and may be found elevated in other disease states.(19) Treatment with Isoniazid and Rifampicin in G2 caused significant increase in level of serum AST and ALK PO4 as compared to G1 (***p-value ≤ 0.001). These results were in agreement with previous study conducted by Chao Wang and Mohd Mujahid Respectively[20,21]. G3 (Isoniazid, Rifampicin and Zinc Sulfate) 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. (22) AST decrease by Zinc Sulfate may be due to antioxidant action by enzyme zinc superoxide dismutase (Zn, SOD) and various metallothioneins production[14]. Metallothioneins induction protect against hepatotoxicity[23]. G3 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 against Lithium induced hepatotoxicity due to anti-peroxidative property of Zinc. Zinc also inhibits LPO (lipid peroxidase) formation and increases GSH (glutathione)[24]. There was only mathematical improvement in Serum ALT levels in G3 but no significant difference was observed among the groups at day 0, 6 and 13 with p-value of 0.6826, 0.4388 and 0.4833 respectively. This mathematical decrease in ALT is due to antioxidant, anti-inflammation, anti-fibrotic activity and liver regeneration capacity. (22) Bilirubin is indicator for bile flow and cholestasis[17]. Bilirubin, an insensitive marker for liver dysfunction, raises only when extensive hepatic injury occurs or by inhibition of biliary transporters. While fractionation of bilirubin level can exclude benign increase due to intravascular hemolysis[19]. Treatment with Isoniazid and Rifampicin in G2 caused significant increase in level of serum total bilirubin as compared to G1 with ***p-value ≤ 0.001. These results were in consistence with previous study conducted by Adnan Jehangir[25]. As experimental study duration was 2 weeks the results are inconsistence with the results of Eunyoung Heo study up till 2 weeks, as a longer study is needed to confirm the preventive effect May be Zinc Sulfate proves to be hepatoprotective against Isoniazid, Rifampicin induced hepatotoxicity by significant reduction in serum bilirubin levels in longer duration studies as mathematical reduction in bilirubin level was obvious in 2 weeks study duration by treatment with Zinc Sulfate. Similarly mathematical increase was observed in serum albumin, globulin, albumin/globulin ratio and total protein parameters in G2, while mathematical improvement was evident from results in G3.

Serum Globulin levels of G1 was 3.100±0.3367 g/dl, 3.271±0.4152 g/dl and 3.314±0.4018g/dl at day 0, 6 and 13 respectively. Serum albumin levels of G2 increased from 3.243±0.5533g/dl to 10.40±16.58 g/dl and 2.986±0.5610 g/dl at day 0, 6 and 13 respectively. In G3 serum globulin level was increased from 3.429±0.3773 g/dl to 3.957±0.2820 mg/dl and 2.929±0.3988 g/dl at day 0, 6 and 13 respectively. Only mathematical difference exists between G2, G3 but no significant difference was observed among the groups at day 0, 6 and 13 with p-value of 0.3824, 0.3300 and 0.2647 respectively.

Serum albumin/globulin ratio levels of G1 was 1.451±0.09008, 1.379±0.1170 and 1.340±0.1183 at day 0, 6 and 13 respectively. Serum albumin/globulin levels of G2 increased from 1.276±0.05972, 1.063±0.09286 and 1.454±0.1813 at day 0, 6 and 13 respectively. In G3 serum albumin/globulin was increased from 1.280±0.05972, 1.063±0.09286 and 1.454±0.1813 at day 0, 6 and 13 respectively. Significant difference was observed among the groups at day 0, 6 and 13 with p-value of *0.0158, ***< 0.0001 and *0.0292 respectively.

Total Protein levels of G1 was 7.629±0.6422, 7.786±0.7105 and 7.743±0.6425g/dl at day 0, 6 and 13 respectively. Total protein levels of G2 increased from 7.343±1.008, 8.529±0.4152 7.286±0.6094 g/dl at day 0, 6 and 13 respectively. In G3 Total protein level was increased from 7.871±0.6676, 8.157±0.2225 and 7.143±0.4429 g/dl at day 0, 6 and 13 respectively. No significant difference was observed among the groups at day 0, 6 and 13 with p-value of 0.4714, *0.0369 and 0.1510 respectively.

Table 1:

Groups	Inducing agents	Protective agents
G1	Distilled Water(0.3ml)	Distilled Water(0.3ml)
G2	Isoniazid(50 mg/kg/day) Rifampicin(100mg/kg/day)	Distilled Water(0.3ml)
G3	Isoniazid(50 mg/kg/day) Rifampicin(100mg/kg/day)	Zinc Sulfate(7mg/kg/day)

Table 2: Comparison of mean ALT Alanine Transaminase (U/L) values of G1, G2 and G3 at day 0, 6 and 13.

ALT	G1	G2	G3	P Value
Day 0	37.29± 7.251	37.86± 8.611	37.14± 5.928	0.6826
Day 6	38.00± 5.477	78.14± 5.460	59.71± 3.302	0.4388
Day 13	39.00± 4.830	104.3± 4.071	63.29± 2.870	0.4833

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

Fig. 1: **G1=** normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Zinc sulfate).

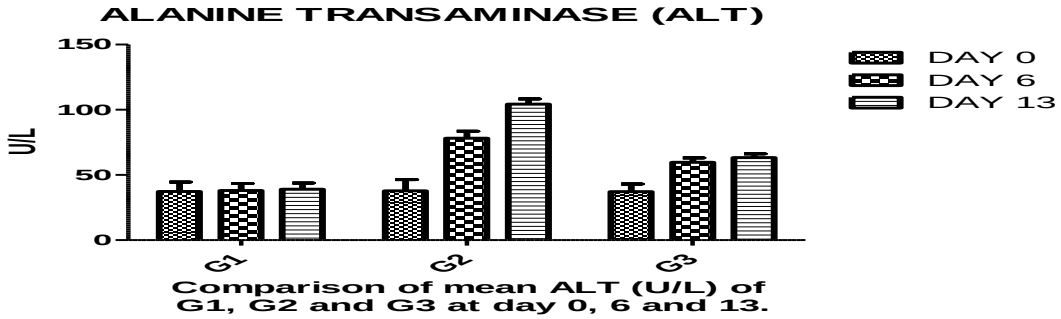


Table 3: Comparison of mean AST Aspartate Transaminase (U/L) values of G1, G2 and G3 at day 0, 6 and 13.

AST	G1	G2	G3	P Value
Day 0	133.4± 17.34	113.6± 21.88	136.7± 20.64	0.0921
Day 6	125.1± 10.11	358.3± 67.56	207.6± 27.43	***< 0.0001
Day 13	125.4± 9.624	728.3± 37.52	330.0± 17.74	***< 0.0001

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

Fig. 2: G1**=** normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Zinc sulfate).

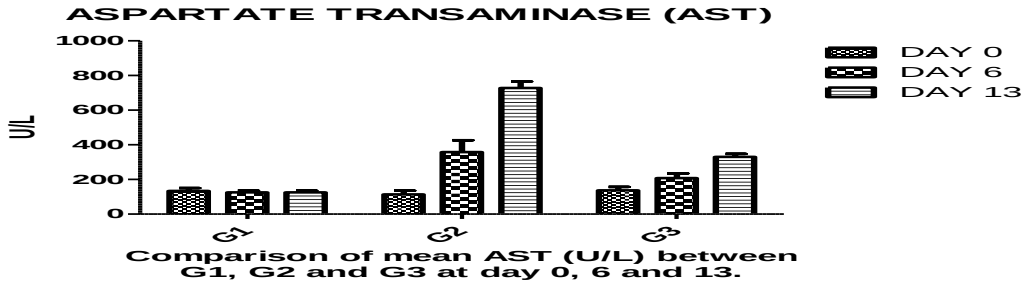


Table 4: Comparison of mean ALK PO_4 Alkaline Phosphatase U/L values of G1, G2 and G3 at day 0, 6 and 13.

ALK PO4	G1	G2	G3	P Value
Day 0	117.0± 23.59	157.4± 42.75	160.9± 39.34	0.0658
Day 6	143.7± 32.77	885.7± 131.5	221.6± 65.66	***< 0.0001
Day 13	145.0± 32.24	1210± 108.9	347.6± 19.65	***< 0.0001

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

Fig. 3: G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Zinc sulfate).

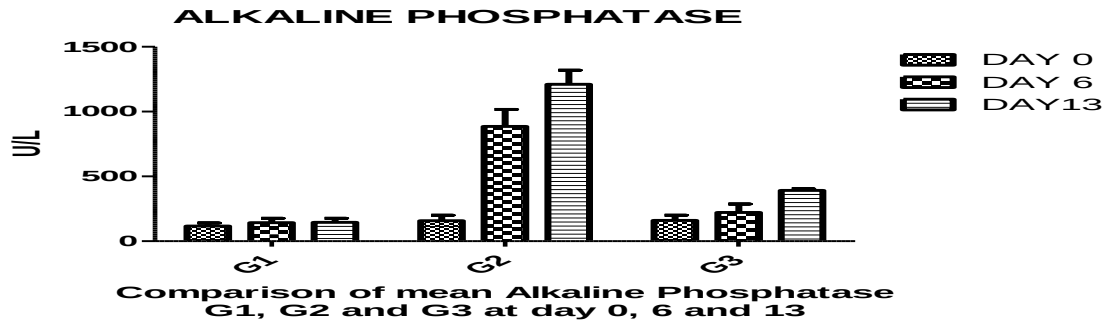


Table 5: Comparison of mean Total Bilirubin (mg/dl) values of G1, G2 and G3 at day 0, 6 and 13.

Total Bilirubin	G1	G2	G3	P Value
Day 0	0.01857±0.04914	0.01429±.03780	0.01429±0.03780	0.9759
Day 6	0.06143±0.05843	1.214± 0.4337	0.8571± 0.1272	***< 0.0001
Day 13	0.04286±0.05345	0.5286±0.1704	0.4429±0.237	***< 0.0001

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

Fig. 4: G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Zinc sulfate).

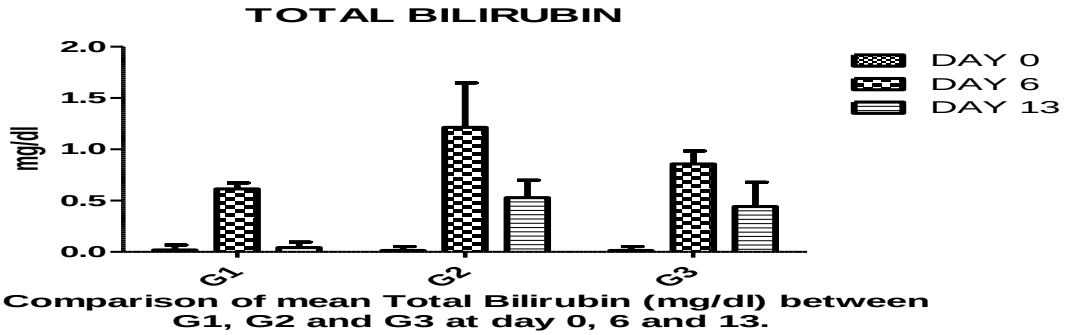
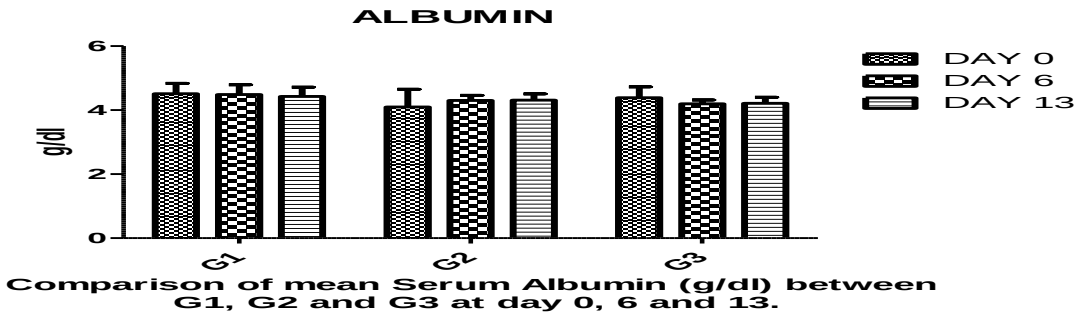


Table 6: Comparison of mean Albumin (g/dl) values of G1, G2 and G3 at day 0, 6 and 13.

Albumin	G1	G2	G3	P Value
Day 0	4.514± 0.3237	4.100± 0.5538	4.386± 0.3436	0.1965
Day 6	4.486± 0.3078	4.300± 0.1633	4.200± 0.1155	0.0612
Day 13	4.429± 0.2984	4.314± 0.1952	4.214± 0.1864	0.2516

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

Fig. 5: G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Zinc sulfate).



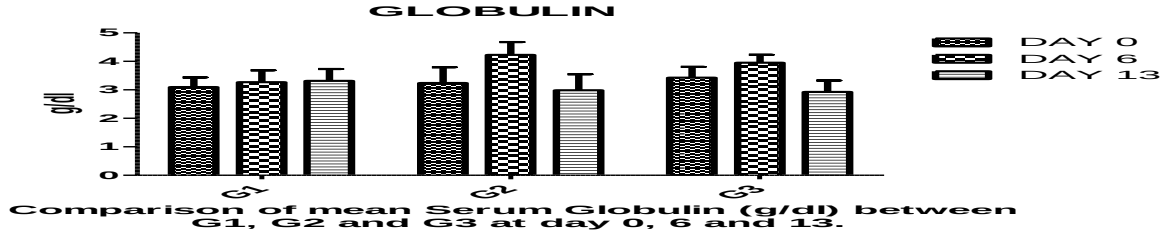
Comparison of mean Serum Albumin (g/dl) between G1, G2 and G3 at day 0, 6 and 13.

Table 7: Comparison of mean Globulin (g/dl) values of G1, G2 and G3 at day 0, 6 and 13.

Globulin	G1	G2	G3	P Value
Day 0	3.100± 0.3367	3.243± 0.5533	3.429± 0.3773	0.3824
Day 6	3.271± 0.4152	10.40± 16.58	3.957± 0.2820	0.3300
Day 13	3.314± 0.4018	2.986± 0.5610	2.929± 0.3988	0.2647

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

Fig. 6: G1= normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3** = (Isoniazid+ Rifampicin+ Zinc sulfate).



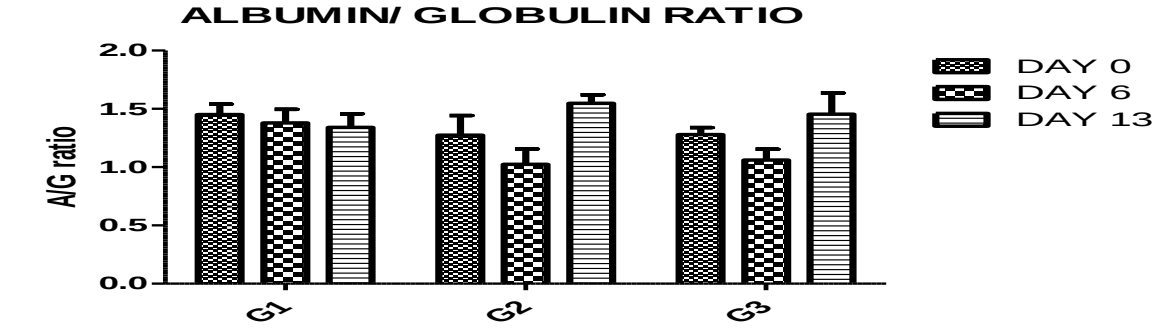
Comparison of mean Serum Globulin (g/dl) between G1, G2 and G3 at day 0, 6 and 13.

Table 8: Comparison of mean Albumin/Globulin values of G1, G2 and G3 at day 0, 6 and 13.

A/G ratio	G1	G2	G3	P Value
Day 0	1.451±0.09008	1.276±0.05972	1.280±0.05972	*0.0158
Day 6	1.379±0.1170	1.063±0.09286	1.063±0.09286	***< 0.0001
Day 13	1.340±0.1183	1.454±0.1813	1.454±0.1813	*0.0292

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

Fig. 7: G1**=** normal control group (distilled water), **G2** = positive control group (Isoniazid+ Rifampicin), **G3 =** (Isoniazid+ Rifampicin+ Zinc sulfate).



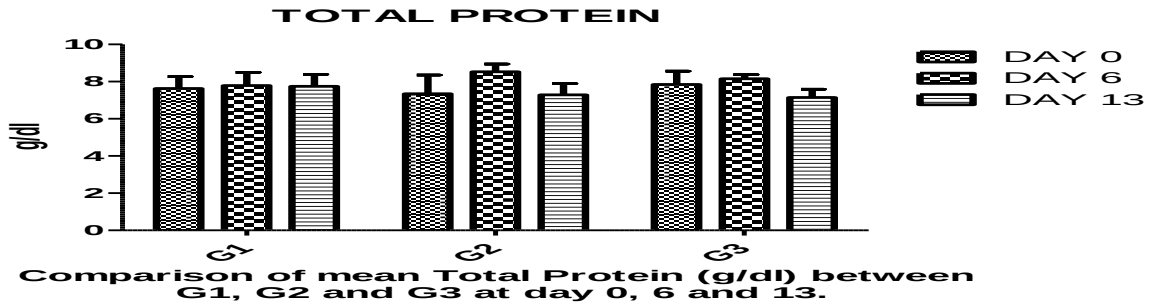
Comparison of mean Serum Albumin/ Globulin between G1, G2 and G3 at day 0, 6 and 13.

Table 9: Comparison of mean Total Protein (g/dl) values of G1, G2 and G3 at day 0, 6 and 13.

Total protein	G1	G2	G3	P Value
Day 0	7.629±0.6422	7.343±1.008	7.871±0.6676	0.4714
Day 6	7.786±0.7105	8.529±0.4152	8.157±0.2225	*0.0369
Day 13	7.743±0.6425	7.286±0.6094	7.143±0.4429	0.1510

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

Fig. 8: G1**=** normal control group (distilled water), **G2 =** positive control group (Isoniazid+ Rifampicin), **G3 =** (Isoniazid+ Rifampicin+ Zinc sulfate).



Comparison of mean Total Protein (g/dl) between G1, G2 and G3 at day 0, 6 and 13.

Table 10: Post hoc Tukey's test for multiple comparisons of means for all the parameters between groups.

Parameters	G1 Vs G2	G1 Vs G3	G2 Vs G3
ALT	***	***	***
AST	***	***	***
ALKPO4	***	***	***
Total Bilirubin	***	**	ns
Albumin	ns	ns	ns
Globulin	ns	ns	ns
Albumin/Globulin	*	ns	ns
Total Protein	ns	ns	ns

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

Acknowledgement: I want to pay my gratitude to Nusrat Firdous, Muhammad Siddique Akhtar and Dr. Humaira Siddique.

REFERENCES

1. Navarro V and Senior J R. Review article drug-related hepatotoxicity. N Engl J Med 2006;354(7):731-9.
2. Tamara A, Simon Z and Milosavljevic T Drug-induced liver injury: do we know everything? WJH 2017;9(10):491-502.
3. Chalasani N, Hayashi P and Bonkovsky H. ACG clinical guideline: the diagnosis and management of idiosyncratic drug-induced liver injury AJG. 2014:950-66.
4. Sangamithira and Abdullah S. Defensive effect of eugenol on isoniazid and rifampicin induced hepatotoxicity in albino rats. Journal of global biosciences 2016;5:4082-89.
5. Kholodenko I,Yarygin K. Review article cellular mechanisms of liver regeneration and cell-based therapies of liver diseases. Bio med research international 2017:1-17.
6. Singh G, Dhadwal N and Harikumar S.I. Experimental models for hepatotoxicity. AJPCR. 2015;8(2):70-4.
7. Elshennawy, Sayed S, Saber Eand Rifaai R. Histopathological and histochemical assessment of the protective effects of zinc on ethanol-induced acute hepatotoxicity in adult albino rats. Journal of cytology & histology. 2015.
8. Prasad A. Discovery of human zinc deficiency: its impact on human health and disease. Reviews from ASN EB 2012 symposia. 2013;4:176-90.
9. Dhawan D. K. and Chadha V. Zinc: A promising agent in dietary chemoprevention of cancer. Indian J Med Res. 2010;132(6):676–82.
10. Trevisan R, Flesch S and Mattos J. Zinc causes acute impairment of glutathione metabolism followed by coordinated antioxidant defenses amplification in gills of brown mussels Pernaperna.
11. King J, Brown K and Gibson R. Biomarkers of nutrition for development (BOND)-zinc review. The journal of nutrition supplement. 2016;146:858–85.
12. Kamil N and Imran-ul-Haque H. Hepatoprotective effect of calotropis procera in isoniazid and rifampicin induced hepatotoxicity. Phcog J 2014;6(5):9-14.
13. Plum L, Rink L and Haase H. Review the essential toxin: impact of zinc on human health Int j environ res public health 2010;7:1342-65.
14. Hasan R. Antioxidant effects of panax ginseng and zinc against CCl4 induced hepatotoxic on rats Global veterinaria 2015; 14(1):103-11.
15. Lian Y, Zhao J and Xu P. Protective effects of metallothionein on isoniazid and rifampicin-induced hepatotoxicity in mice. Plos one. 2013;8(8):1-8.
16. Hamid A, M B and A. Z. Protective effect of some medicinal plants and zinc on some serum parameters and histopathological features of liver, kidney and testis in rats treated with cadmium. Middle east journal of applied sciences 2014;4(3):539-54.
17. Roth A and Lee M. Review article idiosyncratic drug-induced liver injury (IDILI): potential mechanisms and predictive assays. Biomed research international. 2017:1-25.
18. Mirlohi M, Ekrami A and Shirali S. Hematological and liver toxicity of anti-tuberculosis drugs. Electronic physician. 2016; 8(9): 3005-10.
19. Fontana R. Pathogenesis of idiosyncratic drug-induced liver injury and clinical perspectives. Gastroenterology 2014;146(4):914-28.
20. Wang C, Fan R and Zhang Y. Naringenin protects against isoniazid- and rifampicin induced apoptosis in hepatic injury. W J G 2016; 22(44): 9775-83.
21. Mujahid Ma, Hussain T and Siddiqui H. Evaluation of hepatoprotective effect of erythrina indica leaves against antitubercular drug induced hepatotoxicityin expereimental rats. J-AIM 2017: 7-12.
22. Luangchosiri C, Thakkinstian A and Chitphuk S. A double-blinded randomized controlled trial of silymarin for the prevention of antituberculosis drug-induced liver injury. BMC complementary and alternative medicine 2015; 15(334): 2-7.
23. Yong Lian Y,Zhao J and Wang Y. Metallothionein protects against isoniazid-induced liver injury through the inhibition of CYP2E1-dependent oxidative and nitrosative impairment in mice. Food and chemical toxicology 2017;102:32-8.
24. Chadha V, Bhalla Pand Dhawan D. Zinc modulates lithium-induced hepatotoxicity in rats. Liver international ISSN 2007:558-66.
25. Jehangir A, Nagi A.H and Shahzad M. The hepato-protective effect of cassia fistula (amaltas) leaves in isoniazid and rifampicin induced hepatotoxicity in rodents. Biomedica 2010; 26: 25-9.