

ORIGINAL ARTICLE

Hepatoprotective Effect of Aqueous Extract of *Cichorium Intybus* (Kasni) on Pyrazinamide Induced Hepatotoxicity in Male Mice

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ABSTRACT

Objective: To determine the hepatoprotective effect of aqueous extract of *Cichorium intybus* (kasni) roots on Pyrazinamide induced hepatotoxicity in male mice.

Study Design: Experimental randomized control study.

Place and Duration of Study: The study was conducted in the department of pharmacology at Islamic International Medical College Trust (IIMCT), Rawalpindi in collaboration with Riphah Institute of Pharmaceutical Sciences (RIPS), Islamabad and National Institute of Health (NIH), Islamabad. The duration of study was from 14th March, 2015 to 14th March, 2016.

Materials and Methods: The experimental randomized control study was carried out on fifty six male albino Balb/C mice which were divided into four groups each having 14 mice. Group A (control) received normal diet with no medication. Group B was given pyrazinamide in dose of 500mg/kg while group C and D were given pyrazinamide along with low dose (200mg/kg) and high dose (400mg/kg) of *Cichorium intybus* roots (aqueous extract) via gavage tube. At day 0, baseline blood samples were taken and normal values of serum alanine aminotransferase (ALT) were obtained. Mid cycle samples were obtained to ensure the progress of study. Final sampling was done at day 30 through cardiac puncture. Serum was separated in sterile tubes and biochemical estimation was done on the same day. Mean and standard error of mean was calculated. Data was analyzed through SPSS 20 and p value of <0.05 was meditated statistically significant.

Results: The extract of *Chichorium intybus* roots showed improvement in serum alanine aminotransferase (ALT) values in group C and D. ALT value of 5-50UL was considered normal. Group A showed normal value of 24.5 UL. Group B showed severe hepatotoxicity as indicated by the raised value of 143.5UL. The value of ALT in Group C was 86.5 UL and it was 59.9UL in Group D.

Conclusion: Aqueous extract of *Cichorium intybus* roots has significant hepatoprotective activity in high doses as compared to low doses.

Key Words: Alanine Aminotransferase (ALT), *Cichorium Intybus*, Hepatoprotective, Pyrazinamide.

Introduction

Now a days, across the world, tuberculosis (TB) is major health issue.¹ Yearly 2 million deaths are reported from this disease.² Pakistan has been ranked in the top six countries who are bearing the major brunt of TB.³ Pyrazinamide is one of the first line anti tuberculosis drug.⁴ Liver toxicity due to

pyrazinamide is being reported worldwide.⁵ First line anti TB drugs i.e, Isonaizid and Rifampicin show hepatotoxicity but the liver toxicity caused by pyrazinamide is more commonly reported.⁶ The exact mechanism of pyrazinamide induced hepatotoxicity is unknown.⁷ Drug induced hepatotoxicity (DIH) clinically manifests by raised liver function tests (LFTs).⁸ *Cichorium intybus* has been commonly used in Asia and China to improve the liver function.⁹ It is an erect perennial plant commonly known as chichory and kasni.¹⁰ *Chichorium Intybus* inhibits free radical mediated injury which accounts for its hepatoprotective effect.¹¹ Aqueous extract of *Chichorium intybus* roots has hepatoprotective,^{12, 13} anti-hyperglycemic,¹⁴ anti-inflammatory,¹⁵ antimicrobial,¹⁶ antidyslipidemic,¹⁷ neuroprotective,¹⁸ immunomodulatory¹⁹ and gastroprotective effects.²⁰

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The rationale of the present research was to provide cheap and easily available hepatoprotective drug to general population. Our study was intended to explore the hepatoprotective activity of aqueous extract of *Cichorium intybus* roots in pyrazinamide induced hepatotoxicity in male mice.

Materials and Methods

Experimental randomized control study was conducted at Pharmacology department, Islamic International Medical College (IIMC) in collaboration with Riphah Institute of Pharmaceutical Sciences (RIPS) and animal house of National Institute of Health (NIH), Islamabad from 14th March, 2015 to 14th March, 2016. Research proposal was approved from Institutional Review Committee. A total of fifty six healthy male albino Balb/C mice weighing 30-50g and aged of 8 weeks, having normal ALT were included in study. Mice having weight less than 30 g, age less than 8 weeks and female mice were excluded from the study. Mice were kept under room temperature of 22 ± 2 degree Celsius and 12 hour light dull cycle for 1 week. The mice were randomly divided into four groups each containing 14 mice (n=14). Group A, control group was given normal diet and tap water. Group B, drug treated group was given pyrazinamide in dose of 500mg/kg²¹ Group C was given aqueous extract of *Chichorium intybus* roots in dose of 200mg/kg²² and Group D was given aqueous extract of *Chichorium intybus* roots in dose of 400mg/kg²² along with pyrazinamide.

The roots of *Chichorium intybus* were collected from National Institute of Research and Agriculture and was identified by Herbarium department, Quaid-e-Azam University, Islamabad. The fine powder was obtained from the dried roots. After boiling this powder in water for 2 hours, aqueous concentrate was obtained, which is then passed through Whatman No.1 filter paper. Finally the concentrate was formed with a vacuum rotary evaporator and freeze-dried.²³ On day 0, baseline blood samples were taken from 2 mice belonging to each group. To evaluate the progress of study, samples of 2 mice from each group were taken on day 15. On day 30, blood samples from 10 mice of each group were collected. All the sampling was through cardiac puncture by using 3cc syringe. Samples were allowed to clot. Serum was separated by Bench top machine after centrifugation at 3000 rev/min for 5 minutes.²⁴

Serum was separated in sterile tubes and serum ALT was estimated by using ALT kit (Merck) on Chemistry Analyzer, Micro lab 200 (Merck). This parametric data was statistically analyzed by using SPSS 20. Mean and standard error of mean was calculated for all the four groups and post-hoc test was done for comparison between the different groups. Results were considered significant at p value less than 0.05 (p<0.05).

Results

There is significant increase (p<0.001) in serum ALT levels in mice of drug treated group (B) as compared to control group (A) due to pyrazinamide. The levels were significantly reduced in group D who received high dose of *Chichorium intybus* as compared to the group C who received low dose of *Chichorium intybus*. The results are summarized in the following table.

Table I: Mean \pm SEM Values of ALT in All Groups

Groups (n=10)	ALT (5-50 U/L)
Group A	24.50 \pm 9.30
Group B	143.50 \pm 51.866
Group C	86.50 \pm 37.616
Group D	59.90 \pm 34.323
p-value	< 0.001*

* = Significant

ALT=Alanine Aminotransferase

Table II: Post-Hoc Comparison of ALT Between the Groups

Group Comparison	ALT (50-150 U/L)	
	Mean difference	p-value
Group A vs. Group B	- 119.0	0.000*
Group A vs. Group C	- 62.0	0.003*
Group A vs. Group D	- 35.4	0.154
Group B vs. Group C	57.0	0.007*
Group B vs. Group D	83.6	0.001*
Group C vs. Group D	26.6	0.379

* = Significant

ALT=Alanine Aminotransferase

Discussion

Many drugs adversely affect the liver function with their hepatotoxic effects.²⁵ Globally the drugs used for the treatment of tuberculosis are the sole largest cause of acute hepatic failure.²⁶ Amongst the drugs which are used as first line therapy for tuberculosis, incidence of liver toxicity is highest with pyrazinamide.²⁷ The fact that pyrazinamide or its metabolites produce toxic effects in liver is still not known.²⁸ *Chichorium intybus* roots have shown its hepatoprotective role in acute liver toxicity caused

by drugs.²⁹ The free radicle scavenging property of *Chichorium intybus* roots is proposed to be due to its inulin constituent.¹⁰ Estimation of ALT is done based on its high sensitivity and specificity for the evaluation of hepatic functioning.³⁰ To evaluate the hepatoprotective effect of aqueous extract of *Chichorium intybus* roots, our study was conducted with sample size of 56 mice which were divided through random selection in four groups (n=14). Group A (control), group B (drug treated group), group C and D (experimental groups). Significant rise in serum ALT levels were seen in group B who were given pyrazinamide while reduction in serum ALT levels was observed in group C and D which were given *Chichorium intybus* along with pyrazinamide. Our results were in accordance with Li and his colleagues who observed hepatoprotective effect of *Chichorium intybus* on carbontetrachloride (CCL₄) induced hepatotoxicity in rats by measuring serum ALT and ALP levels.⁹ Chen also observed decrease in liver enzymes of mice after treatment with *Chichorium intybus* in tert-butyl hydroperoxide (t-BHP) induced hepatotoxicity.³¹ El-Sayed observed antioxidant effect of chichory in CCL₄ induced hepatotoxicity in rats.³² Study by Atta revealed beneficial hepatoprotective effect of *Chichorium intybus* when used in combination with Zingiber officinale.³³

Conclusion

Aqueous extract of *Chichorium intybus* roots has beneficial hepatoprotective potential both in low and high doses in pyrazinamide induced hepatotoxicity in male mice.

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