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Research Article

Hepatoprotective of *Eclipta Alba* Methanolic Extract in Isoniazid and Rifampicin Proved Oxidative Hepatic Injury

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ABSTRACT
The current study was undertaken to evaluate the preclinical efficacy of methanolic extract of *Eclipta alba* (MEEA) against isoniazid and rifampicin (INH-RIF) induced hepatic injury. Animals were randomly divided in five groups, vehicle (control) or INH-RIF (50 mg/kg, i.p.) or MEEA (200 and 400mg/kg, p.o.) or standard silymarin for 28 days. INH-RIF intoxicated rats displayed significant ($p<0.05$) elevation in serum hepatic markers, lipid peroxidation and decrease in antioxidants like SOD, CAT, Gpx and GSH in liver tissue. Treatment with MEEA (200 and 400mg/kg, p.o.) restored the altered biochemical level to normalcy. Thus, outcome of the study reveals that MEEA showed promising hepatoprotective activity in INH-RIF induced cardiac damage mediated by its membrane stabilizing and antioxidant effect.
Keywords: Eclipta alba, isoniazid, rifampicin, hepatotoxicity, oxidative stress, antioxidants

INTRODUCTION

Tuberculosis (TB) is a worldwide health problem with the highest prevalence rate in India and also imposes huge economic burden (Baskaran, U. L., & Sabina, E. P., 2017) & (Sotgiu, G.,2017). The pharmacotherapy for TB is generally known as ''directly observed treatment, short-course" (DOTS) which encompasses combination of drugs rifampicin (RIF), isoniazid (INH) and pyrazinamide and has a achievement rate of more than 80% approach (Darvin, S. S.,2018). However, the main draw back in the DOTS therapy is, upon chronic administration it causes hepatotoxicity and commonly referred as drug mediated hepatotoxicity (Abbara, A.,2017). Previous studies indicate that RIF and INH alone or in combination elicits hepatotoxicity in patients undergoing treatment (Baskaran, U. L., & Sabina, E. P., 2017). The mechanism of hepatotoxicity provoked by INH-RIF is still obscure, but studies indicate that oxidative stress might be the prime mechanism in INH-RIF (Mitchell, J. R.,1975). Further reports highlights that, Hydrazine (HYZ) a metabolite of INH is converted to toxic compound by CYP450, which leads to hepatotoxicity. RIF, aggravates hepatotoxicity by inducing CYP450, as a result more toxic metabolites are generated from hydrazine (Tostmann, A.,2008). In addition HYZ depletes the reserved glutathione (GSH) level in the liver, precluding to oxidative damage and cell toxicity (Sarich, T. C.,1998)&(Huang, YS, 2003). Mounting herbal plants are recommended for the management of drug induced liver injury and they exhibit significant protection to wide range of hepatotoxins (Singh, D.,2003).

Eclipta alba, is a weed of the family Asteraceae, present in many parts of India and thrives in moist places (Murthy, V.N.,1992). Previous reports show the preventive effect of Eclipta *alba* on CCl_4 provoked hepatotoxicity (Beedimani, R.S.,2015). In this scenario, the current study was conducted to delineate the hepatoprotective of methanolic extract of *Eclipta alba* on anti-tubercular drug isoniazid and rifampicin (INH-RIF) combination mediated liver damage.

MATERIALS AND METHODS

Drugs and Chemicals

Isoniazid, rifampicin and silymarin were procured from Sigma, USA. The other required reagents needed were of highest purity and analytical grade.

Plant material

The whole plant of *E. alba*was procured from the various gardens and nurseries of Palvancha, Bhadradri district, Telangana, India. The collected plant was authenticated by Dr. K.Madhava Chetty, Assistant Professor, Sri Venkateswara University, Chitoor district, Andhra Pradesh. Then, the plant materials were placed under shade for drying and powdered using a mill and stored in an air tight container.

Preparation of extract

Powdered plant material weighing about 250 g of *E. alba* was subjected to extraction using 1000 ml of methanol by simple maceration technique for 72 hours. Distillation was carried to obtain the concentrated extract, 1/4th of its original volume. The final yield obtained was 12% w/w.

Preliminary Phytochemical screening

The phytochemical analysis of methanol extract of *E. alba* reveals the presence of triterpenoids, flavonoids, steroids, tannins, saponins and alkaloids.

Animals

All animal studies were conducted as per the protocol of CPCSEA and Institutional Animal Ethical Committee (IAEC). CPCSEA Reg. No: 1641/PO/E/S/14/CPCSEA. The standard experimental protocols and procedures adopted in this biological evaluation were described below.

Acute toxicity studies

The acute toxicity studies were performed as per the OECD guideline No. 425 by using albino mice.

Isoniazid/ Rifampicin induced hepatotoxicity

Male Wistar rats weighing about 150 ± 10 gm were used in the study. Animals were procured from the Animal facility of, Browns College of Pharmacy, Kammam. The rats were fed with standard diet pellet with free access to water. The rats were restricted to foods before the start of experiment and given access to water. The animals were maintained at standard temperature of 25 ± 2 °C and were adapted to 12 hours light: 12 hours dark cycles.

Study protocol

The rats were divided into five groups, n=6,

Group 1: Normal control rats received vehicle 2% gum acacia suspension for 14 days. (1 ml/ kg b.wt.), p.o for 28 days

Group 2: Rats received INH and co-administered RIF (50 mg/kg; b.wt) p.o daily for 28 days

Group 3: Rats treated with silymarin (100mg/kg; b.wt) p.o for 28 days

Group 4: Rats received 200mg of methanolic extract of E.alba (MEEA) using a vehicle 2% gum acacia p.o for 28 days.

Group 5: Rats received 400mg of methanolic extract of E.alba (MEEA) using a vehicle 2% gum acacia p.o for 28 days.

Meanwhile, group 3-5 rats were treated with INH and co-administered RIF (50 mg/kg b.wt) p.o daily for 28 days, one hour after the drug treatment.

After the final doses of extract and INH-RIF, the access to food was restricted overnight and the animals were anaesthetized using phenobarbital sodium (35mg/kg) intraperitoneally and sacrificed by cervical decapitation. The blood was withdrawn from jugular vein in heparinized tubes and the serum was separated for the measurement of hepatic marker enzymes. The liver tissue was excised, cleaned from adherent tissues, washed in ice cold saline and dried. Then a 100mg weighed tissue was homogenized in cold Tris-HCl buffer (10% w/v) and used for the analyses of various biochemical markers in INH-RIF induced hepatic damage.

Analysis of hepatic markers

Serum biochemical parameters like alanine transaminases (ALT), asparate transaminases (AST), total protein (TP), albumin (ALB), total bilirubin (TB), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) were assayed by biochemical kits supplied by Span Diagnostics Ltd, Gujarat, India.

Estimation of Lipid peroxidation

The lipid peroxidation (LPO) marker, malondialdehyde (MDA) was measured according to the instructions provided in the kit procured from Span Diagnostics Ltd, Gujarat, India.

Estimation of antioxidants

The hepatic level of antioxidants catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) were estimated as per the instructions provided in the kit obtained from Span Diagnostics Ltd, Gujarat, India.

Statistical analysis

The data were represented as mean ± Standard error mean (SEM).The data were analysed by ANOVA followed Tukey's comparison using SPSS version 18.0. $p < 0.05$ was taken as statistically significant.

RESULTS

Acute oral toxicity of *E.alba*

Upon administration of extracts there was no mortality, unwanted clinical reactions, marked reduction in body weight or gross pathological changes seen in rats. LD50 of MEEA was higher than 2000 mg/kg.

Effect of MEEA administration on hepatic markers

In the present study, significant elevation of ($P \leq 0.05$) serum AST, ALT, ALP and LDH was observed, in animals treated with INH-RIF. Supplementation of MEEA (200 and 400 mg/kg)to the rats brought down the increase in serum transaminases, ALP and LDH to near normal levels. Increased levels of TB ($P \leq 0.05$) were observed in INH-RIF. However, treatment with MEEA at the dose of 200 and 400 mg/kg showed significant reduction of serum TB levels. TP and ALB are used to assay liver function. Significant decreases of serum TP ($P \leq 0.05$) and ALB ($P \leq 0.05$), were noticed in the rats in group II. Treatment with MEEA (200 and 400 mg/kg) enhanced the concentration of proteins and ALB. Serum GGT is one of the highly sensitive markers for liver function. Significant increase of serum GGT ($P \leq 0.05$) is observed in group II animals. Treatment with MEEA (200 and 400 mg/kg) showed significant decrease of serum GGT. The results were displayed in table 1 and table 2 respectively.

Effect of MEEA on hepatic lipid peroxidation and antioxidants

In the current study, there was a significant decrease of hepatic SOD, CST, GPx and GSH with a concaminant increase of MDA in rats intoxicated with INH-RIF ($p<0.05$). However, treatment with MEEA (200 and 400 mg/kg) completely brings back the normal levels of these hepatic antioxidants and significantly reduced the hepatic MDA formation (Table 3).

DISCUSSION

The current study was carried to delineate the hepatoprotective potential of *Eclipta alba* methanolic extract on oxidative assault provoked by anti-tubercular drugs isoniazid and rifampicin (INH-RIF) in a murine model. Oxidative damage is the main cause of hepatocellular injury elicited by INH-RIF. The biotransformation of INH leads to the generation of acetyl onium ion which is highly reactive, ketene and acetyl radical and these radicals covalently bind with macromolecules present in liver leading to hepatic damage (Ramappa, V.,2013). Further, it has been shown that Rifampicin actively induces many enzymes involved in the metabolism like cytochrome P450 (CYP3A4) through PXR located in the hepatocytes. Thus CYP3A4activation preludes to rampant metabolism of isoniazid and releases noxious metabolites which in turn accelerates the metabolism of rifampicin and causes hepatotoxicity (Nannelli, A., 2008). During hepatic damage, membrane integrity of hepatic cytoplasmic membrane is damaged as a result of lipid peroxidation generated by free radical from INH-RIF metabolism (Jadhav, V.B., 2010). Due to the distortion of hepatocytes membrane the hepatic markers enzymes present inside are released into the blood stream, which is an indicative of hepatic damage (Baniasadi, S., 2010). In this study, INH-RIF intoxicated rats displayed significant increase in the serum level of AST, ALT, ALP, GGT and LDH. Treatment with MEEA significantly restored the altered liver marker enzymes to normal and thus prevented the hepatic membrane damage which is line with previous report (Indhuleka, A., & Jeyaraj, M., 2019). Total bilirubin is a vital marker for the diagnosis of hepatic injury. Elevated serum bilirubin levels in the event of hepatotoxicity might be due to reduced hepatic clearance of bile, which leads to hepatitis (Ramaiah, S.K., 2007). In our study MEEA treatment significantly reduced the total bilirubin to normal which is in corroboration with earlier reports (Indhuleka, A., & Jeyaraj, M., 2019).

During hepatic damage, protein like albumin level is decreased due to the inability of the liver to synthesize these biomolecules. In this study, INH-RIF intoxicated rats displayed reduced level of serum protein and albumin which is in corroboration with earlier reports (Marasani, A., 2014). Meanwhile treatment with MEEA significantly increased the total bilirubin to normal which is in corroboration with earlier reports (Kumar, K., 2013).

The INH-RIF provoked hepatic injury leads to reduction in antioxidant protective mechanism due to the generation of highly reactive toxic metabolites which preludes to lipid peroxidation and depletion of glutathione stores (Saad, E. I., 2010). In our study, the MDA, a prominent index of lipid peroxidation, is effectively increased in hepatic tissue of rats intoxicated with INH-RIF. However treatment with MEEA effectively reduced the MDA by inhibition the chain termination of lipid peroxidation process (Unnikrishnan,K.P., 2007). Studies have shown that LPO process further decreases the GSH level. Hydrazine, a prime toxic metabolite INH decreases the GSH level by binding to its sulfhydryl group and thus generates noxious free radicals (Chowdhury, A., 2006). In our study, INH-RIF intoxicated rats displayed decreased GSH level as a result of lipid peroxidation induced by anti-tubercular drugs. MEEA treatment significantly increased the GSH and thus restores the antioxidant

defense system (Indhuleka, A., & Jeyaraj, M. (2019). Further, the antioxidant enzymes SOD, CAT, and GPx are significantly decreased in rats insulted with INH-RIF and MEEA treatment effectively increased the antioxidant levels to normal. Thus the hepatoprotective activity rendered by *E.alba*in the present study might be due to the presence of various phytoconstituents like wedelolactone, Eclalbasaponins, α and β-amyrin, Oleanolic and ursolic acids which possess significant antioxidant and free radical scavenging properties (Jahan, R., 2014).

CONCLUSION

On the basis of our findings, methanolic extract of *E.alba* may improve the INH-RIF induced hepatotoxicity by regulating the hepatic marker enzymes, inhibition of lipid peroxidation, improving the status of antioxidants.

ETHICAL CLEARANCE NUMBER: CPCSEA Reg. No: 1641/PO/E/S/14/CPCSEA

SOURCE OF FUNDING: self

CONFLICT OF INTEREST: To find hepatoprotective effect in Eclipta alba against drug induced hepatic injury.

REFERENCES

1. Baskaran, U. L., & Sabina, E. P. (2017). Clinical and experimental research in antituberculosis drug-induced hepatotoxicity: a review. *Journal of Integrative Medicine*, 15(1), 27–36. DOI: 10.1016/S2095-4964(17)60319-4.
2. Sotgiu, G., Sulis, G., & Matteelli, A. (2017). Tuberculosis—a World Health Organization Perspective. *Microbiology Spectrum*, 5(1). DOI:10.1128/microbiolspec.TNMI7-0036-2016
3. Darvin, S. S., Esakkimuthu, S., Toppo, E., Balakrishna, K., Paulraj, M. G., Pandikumar, P et al. (2018). Hepatoprotective effect of lawsone on rifampicin-isoniazid induced hepatotoxicity in in vitro and in vivo models. *Environmental Toxicology and Pharmacology*, 61, 87–94. DOI:10.1016/j.etab.2018.05.006.Epub 2018 May 15.
4. Abbara, A., Chitty, S., Roe, J. K., Ghani, R., Collin, S. M., Ritchie, A et al. (2017). Drug-induced liver injury from antituberculous treatment: a retrospective study from a large TB centre in the UK. *BMC Infectious Diseases*, 17(1), 231. DOI: 10.1186/s12879-017-2330-z.
5. Mitchell, J. R., Thorgeirsson, U. P., Black, M., Timbrell, J. A., Snodgrass, W. R., Potter, W.Z et al. (1975). Increased incidence of isoniazid hepatitis in rapid acetylators: possible relation to hydrazine metabolites. *Clinical Pharmacology & Therapeutics*, 18(1), 70–79. DOI:org/10.1002/cpt197518170.
6. Tostmann, A., Boeree, M. J., Peters, W. H., Roelofs, H. M., Aarnoutse, R. E., Ven, A. J. V. D., et al. (2008). Isoniazid and its toxic metabolite hydrazine induce in vitro pyrazinamide toxicity. *International Journal of Antimicrobial Agents*, 31(6), 577–580. DOI: 10.1016/j.ijantimicag.2008.01.022. Epub 2008 Mar 21.
7. Sarich, T. C., Adams, S. P., & Wright, J. M. (1998). The Role of L-Thyroxine and Hepatic Reductase Activity in Isoniazid-Induced Hepatotoxicity in Rabbits. *Pharmacological Research*, 38(3), 199–207. DOI: 10.1006/phrs.1998.0347.
8. Huang, YS., Chern,HD., Su,WJ., et al. (2003). Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis. *Hepatology*, 37(4), 924–930. DOI: 10.1053/ijep.2003.50144.
9. Singh, D., Cho, W. C., & Upadhyay, G. (2016). Drug-Induced Liver Toxicity and Prevention by Herbal Antioxidants: An Overview. *Frontiers in Physiology*, 6, 363. DOI: 10.3389/fphys.2015.00363. eCollection 2015.
10. Murthy, V.N., Reddy, B.P., Venkateshwarlu, V., Kokate, C.K. (1992). Antihepatotoxic activity of eclipta alba, tephrosia purpurea and boerhaavia diffusa. *Ancient Science Life*, 11(3-4), 182-186.
11. Beedimani, R.S., Shetkar, S. (2015). Hepatoprotective activity of Eclipta alba against carbon tetrachloride-induced hepatotoxicity in albino rats. *International Journal of Basic & Clinical Pharmacology*, 4 (3), 404-409. DOI:10.18203/2319-2003.ijbcp20150045.
12. Ramappa, V., Aithal, G.P. (2013). Hepatotoxicity Related to Anti-tuberculosis Drugs: Mechanisms and Management. *Journal of Clinical and Experimental Hepatology*, 3(1),37–49. DOI: 10.1016/j.jceh.2012.12.001. Epub 2012 Dec 20.
13. Nannelli, A., Chirulli, V., Longo, V. (2008). Expression and induction by rifampicin of CAR- and PXR-regulated CYP2B and CYP3A in liver, kidney and airways of pig. *Toxicology*, 252 (1-3), 105–112. DOI: 10.1016/j.tox.2008.08.004. Epub 2008 Aug 22.
14. Jadhav, V.B., Thakare, V.N., Suralkar, A.A., Deshpande, A.D., Naik. S.R.(2010). Hepatoprotective activity of Luffa acutangula against CCl4 and rifampicin induced liver toxicity in rats: A biochemical and histopathological evaluation. *Indian Journal of Experimental Biology*, 48 (8), 822-829.
15. Baniasadi, S., Eftekhari, P., Tabarsi. P. (2010). Protective effect of N-acetylcysteine on antituberculosis drug-induced hepatotoxicity. *European Journal of Gastroenterology & Hepatology*, 22(10):1235–1238. DOI: 10.1097/MEG.0b013e32833aa11b.

16. Indhuleka, A., & Jeyaraj, M. (2019). Hepatoprotective Effect of Eclipta Alba on Membrane Marker Enzymes against Paracetamol Induced Liver Damage. *Oriental Journal of Chemistry*, 35(3), 1215–1219. DOI: 10.13005/ojc/350343.
17. Ramaiah, S.K. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*, 45(9), 1551-1557. DOI:10.1016/j.fct.2007.06.007.
18. Marasani, A. (2014). Hepatoprotective activity of Bauhinia variegata against Isonized and Rifampicin-induced toxicity in experimental rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6 (4), 177-181.
19. Kumar, K., Katiyar, A. K., Swamy, M., Sahni, Y. P., Kumar, S. (2013). Hepatoprotective effect of Eclipta alba on experimentally induced liver damage in rats. *Indian Journal of Veterinary Pathology*, 37 (2), 159–163.
20. Saad, E. I., El-Gowilly, S. M., Sherhaa, M. O., & Bistawroos, A. E. (2010). Role of oxidative stress and nitric oxide in the protective effects of α-lipoic acid and aminoguanidine against isoniazid–rifampicin-induced hepatotoxicity in rats. *Food and Chemical Toxicology*, 48(7), 1869–1875. DOI: 10.1016/j.fct.2010.04.026.
21. Unnikrishnan,K.P., Anu Fathima., Hashim, K.M., Indira Balachandran. (2007). Antioxidant Studies and Determination of Wedelolactone in Eclipta alba. *Journal of Plant Sciences*, 2(4), 459–464. DOI:10.3923/ips.2007.459.464.
22. Chowdhury, A., Santra, A., Bhattacharjee, K., Ghatak, S., Saha, D. R., & Dhali, G. K. (2006). Mitochondrial oxidative stress and permeability transition in Isoniazid and Rifampicin induced liver injury in mice. *Journal of Hepatology*, 45(1), 117–126. DOI:10.1016/i.jhep.2006.01.027.
23. Jahan, R., Al-Nahain, A., Majumder, S., & Rahmatullah, M. (2014). Ethnopharmacological Significance of Eclipta alba(L.) Hassk. (Asteraceae). *International Scholarly Research Notices*, 2014, 1–22. DOI:10.1155/2014/385969.

Table1: Effect of MEEA and INH-RIF on serum hepatic marker enzymes

Groups	AST (U/L)	ALT(U/L)	GGT(U/L)	ALP(U/L)	LDH(U/L)
Control	37.74±3.89	31.48±3.06	4.94±0.73	260.60±13.15	122.50±9.25
INH-RIF	145.0±2.64*	77.85±4.8*	7.00±0.35*	569.30±32.55*	331.1±28.34*
Silymarin (100mg/kg)+ INH-RIF	68.48±5.18#	38.76±2.52#	5.08±0.33#	294.70±20.74#	178.0±16.19#
MEEA (200mg/kg) + INH-RIF	103.0±11.55#	48.66±0.13#	5.45±0.22#	437.4±32.04*,#	233.8±12.77#
MEEA (400mg/kg) + INH-RIF	77.88±10.94#	45.67±1.52#	5.19±0.21#	369.90±18.37#	170.9±15.50

All values expressed as U/1, in form of mean ± SEM, where n=6. *denotes p≤0.05 when compared between control vs INH-RIF; #denotes p≤0.05 when compared between INH-RIF vs test groups treated with extracts and standard. AST: Asparate transaminases, ALT: Alanine transaminases, GGT: Gamma glutamyl transferase, ALP: Alkaline Phosphatase, LDH: Lactate dehydrogenase.

Table 2: Effect of MEEA and INH-RIF on serum hepatic biochemical markers

Groups	Total Protein	Albumin	Total Bilirubin
Control	6.21±0.22	2.48±0.13	0.36±0.082
INH-RIF	4.48±0.42*	1.75±0.18*	0.85±0.10*
Silymarin (100mg/kg)+ INH-RIF	6.24±0.17#	2.48±0.10#	0.31±0.06#
MEEA (200mg/kg) + INH-RIF	5.29±0.16	2.38±0.19#	0.46±0.07#
MEEA (400mg/kg) + INH-RIF	6.24±0.17#	2.48±0.10#	0.31±0.06#

All values expressed as mg/dl, in form of mean±SEM, where n=6. *denotes p ≤0.05 when compared between control vs INH-RIF; # denotes p≤0.05 when compared INH-RIF vs test groups treated with extracts and standard.

Table 3: Effect of MEEA and INH-RIF on hepatic lipid peroxidation and antioxidants

Groups	SOD	CAT	GPx	GSH	MDA
Control	3.46± 0.03	61.08± 0.62	16.30± 0.27	3.65± 0.03	14.00 ± 0.07
INH-RIF	1.83± 0.04*	45.63± 0.25*	10.57 ±0.16*	1.75± 0.03*	23.15 ± 0.22*
Silymarin (100mg/kg)+ INH-RIF	3.31± 0.03#	57.65± 0.31#	15.77± 0.17#	3.24± 0.02#	14.62 ±0.183*
MEEA (200mg/kg) + INH-RIF	2.35 ± 0.02#	51.15± 0.42#	12.63± 0.19#	2.10± 0.04*	19.83 ± 0.27*
MEEA (400mg/kg) + INH-RIF	3.11± 0.03#	56.05± 0.25#	14.33± 0.29#	2.70± 0.04*	15.40 ± 0.30*

All values expressed in form of mean±SEM, where n=6. *denotes p ≤0.05 when compared between control vs INH-RIF; #denotes p≤0.05 when compared between INH-RIF vs test groups treated with extracts and standard. **Units**: SOD (U/mg of protein); CAT (n moles of H_2O_2 decomposed $min^{-1}mg^{-1}$protein); GST (n moles of CDNB conjugate formed $min^{-1}mg^{-1}$protein); GPx (n moles of GSH oxidized $min^{-1}mg^{-1}$protein); GSH (nmoles/g tissue); MDA (µmoles/g protein).