

Gross Morphometric Effects of Liv-52 on Rifampicin and Isoniazid Induced Hepatotoxicity Among Albino Rats

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ABSTRACT

Background: Gross morphometric parameters of the liver entails weight, width and height. Hepatotoxicity is an injury to the liver due to chemicals or environmental toxins. Rifampicin (RIF) and Isoniazid (INH) are main medicines used in treatment of Tuberculosis and they induce hepatotoxicity. Liv-52 is polyherbal formulation having clinical use in treatment of liver disorders.

Methods: Posttest-only experimental study design was adopted. 24 albino rats between 150g to 250g were randomly allocated into 4 groups of 6 rats each. A negative control group (no intervention). Positive control group; INH 50mg/kgbw and RIF 50mg/kgbw. Low dose Liv-52 group of INH 50mg/kgbw, RIF 50mg/kgbw and Liv-52 155mg/kgbw. High dose Liv-52 group; INH 50mg/kgbw, RIF 50mg/kgbw and Liv-52 207mg/kgbw. After 21 days, the albino rats were sacrificed humanely, liver removed, weighed and the gross morphometric measurements done using a caliper and ruler. The results were presented in tables and figures.

Results: The morphometric measurements recorded a significant increase ($p < 0.0001$) in high dose Liv-52 group compared to positive control group, while low dose Liv-52 recorded slight increment.

Conclusion: The gross morphometric changes on the albino rats indicate Liv-52 prevents hepatotoxicity due to rifampicin and isoniazid therefore, should be administered concurrently to prevent hepatotoxicity.

Keywords: Hepatotoxicity; Morphometric; Liv-52; Albino rats; Liver disorders.

1. Introduction

Hepatotoxicity refers to as harm to the liver or disruption of hepatic function induced by exposure to xenobiotic such as alcohol, drugs, chlorinated solvents, food additives, peroxidized fatty acids, fungal toxins, environmental toxicants and radioactive isotopes [1]. The liver is the major organ for drug elimination and metabolism [2]. Anti-tubercular drugs such as rifampicin (RIF) and isoniazid (INH), Nonsteroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, paracetamol, some anti-cancer drugs and general anesthetic drugs are among the drugs contributing to liver toxicity [3]. Rifampicin (RIF) and Isoniazid (INH) are the two main drugs being used to treat Tuberculosis (TB) for 4-6 months [3]. 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 prevents the mycolic acids formation in the cell wall of bacterial [4]. The ability to cause liver damage and even eventual hepatic failure during INH and RIF's management forms a major challenge [5].

Despite of hepatotoxicity caused by these drugs, they are still first line regimens in the treatment of TB because of their high level of efficacy. The duration of manifestation of liver toxicity varies between 1-25 weeks with an average of 12 weeks. The level of liver toxicity is high in cases of other risk factors such: high alcohol consumption, HIV/AIDS [6]. Additionally, the liver is prone to several diseases including hepatitis, allergic reactions, hepatic encephalopathy and non-alcoholic fatty liver disease among others [1]. Hepatoprotective activity refers to as protecting the liver from the harmful effects of Hepatotoxins or counteracting the alterations in

the antiradical defense mechanisms [7]. Plant extracts can be the best source of such antioxidants and mediate hepatoprotective activity. Several chemical constituents such as coumarins, phenols, monoterpenes, alkaloids glycosides, and xanthenes are found in liver-protective plants [8]. Medicinal plants are vital alternative complimentary sources for hepatoprotective agents, and their safety and efficacy have been shown against liver toxicity [9]. In relation to the scarcity of reliable liver-protective drugs in modern medicine, hepatoprotective drugs obtained from plants seem to have attractive alternatives [6]. Liv-52 is a manufactured polyherbal formulation by Himalaya Drug Company commonly used for the diagnosis or treatment of various liver disorders. The aim of this study was to come up with an affordable drug, with less adverse effect that will prevent liver toxicity caused by anti-tubercular drugs in the treatment of TB. This will facilitate compliance to the medication hence help eradicate the chronic infection.

2. Material and Methods

This was a posttest-only true experimental study design that employed the use of albino rats between 150-250g. They were acclimatized for one week through feeding on standard rodent pellets and water ad libitum. A total of 24 rats were sampled and randomly assigned into 4 treatment groups. Ethical approval was obtained from the relevant sources. Procurement of drugs was done; Isoniazid, Rifampicin and Liv-52. Simple random sampling was used to assign the albino rats to their respective groups where they were fed with water and rat pellets ad-libitum. Weighing of the animals was done between 07:00 hours and 08:00 hours. The drugs were converted into animal equivalent dosage and dissolved in water for injection before administration between 08:00 hours and 09:00 hours. The negative control did not receive any treatment. Positive control group received Isoniazid and Rifampicin only (INH 50mg/kgbw; RIF 50mg/kgbw). LD Liv-52 group received Isoniazid Rifampicin and low dose Liv-52 (INH 50mg/kgbw, RIF 50mg/kgbw and LD Liv-52 155mg/kgbw). HD Liv-52 group received Isoniazid, Rifampicin and high dose Liv-52 (INH 50mg/kgbw, RIF 50mg/kgbw and HD Liv-52 207mg/kgbw). All the groups were sacrificed humanely after 21 days of treatment. The length, width and thickness were obtained by using caliper and ruler.

2.1. Statistical Analysis

The data was entered into excel sheet and then analysis done through SPSS version 25 (IBM). 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.

3. Results

The liver gross morphometric measurements entail weight, length, width and thickness. In this study, there was no gross pathological changes observed on the liver between the control and experimental groups. The liver was observed to have four lobes, dark brown in colour with a smooth texture. A decrease (184.78 ± 0.78) in mean final body weight was observed in positive control group (RIF 50mg/kgbw), compared to 245.39 ± 0.57 in negative control group (Food+water). Similarly, there was a decrease (9.197 ± 0.26) in mean liver weight in positive control group as compared to negative control (11.86 ± 0.20 respectively). A statistical significant difference ($p \leq 0.0001$) was observed in positive control group compared to negative control group.

In the hepatoprotective group, there was a significant ($p \leq 0.0001$) increase in mean final body weight in HD Liv-52 (207mg/kgbw) and slight increase in LD Liv-52 (155mg/kgbw) groups at 236.31 ± 63 and 208.33 ± 83 respectively compared to 184.78 ± 78 in positive control (RIIH 50kg/kgbw) group. Additionally, the mean liver weight in HD Liv-52 group was significantly ($p \leq 0.0001$) higher at 11.40 ± 21 compared to 9.197 ± 26 in positive control group (table 1).

The means of the liver gross morphometric measures were observed to be increasing in both LD Liv-52 (155mg/kgbw) and HD Liv-52 (207mg/kgbw) hepatoprotective groups as compared to positive control (RIIH 50kg/kgbw) group. That is length of 50.82 ± 23 , width of 41.86 ± 23 and thickness of 0.39 ± 01 in LD Liv-52 and length of 54.25 ± 24 , width of 44.68 ± 17 and thickness of 0.50 ± 00 in HD Liv-52 (table 1). There was statistically significant difference ($p \leq 0.0001$) in HD Liv-52 when compared to positive control group.

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Figure 1. Gross appearance of the liver showing four lobes and dark brown in colour

Table 1. Gross morphometric measurements between control and experimental groups

Gross Parameters	Animal Group				df	F	Sig.
	-VE control (Food+ water)	+VE control (RIIH 50mg/ Kg/bwt)	LD Liv-52 (155mg/ kg/bwt)	LD Liv-52 (207mg/ kg/bwt)			
MFBW (grams)	245.39 ± 57	184.78 ± 78	$208.33 \pm 83^{**}$	$236.31 \pm 63^{**}$	3	1500.181	0.0001
MLW (grams)	11.86 ± 20	9.197 ± 26	8.748 ± 38	$11.40 \pm 21^{**}$	3	37.841	0.0001
Length (cm)	57.00 ± 53	44.90 ± 40	$50.82 \pm 23^{**}$	$54.25 \pm 24^{**}$	3	0.042	0.0001
Width (cm)	45.20 ± 33	36.31 ± 19	$41.86 \pm 23^{**}$	$44.68 \pm 17^{**}$	3	0.148	0.0001

Thickness (cm)	0.53±.02	0.35±.01	0.39±.01	0.50±00**	3	0.064	0.0001
Lobes	6	6	6	6			

Key: All values are expressed and presented as the mean± the standard error of the mean (SEM); n=6. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni. Asterisks** represents significant ($p < 0.0001$), RIIH-Rifampicin and Isoniazid Induced Hepatotoxicity, +VE- Positive, -VE- Negative, HD- High Dose, LD- Low Dose, MFBW=Mean final body weight, MLW= Mean liver weight, df-degree of freedom, F- ANOVA value.



Figure 2. Liver gross morphometric measurements

4. Discussions

In this current study, the liver gross appearance was dark brown in colour with a smooth texture after administration of rifampicin and isoniazid. These findings are in line with [10] who also observed similar features, which were attributed to the histological effects of Isoniazid-induced liver toxicity on albino mice. The dark brown pigmentation on the liver could be attributed to damage to liver parenchyma upon the introduction of rifampicin and isoniazid [9,11]. Moreover, [9] observed that besides the change in the pigmentation, a significant (p value < 0.0001) decrease in liver weight and mean terminal body weight have been recorded when antitubercular is used to induce hepatotoxicity among Albino rats. The observation by [9] was consistent with the present study findings

which recorded a decrease ($184.78 \pm .78$) in mean final body weight in positive control (RIIH 50kg/kgbw) group, compared to $245.39 \pm .57$ in negative control (food and water ad-libitum) group.

Similarly, there was a decrease ($9.197 \pm .26$) in mean liver weight in positive control (RIIH 50kg/kgbw) group as compared to negative (food and water ad-libitum) control ($11.86 \pm .20$ respectively). The current study also recorded significant (p value < 0.0001) decrease in the length, width and thickness of the liver. These changes in the length, width, thickness of the liver and terminal body weight could be attributed to hepatotoxic injury to liver parenchyma induced by rifampicin and isoniazid. Similarly, [11] observed a significant (p value < 0.05) decrease in liver weight upon introduction of high dose paracetamol to albino rats.

This study observed that, there was a significant ($p < 0.0001$) increase in mean final body weight in high dose Liv-52 and a slight increase in low dose Liv-52 groups at $236.31 \pm .63$ and $208.33 \pm .83$ respectively compared to $184.78 \pm .78$ in positive control group. The mean liver weight in HD Liv-52 group was significantly (< 0.0001) higher at $11.40 \pm .21$ compared to $9.197 \pm .26$ in positive control group. The increase of measurements in hepatoprotective groups could be due to the hepatoprotective effect of Liv-52 which inhibits oxidation and lipid peroxidation. Similar findings were observed by [11,12] who showed significant ($P < 0.05$) decrease in liver morphometric findings when he introduced different dosage of Liv-52 upon paracetamol-induced liver toxicity.

5. Conclusion

In conclusion, the changes in the liver gross morphometric measurements indicate that Liv-52 was able to prevent liver injury induced by Rifampicin and Isoniazid therapy. There is need to clinically evaluate the pharmacodynamics and pharmacokinetics of high dose Liv-52 in human patients with liver damage attributed to Isoniazid and Rifampicin.

Declarations

Source of Funding

This study did not receive any grant from funding agencies in the public or not-for-profit sectors.

Competing Interests Statement

The authors have declared no competing interests.

Consent for Publication

The authors declare that they consented to the publication of this study.

Ethical Approval

Ethical approval has been obtained from the relevant sources.

References

[1] Gulati, K., Reshi, M.R., Rai, N., & Ray, A. (2018). Hepatotoxicity: Its mechanisms, experimental evaluation and protective strategies. *Am J Pharmacol.*, 1(1): 1004.

- [2] Chojjamts, G., Batsyren, C., Otgontyr, et al. (2018). Role of Liv. 52 DS Tablets as a Hepatoprotective Agent in Tuberculosis Patients Receiving Antitubercular Drugs: A Double Blind Placebo Controlled Study.
- [3] Buabeid, M.A., Arafa, E.A., Rani, T., Ahmad, F.U.D., Ahmed, H., Hassan, W., & Murtaza, G. (2022). Effects of *Solanum lycopersicum* L. (tomato) against isoniazid and rifampicin induced hepatotoxicity in wistar albino rats. *Brazilian Journal of Biology - Revista Brasileira de Biologia*, 84: e254552.
- [4] Tayal, V., Kalra, B.S., Agarwal, S., Khurana, N., & Gupta, U. (2007). Hepatoprotective effect of tocopherol against isoniazid and rifampicin induced hepatotoxicity in albino rabbits.
- [5] Li, S., Tan, H. Y., Wang, N., et al. (2015). The Role of Oxidative Stress and Antioxidants in Liver Diseases. *International Journal of Molecular Sciences*, 16(11): 26087–26124. doi: 10.3390/ijms161125942.
- [6] Sankar M, Rajkumar J, Sridhar D. (2015). Hepatoprotective Activity of Heptoplus on Isoniazid and Rifampicin Induced Liver Damage in Rats. *Indian J Pharm Sci.*, 77(5): 556-62. doi: 10.4103/0250-474x.169028.
- [7] Wang, N., Li, P., Wang, Y., et al. (2008). Hepatoprotective effect of *Hypericum japonicum* extract and its fractions. *Journal of Ethnopharmacology*, 116(1): 1-6.
- [8] Bhawna, S., & Kumar, S.U. (2009). Hepatoprotective activity of some indigenous plants. *Int J Pharm Tech Res.*, 4: 1330-1334.
- [9] Shabbir, M., Afsar, T., Razak, S. et al. (2020). Phytochemical analysis and Evaluation of hepatoprotective effect of *Maytenus royleanus* leaves extract against anti-tuberculosis drug induced liver injury in mice. *Lipids Health Dis.*, 19: 46. doi: <https://doi.org/10.1186/s12944-020-01231-9>.
- [10] Humayun F, Tahir M, Lone KP. (2017). Histologic effects of isoniazid on the liver of albino mice. *Khyber Medical University Journal*, 9(2).
- [11] Rono K, Walter, Kweri J, Kariuk, Kibe G, Kafanya, Thuo Rueben, Kanyoni J, Mwangi (2019). Histological and Morphometric Effects of Liv 52 on Acetaminophen Induced Liver Toxicity in Adult Albino Rats. *IOSR Journal of Pharmacy And Biological Sciences*, 14(4): 44-51.
- [12] Girish C, Koner BC, Jayanthi S, Ramachandra Rao K, Rajesh B, Pradhan SC. (2009). Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. *Fundamental & Clinical Pharmacology*, 23(6): 735-45.