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Original Research Article

Histopathological and biochemical effect of Liv-52 on rifampicin and isoniazid induced liver toxicity in adult albino rats

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ABSTRACT

Background: Rifampicin and Isoniazid are two main medicinal drugs used as regimen in the treatment of Tuberculosis. These drugs induce hepatotoxicity. Liv-52, a polyherbal formulation has been shown to have clinical use in the treatment of liver disorders. The aim of this study was to investigate the histopathological and biochemical effects of Liv-52 on INH and RIF induced hepatotoxicity.
Methods: Adult albino rats weighing 150g to 250g were used. A total of 24 rats were randomly assigned into 4 groups of 6 rats each. Group 1 served as negative control. Hepatotoxicity was achieved by administering 50 mg/kg/day of RIF and INH each as positive control. Hepatoprotective effect was determined by administering Liv-52 concurrently with positive control. Low dose Liv-52 and high dose Liv-52 was administered at (155 mg/kg/day and 207 mg/kg/day) respectively, concurrently with RIF and INH at (50 mg/kg) each orally daily. After 21 days, the albino rats were sacrificed humanely, liver harvested and blood samples taken for estimation of liver serum biomarkers. The livers were processed and stained with Haematoxylin and Eosin for histological examination. Significance levels of ($p \leq 0.05$).
Results: The three selected liver biochemical parameters (ALT, AST and ALP) significantly increased in positive control group relative to negative control. The hepatoprotective groups (especially the HD Liv-52 group) showed significant reduction in the biochemical parameters. The liver histopathological results confirmed the above findings.
Conclusion: High dose Liv-52 significantly prevents hepatotoxicity induced by antitubercular therapy by inhibiting rise liver biochemical parameters and also ameliorating the deranged liver histomorphological features.

Keywords: Hepatotoxicity, Hepatoprotective, Histopathology, Isoniazid, Rifampicin, Deranged

INTRODUCTION

Hepatotoxicity refers to injury or harm to the liver attributed to xenobiotic such as drugs, alcohol, food additives fungal toxins, environmental toxicants and radioactive isotopes.[1,2] The liver plays an essential role in detoxification, metabolism and also maintenance of hemostasis.[3] Anti-tubercular drugs such as rifampicin (RIF) and isoniazid (INH) are among the drugs contributing to liver toxicity.[4] This attributed to its long duration of therapy, 6 months.[5] INH is an anti-mycobacterial drug which has been applied clinically for about 70 years and is still currently being used for TB treatment. It is a bactericide that inhibits the mycolic acids formation in the bacterial cell wall. In a meta-analysis, it has been shown that RIF increases the chances of hepatotoxicity 1.6% to 2.55%.[6] Upon administration of INH, it is metabolized into a biologically active compound by a bacterial catalase-peroxidase enzyme. This prevents mycolic acid formation that is essential in bacterial cell wall synthesis.[7] Hydarazine, a metabolite of INH, is highly associated with hepatotoxicity and

potentially fatal injury to the liver. Furthermore, INH has been shown to induce hepatotoxicity via generation reactive oxygen species leading to oxidative stress.[8] Rifampicin may potentiate Isoniazid metabolism acting as a strong enzyme inducer leading to rise in acehydrazide causing hepatocellular injury.[9,10] The ability to induce liver injury and even hepatic failure in the long run, during INH and RIF's therapy forms a major threat.[11] Despite of hepatotoxicity caused by these drugs, they are still first line regimen in the treatment of TB because of their high level of efficacy. The duration of manifestation of hepatotoxicity ranges between 1-25 weeks with an average of 12 weeks. Increase in liver biomarkers has been shown to occur as early as first seven days and as late as 9th month.[12,13]

Hepatoprotective activity refers to as liver protection from Hepatotoxins.[8] Herbs play major role in management of various disorders of the liver.[14] Medicinal plants are vital alternative complimentary sources for hepatoprotective agents, and their safety and efficacy have been shown against hepatotoxicity. In relation to the scarcity of reliable liver-protective drugs in modern medicine, hepatoprotective drugs obtained from plants seem to have attractive alternatives.[13] Liv-52 is a manufactured polyherbal formulation by Himalaya Drug Company commonly used for the diagnosis or treatment of various liver disorders. These herbs provide vital hepatoprotective effects by preventing the increase of lipid peroxidation and the reduction of antioxidants. These herbs have a vast medicinal application ranging from restoring the metabolic function of the liver in several etiological forms of jaundice such as infective and chronic active hepatitis to drug-induced hepatitis and alcohol induced hepatic damage.[2] The aim of this study aims to evaluate the hepatoprotective effect of Liv-52 which may help prevent liver toxicity caused by anti-tubercular drugs in the treatment of TB. This will facilitate compliance to the medication hence help eradication the chronic infection.

METHODS

Study design, duration and location

Posttest-only true experimental study design was conducted at the study was conducted in Maseno University, Kenya from April 2022 to May 2023.

Inclusion and exclusion criteria

All healthy albino rats in the cage, animals with average weight of 150-250g and animals between 6-8 weeks of age were included in this study. Sick animals in the cage were excluded.

Experimental animals

Adult albino rats were bred in a condition which is microbiologically controlled for all the experimental and control groups. They were obtained from University of Nairobi. The albino rats were bred in cages which hold a maximum of 6 rats per cage. The rats were acclimatized for 1 week in an animal house (26±2°C) with 12h light and dark cycles in the animal house in zoology department, Maseno University. Animal feeds were obtained from Unga Feeds in Kisumu Town. The animals were fed with standard rodent pellets and water provided ad libitum. The albino rats were fed each morning at 0800 hours in their spacious polycarbonate cages. The study research license was obtained from National commission for science technology and innovation (NACOSTI).

Drugs and chemicals

Rifampicin and Isoniazid were obtained from Yala sub-county Referral Hospital as a single tablet (Batch No. NRT2103A). The Liv-52 was purchased from Western Cosmetics. They were converted to animal equivalent dosage and administered for 21 days.

Grouping of the animals and drug administration

A total of 34 rats were randomly assigned into 6 groups each group containing 6 rats. Group I (control) was given food and water only. Group II was treated with INH (50 mg/kg/day) and RIF (50 mg/kg/day) orally. Group III was treated with INH (50 mg/kg/day), RIF (50 mg/kg/day) and low dose (LD Liv-52) at 155 mg/kg/day orally. Group IV was treated with INH (50 mg/kg/day), RIF (50 mg/kg/day) and high dose (HD Liv-52) at 207 mg/kg/day orally. At the end of the experiment, after 21 days, the albino rats were sacrificed humanely, and liver harvested.

Biochemical analysis

Blood samples (3 ml) were obtained from the posterior venacava. The blood samples were centrifuged at 3000 rpm for 10 minutes, sera collected and analyzed using ELISA kits.

Microscopic and photographic examination of histological section

H&E Staining. The right lobe of each liver (5×5×3 mm) was fixed with 4% formaldehyde solution and embedded in paraffin. Next, tissue slices were prepared and stained with hematoxylin for 5 mins, differentiated by exposure to hydrochloric acid alcohol solution for 20 s, and then exposed to a weak ammonia solution for 20 s. After staining with eosin, the slices were dehydrated and made transparent. Finally, the pathological characteristics of the liver tissues were observed under a microscope (Olympus BP). The microscopic examination of the tissue sections prepared from the samples prepared for the study was carried out using an imaging light microscope and then photographs of the sections were taken using a compound microscope equipped with a Novel type camera.

Statistical analysis

The data was entered into excel sheet and then analysis done through SPPS version 25 (IBM). The results are expressed as a mean value ±standard error of mean (SEM). One-way ANOVA with post hoc Bonferroni was used to compare the data obtained from experimental and control groups. Significance levels was p value less than or equal to 0.05 ($p \leq 0.05$) at 95% confidence level.

RESULTS

Liv-52 changes in liver bio-chemical parameters (ALT, AST and ALP) against RIF and INH induced hepatotoxicity

This current study selected three (ALT, AST and ALP) liver biochemical indicators to assess the hepatoprotective of LD Liv-52 and HD Liv-52. In the positive control (50kg/kgbwt) group, it was observed increase in ALT, AST and ALP above the normal ranges. There was statistically significant difference ($p \leq 0.0001$) in positive control compared to negative control (Table 1). There was a slight significant ($p \leq 0.05$) reduction in ALT, AST and ALP in the LD Liv-52 group compared to the positive control group (Table 1). There was a highly significant ($p \leq 0.0001$) reduction in ALT, AST and ALP in the LD Liv-52 group compared to the positive control group (Table 1).

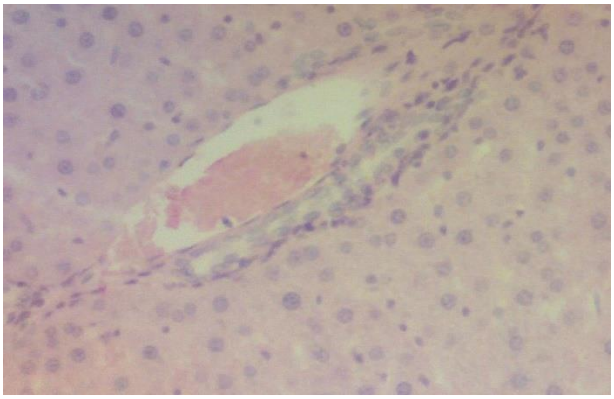

Figure 1: Liver section from negative control, normal histoarchitecture, H & E Mag: X100.

Histopathological changes

Liver sections from negative control group (water+food) showed normal liver histological features. There was no derangement observed, H & E. Mag: X100 (Figure 1). Liver sections treated with 50mg/kg of Rifampicin and Isoniazid showed deranged histomorphological features: areas of necrosis, dilated sinusoid, disrupted central veins, H & E. Mag: X100 (Figure 2).

This was slightly different from liver sections treated with low dose Liv-52 (155 mg/kg) group which showed slight derangement, in that there were minimal areas of necrosis, central veins were minimally disruption and had moderately dilated sinusoids, H & E. Mag: X100 (Figure 3).

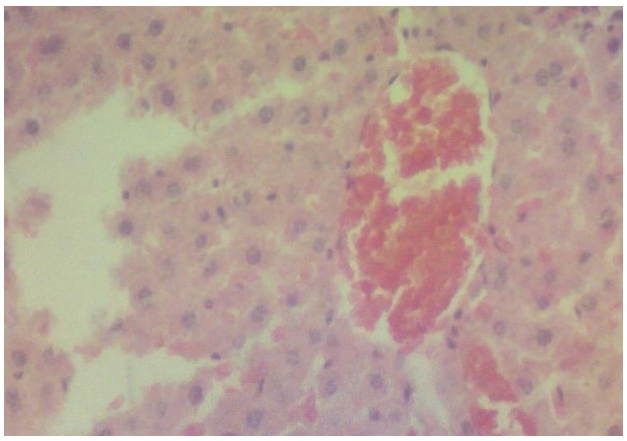

Figure 2: Liver section from positive control, RIF & INH (50 mg/kg). Showing dilated hepatic sinusoids, portal triaditis, disrupted central vein, H & E. Mag: X100.

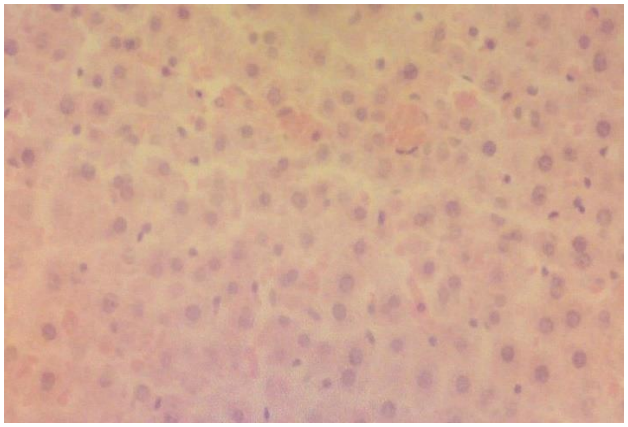

Figure 3: Liver section from LD Liv-52 (155 mg/kg) showing mild to moderate dilated sinusoids, focal necrosis, portal triditis, inflammed cells, H & E. Mag: X100.

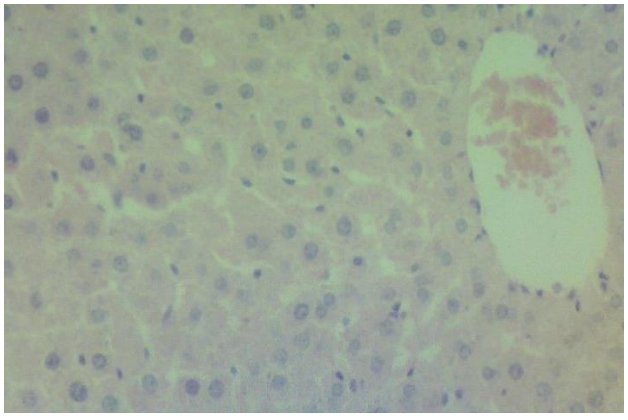

Figure 4: Liver section from HD Liv-52 (207 mg/kg) showing no specific changes, H & E. Mag: X100.

The histomorphological features in liver sections treated with HD Liv-52 (207 mg/kg) appeared normal in that there was no histological derangement from the negative control group, H & E. Mag: X100 (Figure 4).

Table 1: Effects of Liv-52 on co-administration of Liv-52, rifampicin and isoniazid.

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)
Negative control (water+food)	23.1±3.2	83.8±9.2	74.67±5.7
Positive control (50 mg/kg)	42.0±1.9*	153.0±2.1*	131.1±6.3*
LD Liv-52 (155 mg/kg)	29.8±1.4**	80.9±0.9**	128.0±1.4**
HD Liv-52 (207 mg/kg)	24.5±0.9***	70.4±1.8***	117.3±1.8***

All values are expressed and presented as the mean±SEM; N=6. Data analyzed by ANOVA followed by post hoc Bonferroni test. *p<0.0001 vs. negative control; **p<0.05 vs. positive control; ***p<0.0001 vs. positive control, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP).

DISCUSSION

Liver plays an essential role in metabolism and detoxification and is susceptible to injury.[15] The presents study evaluates the efficacy of Liv-52, a polyherbal formulation on hepatotoxicity induced by RIF and INH. Hepatotoxicity induced by RIF and INH is attributed to be due to oxidative stress-induced hepatic injury.[16] Monitoring of liver serum biomarkers is vital in RIF INH therapy.[17] These enzymes are usually released into the blood stream after the cell membrane has been damaged.[15] The serum levels of AST, ALP and AST were observed to decrease upon co-administration of low and high dosage of Liv-52 on RIF and INH induced toxicity. This may be attributed to the protective effect of Liv-52 which prevents damage to membrane integrity of the hepatocytes.[14,18] This protective effect has also been demonstrated in various studies.[19, 20] Although the exact mechanism of Liv-52 on liver serum biomarkers is not known, it is thought to contain active medicinal herbs i.e. Capparis spinosa 32 mg, Mandur bhasma 32 mg, Cichorium intybus 32 mg, Solanum nigrum 32 mg, Cassia occidentalis 16 mg, Terminalia arjuna 32 mg, Tamarix gallica 16 mg and Achillea millefolium 16 mg, which influence the liver functions at the cellular level. These herbs contain anti-inflammatory and anti-oxidant properties.[21] Liver sections in the current study showed significant difference in the histological features between control and experimental groups (Figure 1-4). The liver sections in the group treated with RIF and INH showed disrupted central veins, dilated sinusoids, and inflammation of the portal triad (Figure 2).[22,23,15,16] Although the findings in the current study didn't depict fatty changes in the liver sections, a study by Shabbir et al on antitubercular induced hepatotoxicity showed fatty changes and vacuolations.[24] Studies have attributed the hepatoxicity induced by Isoniazid to secretion of toxic component, hydrazine which causes hepatocyte injury.[25] However, derangement of the histological features was prevented by administration of Liv-52 on the Rifampicin and Isoniazid induced hepatotoxicity treated mice, having a dose-dependent difference between LD and HD Liv-52 groups (Figure 3-4). High dose of Liv-52 (207 mg/kg) provided more effect relative to low dose Liv-52 (155 mg/kg). This may be attributed to modulation of oxidative stress and oxidative enzyme augmentation.[19,20]

Limitations

Some of the blood sample harvested from the rats clotted before centrifugation process hence couldn't be processed for liver biochemical parameter analysis.

CONCLUSION

The present study demonstrate that Liv-52 may have significant protective effect against Rifampicin and Isoniazid induced liver hepatotoxicity. This is due to the presence of anti-oxidant, anti-inflammatory and immunomodulating properties which maintain the integrity of the hepatocyte functioning. The observations in liver biochemical parameters are consistent with the results from histology of the liver, indicating that liv-52 has the capability of structural integrity of the hepatocytes. The study recommends future investigations to explore the pharmacokinetics and pharmacodynamics of Liv-52 in human tissue and samples, so that these findings can be translated to clinical applications.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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