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ORIGINAL ARTICLE

Protective Effect of Chichorium Intybus (Kasni) Roots on Hepatotoxocity Induced by Pyrazinamide in Male Mice

ATTIYA MUNIR[1], AMANAT ALI[2], SAMEER AHMED[2], AMTUL HAFEEZ[3], NAUMAN NASEER[4], MEHWISH TAYYAB[2], TALHA LAIQUE[5]
[1]*Department of Pharmacology, Rawalpindi Medical University, Rawalpindi-Pakistan*
[2]*Department of Pharmacology, HBS Medical and Dental college, Islamabad-Pakistan*
[3]*Department of Pharmacology, Islam Medical College, Sialkot-Pakistan*
[4]*Department of Neonatology, CMH, Rawalpindi-Pakistan*
[5]*Department of Pharmacology, Allama Iqbal Medical College, Lahore-Pakistan*
Correspondence to Dr. Talha Laique, Email: talhalaique51@gmail.com Tel:+92-331-0346682

ABSTRACT

Background: Tuberculosis (TB) is major health issue across the world. Yearly 2 million deaths are reported from this disease. Pakistan has been ranked in the top six countries that are bearing the major brunt of TB.
Aim: To observe hepatoprotective role of aqueous extract of kasni (*Chichorium intybus*) roots on hepatotoxicity caused by Pyrazinamide in male BALB/c mice.
Study Design: Randomized control Trial.
Methodology: Healthy male albino Balb/C mice, fifty six in number were selected randomly and further grouped into four groups (n=14 mice). Group A labelled as control group and maintained on rodent diet with no medication. Group B was labelled as disease control group and only pyrazinamide was administered to mice in this group in dose (500mg/kg BW). Groups C and D designated as exploratory groups. Both group C & D were administered a combination of Anti TB drug, pyrazinamide along with aqueous extract preparations of Kasni roots in low (200mg/kg) & high doses (400mg/kg) respectively. Baseline blood samples were drawn at day zero.
Results: Serum alanine aminotransferase (ALT) and alkaline Phosphatase (ALP) were improved in group C & D given aqueous extract of *Chicorium intybus* roots.
Conclusion: It was concluded that high doses of aqueous extract preparations of kasni (*Chichorium intybus)* roots showed more improvements of serum markers in drug induced hepatotoxicity than low doses of aqueous preparations.
Keywords: Chichorium intybus, Hepatoprotective, Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP),

INTRODUCTION

Tuberculosis (TB) is one of the most common infectious respiratory diseases in all regions of world and one of the most prevalent cause of mortality in underdeveloped countries.[1] One-third of world's population is contaminated with TB.[2]

Drug related hepatotoxicity is now the leading cause of severe hepatic dysfunction. All across the world, anti-TB drugs are the commonest cause of acute liver failure.[3]Among the 1st line Anti TB drugs, isoniazid, rifampicin and pyrazinamide possess hepatotoxic properties.[4] Pyrazinamide is widely associated with hepatitis due to drugs amongst anti-TB medicines[5].

PZA causes toxicity via unknown mechanism.[6] Clinically liver enzymes are elevated in drug induced hepatotoxicity. The use of herbal medicines is common across the world.[7] *Chichorium intybus* is an erect perennial herb grown in many countries as medicinal plant.[8] It is also known as chicory[9]. It has been used in the treatment of jaundice[10]and hepatomegaly[11].

The main constituent present in roots is inulin, possessing antioxidant and hepatoprotective properties.[12] Chicory roots also possess antimicrobial,[13] antidiabetic,[14] antimalarial,[15] anti-inflammatory and antitumor activities[16]. Present study aimed at the protective effects of kasni (*Chichorium intybus*) roots on hepatotoxicity in BALB/c mice induced by Pyrazinamide and aqueous extract preparations of the herb were utilized for the purpose.

The objective of the study was to observe hepatoprotective role of aqueous extract of kasni (*Chichorium intybus*) roots on hepatotoxicity caused by Pyrazinamide in male BALB/c mice.

METHODOLGY

This experimental study was performed at Islamic International Medical College (IIMC), National Institute of Health (NIH) Islamabad and Riphah Institute of Pharmaceutical Sciences (RIPS) for one month after permission from IRB. Fifty six healthy adult male albino Balb/C mice were used through randomized control trial. Eight weeks old healthy male Albino mice weighing 30-50g with normal LFTs are included in the study. Mice weighing more than 50g or less than 30g, mice less than 8 weeks old, female gender and mice that had been used in prior studies or those having any disease are excluded from the study.
Data collection procedure: All experimental mice were acclimatized before they were kept in appropriate experimental conditions. Room temperature was maintained at 21 ± 2 0C. A 12 hour dark and light cycle was provided to Balb/C mice.[17] Total sample was preceded by further division in four groups. Random selection procedure was performed and four groups (n=14) were formulated. Group A labeled as the control group maintained on rodent pellet diet and fresh water without any administration of drug and herb. Group B was marked as Disease control group and Pyrazinamide was given to experimental animals of this group with dose (500mg/kg)[18] body weight. Group C & D were labeled as experimental groups. Aqueous extract preparation of Kasni roots with dose (200mg/kg)[19] body weight & (400mg/kg)[19] were administered to both groups respectively along with the drug Pyrazinamide.

Chichorium intybus roots were collected locally. After collection, roots were identified from Quaid-e-Azam University in Islamabad. Drug Pyrazinamide purchased from Sigma International. *Chichorium intybus* roots were air dried first. After that roots were grounded fine powder. The fine powder of roots was made to boil for 2 hours in water till it concentrated. The concentrated aqueous medium was further filtered by using Whattman filter paper number.1 sieve. Filtrate was collected in a flask. The filtrate was then placed in a vacuum rotary evaporator and through evaporation and then through freeze drying, a viscous aqueous extract preparation was collected[20]. Two mice were taken randomly from each group for baseline blood sampling was on Day 0. Progress of research was evaluated by taking blood samples of 2 mice randomly selected from every group at day 15. At day 30, terminal sampling of blood was done from each group via cardiac puncture and for that purpose 3 c.c syringe was used. Serum was separated through centrifugation at rate of 3000 revolution/min for five minutes by Bench top machine[21]. Sterile tubes were used for cumulation of serum.

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Estimation of serum AST and ALT performed and for this purpose serum marker kits i.e., ALT kit & AST kit from Merck were used on a chemistry analyzer, Micro lab 200 (Merck).

Statistical analysis: Statistical analysis performed on SPSS version 20. After statistical analysis, result report showing ($p<0.05$) were regarded significant.

RESULTS

Comparison of mean values of ALT and ALP between the groups showed that Group D which was given (400 mg/kg) body weight dose of *Cichorium intybus* roots along with the drug Pyrazinamide showed more improvement in liver function than group C administered with herb in a dose (200 mg/kg) body weight along with drug Pyrazinamide. P-value was statistically significant. However, both groups C and D showed improvement in liver function when compared with Group B which was given only Pyrazinamide. P-value was statistically significant as shown in table-1.

Average ALT was 24.5±9.30 u/L in group A, 143.50±51.86u/L in group B, 86.50± 37.61 u/L in group C, 59.90±34.32u/L in group D. The statistical difference between the groups was found to be significant ($p < 0.001$) as shown in Fig-1. Average ALP was 63.40 ± 23.58 u/L in group A, 227.10 ± 88.87 u/L in group B, 145.70 ± 66.782 u/L in group C, 119.80 ± 56.O55 u/L in Group D. The statistical difference b/w groups noted to be significant ($p < 0.001$) as shown in fig-2.

Data analysis between different groups showed significant difference with p-values <0.05 as shown in table-2.

Table-1: Comparison of Total ALT & ALP b/w the groups

Groups	ALT (5-50U/L)	ALP (20-120U/L)
Group A (n=10)	24.50 ± 9.30	63.40 ± 23.585
Group B (n=10)	143.50 ± 51.866	227.10 ± 88.870
Group C (n=10)	86.50 ± 37.616	145.70 ± 66.782
Group D (n=10)	59.90 ± 34.323	119.80 ±56.055
p-value	< 0.001*	< 0.001*

*Statistically significant

Fig-1: Comparison of ALT value between groups

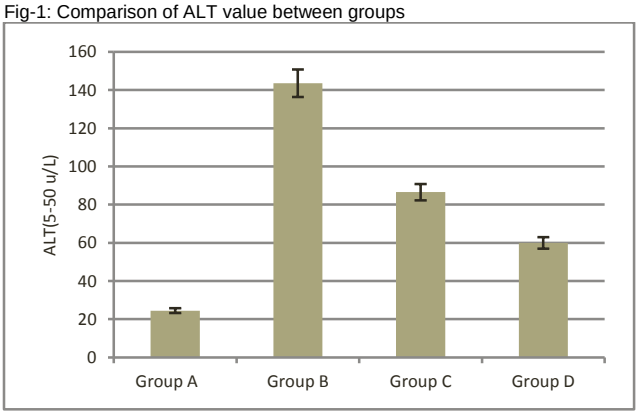

Fig-2: Comparison of ALP values between groups

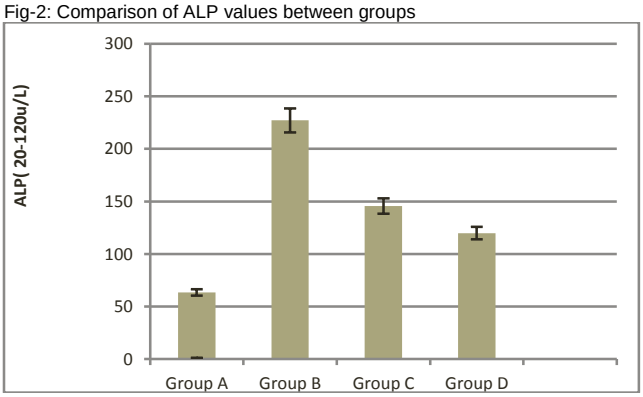

Table 2: Post-Hoc Tukey Analysis between Different Groups

Comparison between the groups	ALT(5-50u/L)		ALP(2-120u/L)	
	Mean difference	*p*-value	Mean difference	*p*-value
Group A vs. Group B	- 120.0	0.000*	- 163.7	0.000*
Group A vs. Group C	- 63.0	0.004*	- 82.3	0.03*
Group A vs. Group D	- 36.4	0.153	- 56.40	0.210
Group B vs. Group C	56.0	0.006*	81.40	0.033*
Group B vs. Group D	84.6	0.001*	107.3	0.003*
Group C vs. Group D	27.6	0.479	25.8	0.797

*****Statistically significant.

DISCUSSION

PZA amongst anti-TB medicines is widely associated with hepatitis.[22]It is unclear whether the drug itself causes toxicity or the metabolites are involved.[17]Herbal remedies are commonly used in liver disorders.[23]The hepatoprotective activity of *Chichorium intybus* roots have been previously reported.[24] Group B in the current study was treated with pyrazinamide at 500mg/kg body weight causing significant rise of serum ALT and ALP markers. Experimental groups C and D were administered aqueous preparations of kasni root extract in low and high dose 200mg/kg & 400mg/kg respectively. ALT and ALP levels were markedly improved in low and high doses. However, aqueous extract administration in high doses i.e., 400 mg/Kg body weight showed more improvement than low dose. Our research results are in congruity with the experiment conducted by El-Sayed et al, 2015 manifesting hepatotoxicity induced by CCL4 and *Chichorium intybus* given for its hepatoprotective effects to improve the ALT & ALP levels.[25] Our experimental results are also in coherence with a research done by Atta that also showed protective effects on liver by *Chichorium intybus* aqueous extract in conjunction with methanolic extract of *Zingiber officinale*[23]. Another study performed by Cha and Park also concluded the protective effects on liver of *Chicorium intybus* root in rats in which fatty liver was induced by orotic acid[26]. In previous studies Isoniazid among anti-TB drugs was used in hepatotoxicity studies while we chose Pyrazinamide to be compared with *Chichorium intybus* roots.

Limitations: Number of limitations like financial constrains, short duration of study, single drug use with no genetic work up hampered our study.

CONCLUSION

It was concluded that beneficial effect of *Chichorium intybus* roots wass evident in hepatotoxicity and the herb has pronounced potential in improving liver function at less & high doses against drug Pyrazinamide induced liver toxicity in male BALB/c mice.

Authors' Contribution: AM&AA: Conceptualized the study, analyzed the data, and formulated the initial draft. **SA&AH:** Contributed to the histomorphological evaluation. **NN**:

Contributed to the analysis of data and proofread the draft, **MT:** Contributed to data collection, **TL**: Contributed to the proofreading the manuscript for intellectual content,
Conflict of Interest: None to declare
Financial Disclosure: None

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