ORIGINAL ARTICLE

DETERMINATION OF HEPATIC HISTOTOPROTECTIVE EFFECT OF COMBINATION OF SM/ SILYMARIN AND ZNSO4/ ZINC SULFATE AGAINST DAMAGE BY RIF/ RIFAMPICIN AND INH/ ISONIAZID IN ALBINO RATS.

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ABSTRACT

Background: To induce hepatotoxicity with RIF and INH in albino rats, to estimate histoprotective effect of combination of ZnSO4 and Silymarin.

Objective: To evaluate the hepatic histoprotective activity of zinc sulfate in combination with silymarin against rifampicin- and isoniazid-induced hepatic damage in albino rats.

Methods: The study was conducted at the Department of Pharmacology, King Edward Medical University Lahore and UVAS, Lahore after approval from IRB committee vide no. 18/ PEC/RC/KEMU. The 'Animal Experimental Study' was carried out of 14 days' duration. A sample size of 28 healthy albino wistar rats was taken and divided into four equal groups. GA was normal control group and given water once a day orally. GB was toxic control group and was given inducing agents RIF and INH (100 and 50 mg/kg/day) orally for 14 days. Whereas, rats in GC and GD received inducing agents along with combination of ZnSO4 + SM (3.5 + 100 mg /kg/day) and (7 + 200 mg /kg/day) respectively once daily for 14 days. Histoprotective effects evaluated by various histopathological parameters.

Results: Significant change (p-value 0.05) between positive control and hepatoprotective groups was observed for parameters like hepatic cell enlargement, lobular lymphocytic infiltrate, portal lymphocytic infiltrate, kuppfer cells, sinusoidal distention, lobular and portal plasma cellular infiltrate, steatosis, neutrophils, inflamed portal area, congestion and mast cells. Comparing GC and GD significant improvement was seen in parameters like lymphocytic lobular infiltrate, steatosis, hepatocytes enlargement and portal inflammation.

Conclusion: The current study has shown hepatoprotection of ZnSO4, SM and their combination in different doses as evidenced by improvement in histological parameters

Keywords: Hepatotoxicity, Histoprotection, INH/ Isoniazid, RIF/ Rifampin, ZnSO4/ Zinc sulfate, SM/ Silymarin.

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INTRODUCTION

Drug-induced liver damage refers to the damage caused by the use of medications to the liver. Liver toxicity is defined as disruption of liver functions by xenobiotics. Various drugs can lead to liver injury, INH and RIF are reliable drugs against tuberculosis (TB). Both are liver metabolized drugs and are toxic as well.[1] TB a communicable infectious disease rampant world over. First line of treatment used for TB is associated with liver injury known as drug induced liver damage.[2] Throughout the treatment course,

approximately 5–33% patients suffered from liver toxicity. INH (1st line anti-TB), is known to induce liver damage which hampers the therapy as well as contributes to increased mortality.[3] The most important adverse effects related to INH are neuropathy and liver toxicity. A meta-analysis of 35 studies showed 2.6% frequency of liver injury and 0.02% mortality associated with INH.[4] INH is usually used with RIF as 1st-line treatment. Both are associated with liver problems and are significant concern throughout the globe. Metabolites of INH and RIF increases production of oxidative species (ROS) and damage, inflammation, lipid peroxidation and apoptosis of liver cells. Microsomal (CYP450) enzyme metabolize RIF into liver toxic species which results in enhanced synthesis of ROS, which initiate lipid membrane oxidative damage and hepatic insults.[5] Induction of microsomal enzyme CYP2E1 results in metabolism of INH to hydrazine, which causes liver damage. INH is also metabolized by to (Hz) hydrazine and (AcHz) acetylhydrazine, by N-acetyltransferase, which are further metabolized by cytochrome P450 2E1 to liver toxic substances.[6]

Multiple herbal drugs and supplements are in use to reduce liver toxic effects of drugs.[7] Silymarin SM an extract from *Silybum marianum* (milk thistle), reduces oxidative stress to liver and inhibits inflammatory, apoptotic cytokines and fibrosis. Most prominent and biologically active constituent is Silybinin having potent anti- ROS activity. Silymarin decreases oxidative damage by decreasing ROS production.[8] It can increase glutathione levels in the liver. Silymarin a natural compound prevents lipid peroxidation, stabilizes membrane and inhibit the production of cytokines. Hepatic fibrosis is associated to chronic inflammation. Silymarin inhibit fibrosiss by blocking inflammation and production of mediators like NF-B, prostaglandin E2 (PGE2) and leukotriene B4 (LTB4).[9]

Zinc (Zn) a nutritional supplement, decreases oxidative stress by induction of metallothioneins. Zinc also possess anti-oxidant activity by activating antioxidant system like glutathione and catalase.[10] One in ten proteins in the body contain zinc, more than 300 enzymes and 1000 transcription factors needs Zinc for their functioning.[11] Zinc is very important for cell division, its differentiation and signaling. It plays pivotal role for proper liver functioning. Its metabolism is controlled by liver. Decreased Zn stores have been reported in liver diseases (acute as well as chronic). Zinc plays catalytic role in various metalloenzymes (more then 300) and regulates cellular processes like expression of genes and signal transduction. Zinc is also important for normal hepatic metabolism. Its deficiency occurs in progressive liver dysfunctions and cirrhosis.[12] Zn deficiency can lead to cirrhosis of the liver.[13] Keeping all these factors in mind we have decided to evaluate and compare the histoprotective effect of combination of ZnSO4, Silymarin against INH and RIF (ATDs) induced liver toxicity.

METHODS

An experimental study on animal model was conducted in University of Veterinary and Animal Sciences Lahore. The technique for sampling used was **s**imple random sampling by lottery method. 28 albino male rats weighing 150-200 g (7 rat in one group) were used in this study, and sample size was calculated by utilizing 1% level of significance (α), 95% power of test (β) with expected mean value SM and ZnSO4 in half doses as 346.6±17.7 and full doses as 268.2±31.13.

$$N=\frac{\sigma^2(Z_1-\alpha+Z_1-\beta)^2}{(\mu_0-\mu\alpha)^2}$$

σ^2 = Variance
$Z_{1-\beta}$ = power of test 95%
$Z_{1-\alpha}$ = confidence level 99%
μ_0 = population mean 1 = 346.6
$\mu\alpha$ = population mean 2 = 268.2
Sample size in one group = n = 7
(Sample size was calculated from the values derived from results of preliminary report).

This study was performed on 28 albino male rats of 150-200 g and were divided in group **GA, GB, GC and GD**. All rats were marked for identification. Rats were given rodent chow and were kept in cages under natural day and night cycle, at temperature 23±2 º C. Rats were weighed weekly for drug dose adjustment.

Table- 1: Drugs and their dosages for GA, GB, GC and GD.

GROUPS (G)	HEPATOTOXIC AGENTS	HEPATOPROTECTIVE AGENTS
	Day 0 to day 13 (total 14 days)	
GA	0.3ml water	0.3ml water
GB	RIF and INH (100+ 50 mg/kg/day) (14)	0.3ml water
GC	RIF and INH (100+ 50 mg/kg/day)(14)	SM(15)+ZnSO4(16)(100+3.5 mg/kg/day)
GD	RIF and INH (100+ 50 mg/kg/day)(14)	SM(15)+ ZnSO4 (16) (200+ 7 mg/kg/day)

Rat's livers were dissected out after cervical dislocation on 14th day of the study. All hepatic specimens were fixed in 10% formalin. Tissues were dehydrated in ethanol by passing through processor. All tissues were washed with Xylene and embedded in paraffin wax. 5 um sections were prepared. Slides were prepared, dewaxing was done and hydrated by passing alcohol. Then stained with hematoxylin and eosin and washed in tap water for 2-3min. The stained sections were mounted and examined for liver parenchymal changes

like hepatic cell enlargement, lobular lymphocytic infiltrate, portal lymphocytic infiltrate, kuppfer cells, sinusoidal distention, lobular and portal plasma cellular infiltrate, steatosis, neutrophils, inflamed portal area, congestion and mast cells.[17,18] Data was analyzed by Graph pad prism-5 for quantitative data and SPSS-20 for qualitative data. Qualitative data was analyzed by applying Chi-square test (P-value of ≤ 0.05 significant) were expressed as frequencies and percentages.

RESULTS

Total 28 liver tissue were observed for various histopathological parameters. The histopathological parameter's frequencies + percentages have been mentioned in table 2. The maximum values were found out for lobular lymphocytic infiltrate 57.14%, portal lymphocytic infiltrate 75%, hepatic cell enlargement 50%. Minimum values (0, 0%) was seen for bridging/ confluent necrosis, perivenular necrosis and fibrosis. Chi-square test showed significant values for sinusoidal distension, portal and lobular lymphocytic infiltrate, hepatic cell enlargement, lobular and portal plasma cellular infiltrate, steatosis, neutrophils, inflamed portal area, congestion, mast cells and kuppfer cells, with p-value (.000) and it is non-significant for bridging/ confluent necrosis, perivenular necrosis and fibrosis as mentioned in table- 3.

DISCUSSION

Our study has demonstrated that both RIF and INH combination cause significant histological damage in GB. The results are consistent with study conducted by Libamila, H.in 2023.[19] RIF +INH combination can damage hepatic cell defensive mechanisms, by enzymatic as well as non-enzymatic ways. Acetylhydrazine, and isonicotinic acid are formed by N-acetyl transferase during the acetylation of isoniazid in liver. Acetylhydrazine is further metabolized to hydrazine and diacetylhydrazine on hydrolysis. These metabolites cause irretrievable cell damage. RIF gets metabolized to desacetyl-RIF in liver. Desacetyl-RIF further hydrolyze to form 3-formyl RIF which can cause liver cellular injury.

Table 2: GA, GB, GC and GD (frequency and percentage of histopathological parameters)

Histopathological parameters		Groups				Total frequency	Total %age
		GA	GB	GC	GD		
Lobular lymphocytic infiltrate	Present	0	7	7	2	16	57.14%
	Absent	7	0	0	5	12	42.857%
Portal lymphocytic infiltrate	Present	0	7	7	7	21	75%
	Absent	7	0	0	0	7	25%
Lobular and portal plasma	Present	0	7	0	0	7	25%
cellular infiltrate	Absent	7	0	7	7	21	75%
Perivenular necrosis	Present	0	0	0	0	0	0%
	Absent	7	7	7	7	28	100%
Bridging/ confluent necrosis	Present	0	0	0	0	0	0%
	Absent	7	7	7	7	28	100%
steatosis	Present	0	7	3	1	11	39.285%
	Absent	7	0	4	6	17	60.714%
fibrosis	Present	0	0	0	0	0	0%
	Absent	7	7	7	7	28	100%
Neutrophils	Present	0	7	0	0	7	25%
	Absent	7	0	7	7	21	75%
Sinusoidal distension	Present	0	7	0	0	7	25%
	Absent	7	0	7	7	21	75%
Hepatic cell enlargement	Present	0	7	7	0	14	50%
	Absent	7	0	0	7	14	50%
Inflamed portal area	Present	0	7	7	0	14	50%
	Absent	7	0	0	7	14	50%
congestion	Present	0	7	0	0	7	25%
	Absent	7	0	7	7	21	75%
Prominent kuppfer cells	Present	0	7	0	0	7	25%
	Absent	7	0	7	7	21	75%
Mast cells	Present	0	7	0	0	7	25%
	Absent	7	0	7	7	21	75%

Table 3: Chi- square test: Histopathological parameters for GA, GB, GC and GD.

Histopathological Indices	Chi square test	Values Pre Pearson Chi-Square N of Valid Cases	Asymp. Sig. (2-sided)
Lobular lymphocytic infiltrate	a. All 8cells (100.0%) demonstrated frequencies less than5; with lowest expected value was 3.00.	22.167 [a] 28	.000
Steatosis	a. frequencies for all eight cells (100.0%) were <5, with a minimum expected value of 2.75.	17.219 [a] 28	.001
Inflamed portal area	a. all 8cell (100.0%) have expected count <5.the minimum expected count is 3.50.	28.00 [a] 28	.000
Hepatic cell enlargement	a. eight cell (100.0%) have expected count less than 5.the minimum expected value is 3.50.	28.00 [a] 28	.000
Portal Lymphocytic infiltrate	a. four cell (50.0%) have expected count < 5.the minimum expected count is 1.75.	28.00 [a] 28	.000
Lobular and portal plasma cellular infiltrate	a. Expected frequencies were <5 in 4/8 cells, with a 1.75 as minimum value.	28.00 [a] 28	.000
Neutrophils	a. 4/8 cells had values <5; the minimum expected count observed was 1.75.	28.00 [a] 28	.000
Sinusoidal distension	a. Four out of eight cells exhibited the frequencies below 5; the minimum expected count was 1.75.	28.00 [a] 28	.000
Congestion	a. Expected value of frequencies were <5 in four of eight cells, with 1.75 as the minimum expected value.	28.00 [a] 28	.000
Kuppfer cells	Four of eight cells had expected counts <5; the expected value is 1.75.	28.00 [a] 28	.000
Mast cells	The minimum expected count is 1.75. a. four of eight cells (50.0%) have count <5	28.00 [a] 28	.000
Perivenular necrosis	a. No statistics are computed because variable remains constant.	a 28	
Bridging/ confluent necrosis	a. Statistics are not computed because bridging/ confluent necrosis is being constant.	a 28	
Fibrosis	a. No statistical analysis was computed because fibrosis exhibit no variability	.a 28	

P < 0.05 is considered to be significant

Decrease in scavenging activity of the liver cells causes the increase in levels of oxidative radicals. Increase in free radicals leads to increased oxidative insults.[20] GC and GD showed significant improvements as compared to GB. Both SM + ZnSO4 treated groups shows hepatoprotection by improvement in histopathological parameters. SM treated group shows reduction in oxidative stress and a marked reduction in inflammatory species.[21] SM showed antioxidant, antifibrotic, hepatoprotective, immunostimulating, and anti-inflammatory effects.[22]

Zinc supplements decreases oxidative damage by metallothioneins induction. An anti-oxidantive effect is produced by antioxidant system enzymes like catalase and glutathione. It also important in sulfhydryl group stabilization against oxidation.[23] This study has shown that the incidence of fibrosis was not significant in all groups treated with INH and RIF. The results are in consistant

with Sude Eminzade study.[24] According to Sergio Duarte review prominent feature of liver is its ability to regenerate by induction of anti-fibrotic agent. Only injury for long duration can lead to fibrosis.[25]

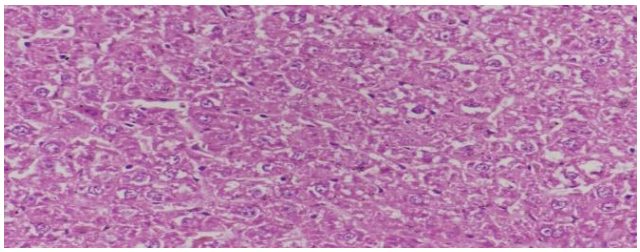

Figure- 1: GA (Distilled water) liver histology (40X)

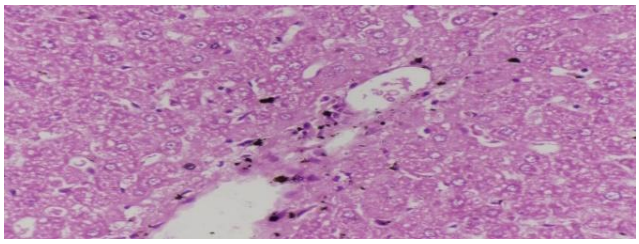

Figure- 2: GB (INH+ RIF) liver histology (40X)

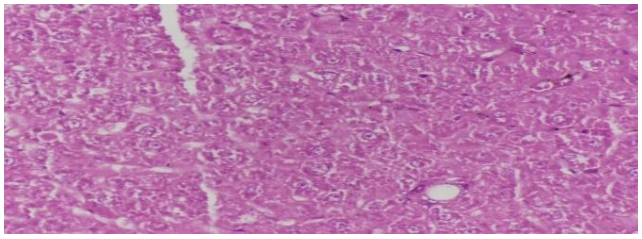

Figure- 3: GC (INH+ RIF+ 1/2SM+ 1/2 ZnSO4) liver histology (40X)

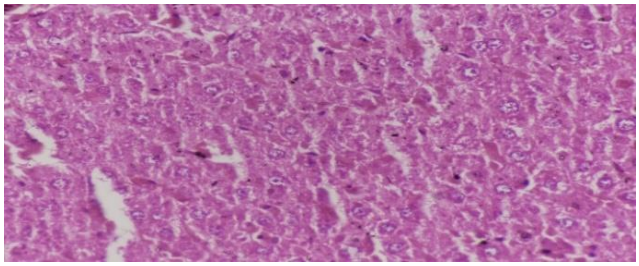

Figure- 4: GD (INH+ RIF+ SM+ ZnSO4) liver histology (40X)

CONCLUSION

The current study has shown the liver protective effect of ZnSO4 + SM combination at different doses as evidenced by improvement in histological parameters (lobular lymphocytes, lobular and portal plasma cellular infiltrate, congestion, steatosis, hepatic cell enlargement, inflamed portal area, mast cells, sinusoidal distension, neutrophils and kuppfer cells). This study will help in development of new hepatoprotective formulation containing ZnSO4 and SM which will help to improve health of patients with various liver diseases and hepatotoxic drugs damaging liver.

ETHICAL APPROVAL

Ethical approval of article was granted by the Ethics Committee of King Edward Medical University, Lahore

AUTHOR'S CONTRIBUTIONS

WS: Conceived idea, design, manuscript writing
HP: Conceptualization of research, data analysis
JF: Data collection, data analysis & interpretation
AEZ: Critical analysis, Manuscript writing
All Authors: Approval of the final version of the manuscript to be published

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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