ORIGINAL ARTICLE

Hepatoprotective Effect of Aqueous Extract of *Chichorium Intybus* Roots on Isoniazid Induced Hepatotoxicity

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ABSTRACT

Objective: To determine the hepatoprotective effect of aqueous extract of *Chichorium intybus* roots in isoniazid induced hepatotoxicity in adult male mice.

Study Design: Experimental study.

Place and Duration of Study: Study was conducted from 15th of January to 15th of March 2015 at National Institute of Health Sciences (NIH) in collaboration with Riphah Institute of Pharmaceutical Sciences (RIPS).

Materials and Methods: Forty four Balb/c albino mice were divided randomly in to two groups, Group A (n=12) a control group and Group B (n=32) ,was given isoniazid 50mg/kg body weight orally once daily along with normal diet and water for 30 days to develop hepatotoxicity. Initially 2 mice from both groups were taken to check the ALT level on day 0. Isoniazid induced hepatotoxicity was confirmed by raised serum ALT levels in a mid-cycle sample of 10 mice from the Group B on day 30 mice (n= 10). After development of hepatotoxicity mice from Group B were further divided into two groups C and D. Group B1 (n=10) were given aqueous extract of *Chichorium intybus* roots at a dose of 200mg/kg/day and Group B2 (n = 10) at a dose of 400mg/kg/day orally for a duration of 30 days. On day 60 serum ALT of all the mice of Group B1, Group B2 was estimated to determine the hepatoprotective effect of aqueous extract of *Chichorium intybus* roots in Group C and D.

Results: Isoniazid produced severe hepatotoxicity as depicted by raised alanine aminotransferase (ALT) levels. ALT levels were decreased in Group B1 and B2.

Conclusion: Aqueous extract of *Chichorium intybus* roots has significant hepatoprotective effects.

Key Words: *Chichorium Intybus, Drug Induced Liver Injury (DILI), Hepatoprotoxicity, Isoniazid.*

Introduction

Across the globe most of cases of tuberculosis occur due to Mycobacterium tuberculosis.[1] Before the invention of antibiotics tuberculosis was a leading cause of death in both Eastern and Western nations.[2]According to the statistics Pakistan is ranked 4th amongst the multi-drug resistant cases of tuberculosis, approximately 3 million deaths per annum have been recorded with increased frequency of new cases.[3] All major drugs used for the treatment of tuberculosis i.e. isoniazid, rifampicin and pyrazinamide have hepatotoxic effects.[4] Drug induced liver injury (DILI) caused by the anti-tuberculous drugs varies from 2.0-28%.[5] Drug induced liver injury is clinically manifested by the raised liver enzymes. The most sensitive hepatic injury indicator alanine aminotransferase (ALT) level was measured to see the hepatotoxicity in all groups.[6] Isoniazid is the main antibiotic used for longer duration for the treatment of tuberculosis.[7] Acetyl hydrazine, a metabolite of isoniazid which on bio-activation leads to hepatotoxicity.[8] Plants have been a source of medicinal importance throughout the history.[9] *Chichorium intybus* commonly known as chicory, has been used as a medication in gastrointestinal and inflammatory diseases, whole plant has got valuable phytochemicals in it however roots contain essential components of therapeutic significance.[10] *Chichorium intybus* roots has got hepatoprotective,antioxidant[11,12] anti-inflammatory[13] antimicrobial[14,15] anti-hyperglycemic[16,17] immunostimulant[18,19] tumor inhibitory properties.[20] Traditional medicines and herbs have been used locally in the market and scientific study has not be explored to see the active

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principles and phytochemicals. Current research was aimed to see the active principle in the herb and to support it biochemically. Rationale was to explore the scientific evidence of the active ingredients helpful in preventing DILI in patients on antituberculous drugs. The objective of the present study was to explore the hepatoprotective effect of aqueous extract of *Chichorium intybus* roots in dose dependent manner on isoniazid induced hepatotoxicity.

Materials and Methods

An experimental randomized control study was carried out at Riphah Institute of Pharmaceutical Sciences (RIPS) and National Institute of Health Sciences (NIH), Islamabad. Forty four Balb/c male and healthy albino mice weighting 30-50 grams with normal ALT levels were taken for the study and were acclimatized for one week in the NIH animal house under standard facilities and were given normal diet and water ad libitum.

Initially, 44 mice were randomly divided in to two groups, Group A (n=12) which was given normal diet and tap water ad libitum, Group B (n=32) was given isoniazid 50mg/kg body weight orally once daily along with normal diet and water for 30 days to develop hepatotoxicity.[21,22] On day 0 blood samples of two mice from each group were taken through cardiac puncture. After 30 days mid cycle samples of 10 mice from Group B were taken, ALT levels were performed to see establishment of hepatotoxicity. After confirmation of hepatotoxicity mice from Group B were further divided in to two groups, Group B1 n=10 which was given aqueous extract of *Chichorium intybus* roots at a low dose of 200mg/kg/day[23] and Group B2 which was given aqueous extract of *Chichorium intybus* roots at a high dose of 400mg/kg/day [23] orally for a duration of 30 days. On termination day i.e. day 60th blood samples were taken from the both experimental Groups B1 and B2 for evaluation of ALT levels.

Chichorium intybus was identified by herbarium department, Quaid-e-Azam University, Islamabad. Aqueous extract of Chichorium intybus roots was prepared at RIPS, Islamabad by using fine homogenized powder of dried chicory roots which were mixed with distilled water, the whole solution was boiled for 2 hours and after cooling was sifted through filter paper. The aqueous extract was formed by using vacuum rotary evaporator and was frozen dried.[24]

Results were compiled and data was entered into SPSS 17 was used for statistical analysis. Tuckey's multiple comparison test to observe group mean differences. A p-value of <0.05 was considered as statistically significant.

Results

Serum ALT levels were significantly raised (p<0.01) in Group B treated with isoniazid as compared to Group A. *Chichorium intybus* roots extract significantly reduced (p<0.01) serum ALT level in Group B1 and Group B2 in comparison to Group B.

Table I: Tukey's multiple comparisons test between study Groups

Groups	Mean Difference	Significant	Summary
Group A vs Group B	-156.8	Yes	****
Group A vs Group B1	-59.47	Yes	**
Group A vs Group B2	-44.13	Yes	*
Group B vs Group B1	97.33	Yes	****
Group B vs Group B2	112.7	Yes	****
Group B1 vs Group B2	15.33	No	Ns

ANOVA summary
F 35.52
P value <0.0001
P value summary ****
Are differences among means statistically significant? (P <0.05) Yes

Discussion

In the present study mice were treated with isoniazid at 50mg/kg resulted with significant elevation in serum ALT levels. Group B1 and B2 received aqueous extract of *Chichorium intybus* roots resulted in significant improvement of ALT levels in a dose dependent manner. Our study in accordance with study carried out by El-Sayed et al in 2015 which showed antioxidant activity of *Chichorium intybus* in CCl4 induced hepatotoxicity.[14] similarly our study is in correlation with another study performed by Atta et al. showing hepatoprotective effect of *Chichorium intybus* extract when given with methanolic extract of Zinger Officinale.[25] Similar results have been

found in the study performed by Li et al. on hepatoprotective effect of *Chichorium intybus* in CCl4 induced hepatotoxicity in rat model.[26]

Previously studies have been done on exploring hepatoprotective effect of *Chichorium intybus* in combination with medical compounds like silymarin and other herbal compounds and extracts. No dose dependent study was done individually on aqueous extract of *Chichorium intybus* roots extract which guides us about the submaximal, ceiling effect and toxicity. Our study confirms the individual hepatoprotective effect of aqueous extract of *Chichorium intybus* roots.

Further studies are needed to determine molecular mechanism of inulin which is the major active principle of the *Chichorium intybus* roots. In addition a higher dose and different routes of administration can be tried to see the same effect.

Conclusion

Aqueous extract of *Chichorium intybus* roots have significant hepatoprotective effect on isoniazid induced hepatotoxicity.

REFERENCES

1. Hurtado AM, Hill KR, Rosenblatt W, Bender J, Scharmen T. Longitudinal study of tuberculosis outcomes among immunologically naive Aché natives of Paraguay. American Journal of Physical Anthropology. 2003; 121: 134-50.
2. Tiemersma EW, Van Der Werf MJ, Borgdorff MW, Williams BG, Nagelkerke NJD. Natural History of Tuberculosis: Duration and Fatality of Untreated Pulmonary Tuberculosis in HIV Negative Patients: A Systematic Review. PLoS ONE. 2011; 6: e17601.
3. JP N. Tuberculosis and HIV infection. Tuberculosis: Epidemiology and control.New Dehli: World Health Organization Reginaol Office for South-East Asia. 2002; 120: 84-1000.
4. Yew WW, Leung CC. Antituberculosis drugs and hepatotoxicity. Respirology. 2006; 11 :699-707.
5. Tostmann A, Boeree MJ, Aarnoutse RE, De Lange W, Van Der Ven AJ, Dekhuijzen R. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. Journal of gastroenterology and hepatology. 2008; 23: 192-202.
6. Adhvaryu MR, Reddy N, Parabia MH. Effects of four Indian medicinal herbs on Isoniazid-, Rifampicin-and Pyrazinamide-induced hepatic injury and immunosuppression in guinea pigs. World journal of gastroenterology. 2007; 13: 3199-205.
7. Control CfD. Core curriculum on tuberculosis: What the clinician should know. 5th edition ed: US Department of Health & Human Services Atlanta; 2011. p. 24
8. Metushi I, Cai P, Zhu X, Nakagawa T, Uetrecht J. A Fresh Look at the Mechanism of Isoniazid-Induced Hepatotoxicity. Clinical Pharmacology & Therapeutics. 2011; 89: 911-4.
9. Vogel G, Tuchweber B, Trost W, Mengs U. Protection by silibinin against Amanita phalloides intoxication in beagles. Toxicology and applied pharmacology. 1984; 73: 355-62.
10. Street RA, Sidana J, Prinsloo G. Cichorium intybus: Traditional uses, phytochemistry, pharmacology, and toxicology. Evidence-Based Complementary and Alternative Medicine. 2013; 26: 2013.
11. Hassan HA, Yousef MI. Ameliorating effect of chicory (Cichorium intybus L.)-supplemented diet against nitrosamine precursors-induced liver injury and oxidative stress in male rats. Food and Chemical Toxicology. 2010; 48: 2163-9.
12. Kim TW, Yang KS. Antioxidative effects ofCichorium intybus root extract on LDL (Low Density Lipoprotein) oxidation. Arch Pharm Res. 2001; 24: 431-6.
13. Cavin C, Delannoy M, Malnoe A, Debefve E, Touche A, Courtois D, et al. Inhibition of the expression and activity of cyclooxygenase-2 by chicory extract. Biochemical and biophysical research communications. 2005; 327: 742-9.
14. El-Sayed YS, Lebda MA, Hassinin M, Neoman SA. Chicory (Cichorium intybus L.) Root Extract Regulates the Oxidative Status and Antioxidant Gene Transcripts in CCl(4)-Induced Hepatotoxicity. PLoS ONE. 2015; 10: e0121549.
15. Liu H, Wang Q, Liu Y, Chen G, Cui J. Antimicrobial and Antioxidant Activities of Cichorium Intybus Root Extract Using Orthogonal Matrix Design. Journal of Food Science. 2013; 78: 258-63.
16. Pushparaj P, Low H, Manikandan J, Tan B, Tan C. Anti-diabetic effects of Cichorium intybus in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 2007; 111: 430-4.
17. Lee KT, Kim JI, Park HJ, Yoo KO, Han YN, Miyamoto Ki. Differentiation-inducing effect of magnolialide, a 1 beta-hydroxyeudesmanolide isolated from Cichorium intybus, on human leukemia cells. Biological & pharmaceutical bulletin. 2000; 23: 1005-7.
18. Kim JH, Mun YJ, Woo WH, Jeon KS, An NH, Park JS. Effects of the ethanol extract of Cichorium intybus on the immunotoxicity by ethanol in mice. International immunopharmacology. 2002; 2: 733-44.
19. Amirghofran Z, Azadbakht M, Karimi MH. Evaluation of the immunomodulatory effects of five herbal plants. Journal of Ethnopharmacology. 2000; 72: 167-72.
20. Hazra B, Sarkar R, Bhattacharyya S, Roy P. Tumour inhibitory activity of chicory root extract against Ehrlich ascites carcinoma in mice. Fitoterapia. 2002; 73: 730-3.
21. Pal R, Valphei K, Singh K, Rana S. Garlic confers hepatoprotection in isoniazid rifampicin induced hepatic injury. Ind J Gastro. 2003; 1: 100.
22. Attri S, Rana S, Vaiphei K, Sodhi C, Katyal R, Goel R, et al. Isoniazid–and rifampicin–induced oxidative hepatic injury–protection by N–acetylcysteine. Human & experimental toxicology. 2000; 19: 517-22.
23. Butt K, Yunas S, Sheikh RM. Hepatoprotective effect of Cichorium intybus on paracetamol induced liver damage in albino rats. Libyan Agric Res Center J Int. 2012; 3: 60-3.
24. Cha JY, Park CK, Cho YS. Hepatoprotective effect of chicory (Chicorium intybus) root extract against orotic acid-induced

fatty liver in rats. Food Sci Biotechnol. 2010; 19: 865-71.
25. Atta A, Elkoly T, Mouneir S, Kamel G, Alwabel N, Zaher S. Hepatoprotective effect of methanol extracts of Zingiber officinale and Cichorium intybus. Indian journal of pharmaceutical sciences. 2010; 1; 72: 564.
26. Li GY, Gao HY, Huang J, Lu J, Gu JK, Wang JH. Hepatoprotective effect of Cichorium intybus L., a traditional Uighur medicine, against carbon tetrachloride-induced hepatic fibrosis in rats. World Journal of Gastroenterology: 2014; 20: 4753-60.