Original Article

Protective Role of Stem-Bark Ethanolic Extract of Sumbloo (Berberis Lycium Royale) against Rifampicin Induced Liver Damage in BALB/C Mice

Saima Rafique[1], Aysha Afzal[1], Sabeen Shakir[3], Khalida Ajmal[1], Lubna Ghazal[2] and Lubna Ehtizaz[2]

ABSTRACT

Objective: To explore protective impact of stem-bark ethanolic extract of Sumbloo (Berberis Lycium Royale) against Rifampicin induced hepatotoxicity in BALB/c Mice.

Study Design: Explorative randomized control study

Place and Duration of Study: This study was conducted at Animal Chamber of National Institute of Health (NIH), Islamabad in collaboration with Islamic International Medical College (IIMC), Army Medical College (AMC) & Riphah Institute of Pharmaceutical Sciences (RIPS) from April 2014 to May 2014.

Materials and Methods: This experimental study was performed on 56 male BALB/c mice. They were arbitrary split up into 4 groups (n=14). Control Group A was kept on rodent pellet diet and water only. Drug treated group Group B was loaded with Rifampin 50mg/kg BW whereas Less dose group C & High dose ethanolic extract Group D were given Rifampicin low dose (150mg/kg BW) and high dose (200mg/kg BW) of Ethanolic extract of stem bark of the Sumbloo via gavage tube respectively. Baseline blood samples collected at day 0 and normal values of serum Alanine aminotransferase (ALT) were estimated. Mid-cycle blood sampling taken at day 15 to evaluate the progress of experimental study. Final blood sampling performed on day 30 by intra-cardiac puncture. Serum was stored in sterile tubes at 4^{o}C for analysis of serum Alanine aminotransferase(ALT). Data analysis performed on SPSS version 20 and p-value (<0.05) regarded as statistically significant.

Results: Ethanolic extract of stem bark of Sumbloo depicted marked improvement of serum Alanine aminotransferase (ALT) values in group C & D. Serum ALT value of (17-77 U/L) was labeled as normal. Group A depicted normal value of (54 U/L). Group B manifested severe hepatoxicity as shown by very high value of (186.1 U/L). Serum value of ALT measured in Group C was (96.6 UL) & (56 UL) in Group D.

Conclusion: Ethanolic extract of stem-bark of Sumbloo (Berberis lycium Royale) possesses remarkable protective potential for liver toxicity in high doses as compared to less dose.

Key Words: Berberis lycium Royale, Alanine Aminotransferase (ALT), Hepatoprotective, Rifampicin.

Citation of article: Rafique S, Afzal A, Shakir S, Ajmal J, Ghazal L, Ehtizaz K. Protective Role of Stem-Bark Ethanolic Extract of Sumbloo (Berberis Lycium Royale) against Rifampicin Induced Liver Damage in BALB/C Mice Med Forum 2020;31(9):50-53.

INTRODUCTION

Tuberculosis (TB) has remained among top leading causes of morbidity & mortality due to infecting one-third of population worldwide.[1]

[1.] Department of Pharmacology / Pathology[2], Wah Medical College, Wah Cantt.
[3.] Department of Pharmacology, Rawal Institute of Health Sciences, Islamabad.

Correspondence: Samia Rafique, Assistant Professor of Pharmacology, Wah Medical College, Wah Cantt.
Contact No: 0300-8546013
Email: samiarafiqu34@gmail.com

Received: January, 2020
Accepted: July, 2020
Printed: September, 2020

Mycobacterium tuberculosis is known as the culprit of this devastating disease and has proven to be one of the most notorious microorganism since ancient times.[2]

Rifampicin firstly discovered in 1965 and now it is one of the first line treatment for tuberculosis. It has a remarkable potential of sterilizing activity and shortening the treatment span.[3] Rifampicin binds with DNA dependent RNA polymerase of Mycobacterium tuberculosis and inhibits it. It blocks RNA synthesis of the mycobacterium leading to its death. Its MIC is 1 g/ml.[4] Alongwith its useful anti-bacterial activity, it also causes side effect of hepatotoxicity due to being a strong inducer of various metabolic enzyme pathways of the body particularly of cytochrome P450 (CYP3A4) system.[5] PPARγ signaling mechanisms and oxidative stress were also found to be greatly linked to Rifampicin-induced toxicity to liver tissue.[6] Liver injury

caused by drugs is clinically manifested by raised serum liver function tests (LFT's).[7]

Berberis lycium Royle is well known for its hepatoprotective activity.[8]It is known by its various regional names like ziarlargay, sumbloo & Barbery.[9] Major constituent of the stem bark is found to be Berberine (4.2%) which has significant hepatoprotective potential. [10,11] It has been used for diabetes due to its hypoglycemic activity.[12] It has found to be very useful for different ailments like headache, rheumatism, jaundice, whooping cough ear and eye infections.[13]

Rationale of this experimental randomized trial was to investigate the protective activity of the Ethanolic extract of stem-bark of Sumbloo (Berberis lycium Royle) in dose dependent manner on rifampicin induced hepatotoxicity in male BABL B/c mice.

MATERIALS AND METHODS

The experimental randomized control trial has been conducted in research institute, National Institute of Health (NIH), Islamabad following ethical acceptance by RARE (Riphah Academy of Research & Education) from 10th April 2014 till 10th May 2014. Male BALB/c Mice, weighing 30-50 grams, age 7-8 weeks with normal serum Alanine aminotransferase (ALT) levels were housed in NIH under standardized conditions in wire topped cages at temperature (21-22°C) and acclimatized for a period of one week.

Grouping of Animals:

A total of fifty-six healthy BALB/c mice were arbitrary put into 4 groups. Control Group A: (n=14) was kept on normal diet & fresh water per mouth only. Drug treated Group B: (n=14) was given Rifampicin 50mg/kg BW.[14] Low dose Group C: (n=14) was given Rifampicin 50mg/kg BW[14] and 150 mg/Kg BW of Ethanolic extract wherease High dose Group D: (n=14) given Rifampicin 50mg/kg BW[14] & 200 mg/Kg BW of ethanolic extract of stem-bark of Sumbloo (Berberis lycium Royle). The drugs & herb were given through gavage tube once daily orally for 1 month.

Ethanolic extract preparation:

Stem bark of Sumbloo (Berberis lycium Royle) procured from a village of Charsadda and authenticated by renowned botanist from Peshawar University. It was then dried and crushed into fine powder form. For ethanolic extract preparation, 1kg of dried crushed fine powder was firstly soaked in ethanol at temperature (24ºC) for 72 hours. Coarse filtration of ethanol soaked powder was done with the help of muslin cloth whereas fine filtration was performed by using Whatman filter paper no.1. Filtered filtrate initially kept in open air for evaporation and later it was evaporated by using rotary evaporator at temperature (41ºC) under reduced pressure. After evaporation in rotary evaporator, a dark choclate brown color extract with semi-solid and sticky consistency was obtained. Finally, ethanolic extract put in transparent air tight small glass jars in refrigerator with (2-8ºC) temperature for future research use. Ethanolic extract yield was around 20% (20g was obtained from 100g).[15]

Blood Sample Collection:

Baseline blood samples of two mice from every cluster were taken at day zero. Mid-study cycle sample of two mice from each cluster were taken at day 15th in order to evaluate progress of research and final blood samples were collected at 30th day of experimental study for measurement of serum Alanine Aminotransferase (ALT). Blood samples were collected through cardiac puncture and poured in sterile serum tubes. Blood samples were kept at room temperature, separation of serum was done after doing centrifugation (3000 rpm) using bench top centrifuge machine and stored in serum cups.[16] Serum Alanine Aminotransferase (ALT) was measured by using ALT kit (Merck) with Lot No. 505 on Micro lab 200 (Merck) as per IFCC principles.

Statistical analysis:

Analysis of data was done by using SPSS version 20. Final results of the serum analysis were expressed as Means & Standard Error of Means. Post-hoc tukey test was used for doing comparison b/w different groups. P <0.05 was taken as statistically significant.

RESULTS

Table No.1: Mean ± SEM Values of ALT

Animal group no. (n=10)	ALT(17-77 U/L)
Group A	54±5.99
Group B	186.1±66.32
Group C	96.6±15.34
Group D	56 ± 4.89
p-value	<0.001*

*= p - value Significant

(ALT= Alanine aminotransferase)

Table No.2: Post-Hoc Comparison of ALT

Group Comparisons	ALT(17-77 U/L)	
	Mean Difference	p - value
Group A vs. Group B	-132.1	<0.001*
Group A vs. Group C	-42.6	0.079
Group A vs. Group D	4	1
Group B vs. Group C	89.5	<0.001*
Group B vs. Group D	134.1	<0.001*
Group C vs. Group D	45.6	0.048*

Serum Alanine Aminotransferase (ALT) levels were significant raised in drug treated group (B) (p<0.001) as compared to the control group (A) owing to Rifampicin induced hepatotoxicity. Serum ALT levels remarkably restored in group D given high dose of ethanolic extract of the Sumbloo as compared to group C which received low dose of ethanolic extract of the herb. Results are summarized in the given table.

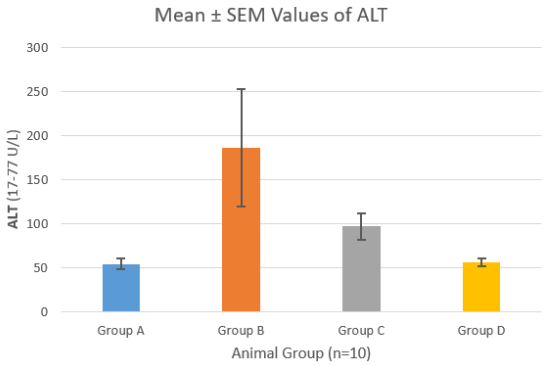

Figure No.1: Comparison of ALT values b/w the groups

DISCUSSION

The experimental study was carried out to detect Rifampicin induced hepatotoxicity by quantitative estimation of serum Alanine Aminotransferase (ALT) and to observe hepatoprotective potential of low and high doses of ethanolic extract of Sumbloo in vivo in male BALB/c mice.

Rifampicin caused highly deranged serum Alanine Aminotransferase (ALT) due to its hepatotoxic potential in Group-B animals with statistically significant difference in values as compared to Group A animals (Control group). Serum Alanine Aminotransferase (ALT) was increased to (186.1±66.32) in drug treated Group-B animals from (54±5.99) in normal Control Group A. Similar results were found by Kim JH et al while doing research study on investigating mechanisms involved in rifampicin induced liver injury during 2017.[6] Ramappa V et al in 2013 found similar results on the study done regarding mechanisms and management of hepatotoxicity related to anti-tuberculosis drugs.[5]

Similar findings were recorded by Issabeagloo in 2012 by producing hepatotoxicity in rats with combination of isoniazid & rifampicin.[17]

Drug induced liver injury is caused by free radical damage and oxidative stress to hepatocytes.[18] Rifampicin causes hepatotoxicity due to enzyme induction of major metabolic pathways including mainly cytochrome P450 (CYP3A4) system leading to increase oxidative stress & hepatotoxicity. [5,6] It is documented that reactive oxygen species (ROS) and free radicals are mainly responsible for inflammatory responses and tissue damage. Antioxidants known as scavenging compounds and play a protective role in reducing the oxidative injury to tissues from free radicals.[19] Since ancient times, herbal medicine is known for its better safety impact.[20] Berberis lycium Royle, is very well known for its hepatoprotective and anti-oxidant activity.[8] It is frequently used for the cure of different diseases. Berberis lycium Royle contains a major alkaloid compound known as berberine.[21] Berberine is highly efficacious in boosting up the immune system of the body.[22] Our research designed to explore the protective potential of low and high doses of ethanolic extract of stem bark of Sumbloo.

This research study results have shown that ethanolic extract of stem bark of Sumbloo possesses remarkable hepatoprotective potential ($p < 0.001$) in dose-dependent manner. Higher dose of ethanolic extract (200mg/kg BW.) reduced serum Alanine Aminotransferase (ALT) level significantly as compared to low dose of ethanolic extract (150mg/kg BW.). A greater upsurge was observed in serum Alanine Aminotransferase (ALT) level in drug treated group B given Rifampicin as compared to Control group A mice which was given no drug. Raised serum serum Alanine Aminotransferase (ALT) level is a specific diagnostic marker of hepatotoxicity.[23] Combined administration of Rifampicin alongwith low and high dose of ethanolic extract of stem-bark of Sumbloo in group C and D restored serum Alanine Aminotransferase (ALT) level to normal mainly due to its anti-oxidant activity. Our experimental results were found in correlation with the work done by Ahmed and his fellows in 2008 on crude powder & methanolic extract of Berberis lycium Royle by inducing paracetamol hepatotoxicity in rabbits.[24]Our experimental results were also found in similarity with comparative study done by Ahmed in 2009 while performing research on Biochemical studies of Berberis lycium Royale and Analysis of its Extracts for their Bioactivity showing greater antimicrobial & wound healing activities of ethanolic & methanolic extract of root bark than its aqueous extract.[25]

CONCLUSION

Ethanolic extract of stem-bark of Sumbloo (Berberis lycium Royle) has significant protective potential for liver toxcity in high doses than low doses in Rifampicin induced hepatotoxicity in male BALB/c mice.

Author's Contribution:

Concept & Design of Study:	Saima Rafique
Drafting:	Aysha Afzal, Sabeen Shakir
Data Analysis:	Khalida Ajmal, Lubna Ghazal, Lubna Ehtizaz
Revisiting Critically:	Saima Rafique, Aysha Afzal
Final Approval of version:	Saima Rafique

Conflict of Interest: The study has no conflict of interest to declare by any author.

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