Original Article



# Liver Toxicity Incited by Rifampicin & its Remedy by Aqueous Extract Preparation of Stem Bark of Barberry (Berberis Lycium Royale) in Male Mice Model

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## ABSTRACT

**Objective:** To investigate the liver toxicity produced by Rifampicin and its amelioration by using aqueous extract of stem-bark of Barberry (Berberis Lycium Royale*)* in male mice.

**Study Design:** Randomized control trial study.

**Place and Duration of Study:** This study was conducted at the animal abode of National Institute of Health (NIH), Islamabad in cooperation with Riphah Institute of Pharmaceutical Sciences (RIPS), Islamic International Medical College (IIMC) & Army Medical College (AMC) for 1 month from April 2014 to May 2014.

**Materials and Methods:** This research study was conducted on 56 male mice. Animals indiscriminately allotted into 4 groups (n=14). Group I: Control group fed on rodent pellet food and tap water. Group II: Drug toxicity induced group put up on Rifampin 50mg/kg BW. Group III: Lower dose aqueous extract group given Rifampicin & (150mg/kg BW) aqueous extract of stem bark of Barberry. Group IV: High dose aqueous extract group kept on Rifampicin & (200mg/kg BW) of aqueous extract of Barberry via gavage tube orally. Baseline blood samples stockpiled at day 0 and serum values of Alanine aminotransferase (ALT) were recorded. Progress of experimental research was evaluated by serum measurement of Alanine aminotransferase (ALT) at day 15. At the end of experimental research on day 30 blood samples were collected by intra-cardiac puncture technique. Serum separated from blood samples were preserved in sterile containers at temperature of $4^{o}$C for analysis of serum Alanine aminotransferase (ALT). SPSS version 20 utilized for analysis of statistical data & p-value (<0.05) regarded as significant.

**Results:** Serum Alanine aminotransferase (ALT) level (17-77 U/L) was recognized as normal range. Group I showed normal level of (56 U/L). Group II displayed extreme drug hepatotoxicity with very high value of (188.3 U/L). Serum value of ALT measured in Group C was (96.6 UL) & (56 UL) in Group D. Aqueous extract of stem-bark of Barberry restored serum Alanine aminotransferase (ALT) levels in group III (98.8 UL) & more remarkable improvement in Group IV with level of (58 UL).

**Conclusion:** Aqueous extract of stem-bark of Barberry (Berberis Lycium Royale) proved amazing protective activity for rifampicin induced liver toxicity in high doses in comparison to less doses.

**Key Words:** Aqueous extract, Barberry, Hepatoprotective, Rifampicin, Alanine Aminotransferase (ALT)

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## INTRODUCTION

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Tuberculosis (TB) prevailed as one of the devastating disease in the history of mankind by affecting one-third of the human population all over the world. In 2018, around 10 million persons suffered from TB, and 1.5 million demise occurred because of tuberculosis.[1]The microbe Mycobacterium tuberculosis is responsible for pathogenesis of tuberculosis. [2] The first line anti-TB drug Rifampicin was discovered in 1957 and approved for therapeutic use in 1968 due to its tremendous sterilizing activity and reducing time span of treatment.[3] Rifampicin acts by binding to DNA dependent RNA polymerase and blocking its action. It stops RNA synthesis of Mycobacterium tuberculosis leading to its demise.[4] t has verified to be one in all the foremost helpful initial line anti-TB agent, however it conjointly causes severe hepatotoxicity because of being a

powerful inducer of varied metabolic accelerator pathways of the body significantly of haemoprotein P450 (CYP3A4) system.[5]

PPARγ signal mechanisms and aerophilic stress were conjointly found to be greatly coupled to Rifampicin-induced toxicity to liver.[6] Medications resulting liver toxicity can be depicted by increased serum markers of liver i.e liver function tests (LFT's).[7] Berberis lycium Royle is famous for its hepatoprotective potential.[8] It is renowned by its numerous regional names like Barberry, Kashmal, Sumbloo, Ishkeen & ziarlargay.[9] Major component of stem-bark of Barberry is Berberine (4.2%) that has vital hepatoprotective effect.[10, 11] Barberry has wide implication in treatment of diabetes.[12] It's been used for polygenic disorders like jaundice, rheumatism, ear & eye infections.[13] The rationale of this randomized control trial intended to investigate hepatoprotective role of the aqueous extract preparation of stem-bark of Barberry (Berberis lycium Royle) in dose dependent manner against rifampicin induced hepatotoxicity in male mice.

# MATERIALS AND METHODS

This randomized control trial study has been conducted in research & analysis institute, National Institute of Health (NIH), Islamabad after getting ethical approval by RARE (Riphah Academy of Research & Education) from 12th April 2014 until 12th May 2014. Male mice of weight 28-50 grams, of age 6-8 weeks having normal serum Alanine aminotransferase (ALT) levels were kept in NIH underneath environment with standardized conditions in wire lidded cages at temperature of (21-24°C) and accustomed for one week.

**Animals' Groups:** Group I was considered control group fed on rodent pellet food and tap water. Group II: Drug toxicity induced group kept on Rifampin 50mg/kg BW[14]. Group III: Low dose aqueous extract group given Rifampicin & (150mg/kg BW) aqueous extract of stem bark of Barberry. Group IV: High dose aqueous extract group put on Rifampicin & (200mg/kg BW) of aqueous extract of Barberry via gavage tube orally.

**Aqueous extract preparation:** Bark of the stem of genus Berberis Lycium Royle (Barberry) stockpiled from one of the small town of Charsadda i.e Prang, Charsadda. A renowned botanist Ghulam Jillani of Peshawar University authenticated it. It was completely washed with water and dried in shade. Dried Barberry was crushed into fine powder by using electric grinder and stored in non-metallic jar. Distilled water was used for soaking fine Barberry powder for 72 with intermittent stirring. Filtration of soaked Barberry powder was done by Whatmann's filter paper no.1. Barberry's filtrate vaporized at 56 $^0$C by using the rotary evaporator at premises of research lab of Riphah Institute of Pharmaceutical Sciences (RIPS), Islamabad. Aqueous extract acquired found to be dark brown in color thick sticky paste in consistency. Air tight small glass bottles were used for its storage in refrigerator at 2-6$^o$C for future use in research. 25% yield was obtained of aqueous extract of Barberry as compare to original dried plant material.

**Collection of Blood Samples:** Two mice from each cluster were used for collection of blood samples by cardiac puncture at day zero as baseline of serum markers. Mid-study cycle blood sample of 2 mice was drawn at day fifteenth from every cluster so as to gauge progress of research. Finally, blood samples of all remaining mice were drawn at the last thirtieth day of research for measuring of serum Alanine Aminotransferase (ALT). Cardiac puncture technique was applied for drawing blood samples and transferred to sterile red cap serum tubes. Bench top centrifuge machine was used for segregation of serum from blood samples by doing centrifugation at (3000 rpm). Segregated serum was transferred for storage in serum cups. [16] ALT kit from Merck company having Lot No. 505 was used for estimation of serum Alanine Aminotransferase (ALT) on Micro lab 200 (Merck) following International Federation of Clinical Chemistry (IFCC) principles.

**Statistical analysis:** Data analysis done on software SPSS 20. Means and Standard Error of Means (S.E.M) calculated from final results of the serum analysis. Comparison between the different groups was done by applying Post-hoc analysis. P-value <0.05 regarded as significant.

# RESULTS

Serum Alanine Aminotransferase (ALT) levels were highly raised in drug treated group II in comparison to control group I due to hepatic damage produced by Rifampicin (p<0.001).

**Table No.1: Mean ± SEM Values of ALT**

| Animal group no. (n=10) | ALT(17-77 U/L) |
|---|---|
| Group I | 56±7.99 |
| Group II | 188.1±68.32 |
| Group III | 98.6±17.34 |
| Group IV | 58 ± 6.89 |
| p-value | <0.001* |

*= p - value Significant (ALT= Alanine aminotransferase, S.E.M= Standard Error of Means)

**Table No.2: Post-Hoc Comparison of ALT**

| Group Comparisons | ALT(17-77 U/L) | |
|---|---|---|
| | Mean Difference | p - value |
| Group I vs. Group II | -134.3 | <0.001* |
| Group I vs. Group III | -44.8 | 0.079 |
| Group I vs. Group IV | 6 | 3 |
| Group II vs. Group II | 91.7 | <0.001* |
| Group II vs. Group IV | 136.3 | <0.001* |
| Group III vs. Group IV | 47.8 | 0.050* |

Serum ALT levels were strikingly normalized in group IV kept on high dose of aqueous extract of Barberry in comparison to group III which were given low dose of aqueous extract of the herb. Results have been briefed in the given table I. Post hoc comparison of ALT is also shown in table 2.

# DISCUSSION

This randomized control trial was conducted to identify the degrees of hepatotoxicity produced by Rifampicin by measuring serum Alanine Aminotransferase (ALT) levels and to explore hepatoprotective activity of low & high doses of aqueous extract of Barberry in vivo in male mice. Serum Alanine Aminotransferase (ALT) levels were highly deranged in Group-II animals due to hepatotoxic nature of rifampicin with statistically significant difference in values between Group I & Group II. Serum Alanine Aminotransferase (ALT) levels were highly raised to (188.1±68.32) in Group-II mice from (56±7.99) in Group-I. Kim JH et al in 2017 observed alike results during research on investigating mechanisms involved in rifampicin induced liver injury.[6] Analogous findings were detected by Ramappa V et al in 2013 while studying mechanisms & management of hepatotoxicity related to anti-tuberculosis drugs.[5] Comparable results were noticed by Issabeagloo in 2012 by triggering hepatotoxicity in rats with combination of rifampicin & isoniazid.[17]Free radical ions and oxidative stress produced by the drug leads to severe damage to hepatocytes.[18] Hepatotoxicity produced by rifampicin is because of induction of many oxidizing enzymes specifically of cytochrome P450 (CYP3A4) enzyme system resulting in increase in oxidative trauma & hepatotoxicity. [5,6] It is widely accepted regarding reactive oxygen species (ROS) & free radicals being main culprit of inflammatory cascade and subsequently tissue damage. Antioxidants are scavenging compounds by nature and play a vital role in reducing oxidative stress to the tissues from free radical ions.[19] Herbal medicine are known for their excellent safety profile since earliest times.[20] Berberis lycium Royle, (Barberry) is widely recognized for its anti-oxidant & hepatoprotective activity.[8] Barberry is commonly used for different ailments. Major constituent of Barberry is berberine.[21] It is tremendously effective in improving body's immune system.[22] Our randomized control trial was planned to investigate the hepatoprotective activity of lower & higher doses of aqueous extract of stem bark of Barberry. This randomized control trial manifested that aqueous extract of Barberry have astonishing hepatoprotective potential ($p < 0.001$) in dose-dependent manner. Higher dose of aqueous extract (200mg/kg BW) significantly dropped serum Alanine Aminotransferase (ALT) levels in comparison to low dose of aqueous extract (150mg/kg BW.). A higher peak was depicted in serum Alanine Aminotransferase (ALT) levels in group II kept on Rifampicin as compare to Group-I without administration of any drug. Increased serum Alanine Aminotransferase (ALT) level is a key diagnostic criteria of hepatic damage.[23] Concurrent administration of Rifampicin besides with lower and higher doses of aqueous extract of stem-bark of Barberry in group III and IV re-establish serum Alanine Aminotransferase (ALT) to normal level owing to its anti-oxidant potential. Khan & his colleagues in 2011 observed heaptoprotective effects of aqueous extract of Berberis Lycium Royale (Barberry) in combination with Gallium aparine & Pistacia Integerrima in Ccl4 treated rabbits.[24] Similar findings established in the research done on hepatoprotective activity of Berberis Lycium in 6 herbal formulations alongwith Livokin (Herbo-med, Kolkata) on hepatotoxicity produced by paracetamol in mice model.[25]

# CONCLUSION

The remunerative effect of aqueous extract of stem bark of Berberis Lycium Royale (Barberry) is proven in hepatotoxicity and this herb has promising potential in ameliorating liver function at low & high doses in drug Rifampicin induced liver damage in male mice.

**Recommendations:**

- Other serum LFT's should also have been performed.
- Histopatholgical studies of liver should also be planned for future research.
- Active components of stem bark of Barberry (Berberis lycium Royle) extracts should be isolated and evaluated regarding their hepatoprotective potential.

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**Author's Contribution:**

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|---|---|
| Concept & Design of Study: | Saima Rafique |
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**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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