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Hepatoprotective Effects of Silymarin and Walnut Leaf Extract in Isoniazid and Rifampicin Induced Hepatotoxicity

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ABSTRACT

Objective: To evaluate the hepatoprotective effects of Silymarin and walnut leaf extract in isoniazid-rifampicin induced hepatotoxicity in Sprague-Dawley rats.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad Pakistan, from Jun to Jul 2023.

Methodology: A total of 50 rats were divided in 5 groups containing 10 rats each. Group-A was control group, in Group-B hepatotoxicity was induced by isoniazid 50mg/kg/day and Rifampicin 100 mg/kg/day co-administration daily for 14 days, Group-C was given isoniazid 50mg/kg/day and rifampicin 100 mg/kg/day and 200 mg/kg/day silymarin for 14 days , Group-D was given isoniazid 50mg/kg/day and rifampicin 100 mg/kg/and walnut leaf extract was administered at a dosage of 0.2 g/kg one hour before isoniazid, Group-E was given isoniazid 50mg/kg/day and rifampicin 100 mg/kg/day, silymarin 200 mg/kg/day and walnut leaf extract 0.2 g/kg orally for 14 days. On day fifteen, terminal blood sampling was done for estimation of serum Aspartate aminotransferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP).

Results: Isoniazid and Rifampicin produced severe hepatotoxicity, depicted by raised mean serum AST (168.20±4.85), ALT (233.60±29.98) and ALP (288.30±19.51). Mean AST (53.30±7.32), ALT (51.80±7.21) and ALP (133.70±7.42) levels were significantly decreased in Group-E, followed by groups C and D.

Conclusion: The combination of silymarin and walnut leaf extract is more effective in preventing liver damage as compared to their separate effects.

Keywords: Drug-Induced Liver Injury, Isoniazid, Rifampicin, Silymarin, Tuberculosis.

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INTRODUCTION

Tuberculosis (TB) is caused by infection with Mycobacterium tuberculosis, and globally continues to be a leading cause of mortality. Annually, over 10 million people contract tuberculosis, leading to approximately 2 million deaths each year.[1] In 2020, Pakistan held the sixth position among countries contributing significantly to the worldwide gap in TB notifications. The reported incidence in Pakistan was 259 per 100,000 new TB cases annually, with approximately ony 48% of these cases receiving treatment.[2] Anti-TB drugs are divided into, first-line and second-line drugs. First-line drugs include rifampicin (RIF), isoniazid (INH), ethambutol (E), and pyrazinamide (Z). Anti-TB drug-induced liver injuries (ATLIs), are the most frequent of these medications when taken in combination.[3] INH induced toxicities include neuropathy and hepatotoxicity. INH-induced hepatotoxicity has no cure other than closely watching patients for liver damage. Rifampicin also causes hepatotoxicity by accelerating the metabolism of isoniazid.[4,5]

Drug-induced liver injury (DILI) is the leading cause of acute liver failure, characterized by liver or biliary system damage resulting from the administration of hepatotoxic medications.[6]

Juglans regia commonly known as the walnut, is the most extensively cultivated tree globally.[7] Walnut leaf extract has a strong hepatoprotective effect. Antioxidant properties of walnut leaf extract, which may be due to its flavonoids, polyphenols, and other components, prevent free radical injury.[8] Silymarin, a hepatoprotective substance and bioactive component of Silybum Marianum (SMR).The use of SMR in the herbal therapy of chronic liver disease and its remarkable clinical efficacy have earned it widespread recognition.[9]

This study aimed to investigate the hepatoprotective properties of both walnut leaf extract and silymarin individually, as well as their combined and comparative effects.

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METHODOLOGY

This laboratory-based experimental study was conducted in Department of Pharmacology and Therapeutics, Shaheed Zulfiqar Ali Bhutto Medical University in collaboration with Riphah International University for preparation of extract and provision of animals from National Institute of Health (NIH), Islamabad, Pakistan, after receiving approval from Institutional Ethical Review Committees (No.F.1-1/2015/ERB/SZABMU/1094 and F.No.20-4/ASandRB-M/SZABMU/2023) from June to July 2023.

Inclusion Criteria: Sprague-Dawley Male rats with body weight of 200–230gm were included.

Exclusion Criteria: Inactive Rats and rats having a prominent deformity were excluded.

The study involved 50 healthy male Sprague-Dawley rats, weighing 200–230 gm. The rats had unrestricted access to tap water, and the standard feed provided to them was prepared at the National Institutes of Health (NIH). The lighting conditions were set to follow a 12-hour cycle, alternating between light and darkness. The leaves of Juglans regia (Walnut), obtained from the surrounding area of Muzaffarabad, Azad Kashmir, were carefully dried in shade and then finely ground into a powder. A total of 1,000 grams of the powdered J. regia leaves were soaked in 70% ethanol for a period of 24 hours. To ensure thorough extraction, the process was repeated three times using fresh 96% ethanol, with each extraction lasting 24 hours at room temperature. Subsequently, the resulting solution was filtered through Whatman #1 paper and further subjected to evaporation in a rotary evaporator at a temperature of 55°C. The obtained extract was carefully stored in a sealed glass bottle, ensuring an airtight environment and protection from light. To maintain its integrity, the bottle was refrigerated at a temperature range of 2 to 8 degrees Celsius.[4] To induce hepatotoxicity, a combination of Isoniazid at a dosage of 50 mg/kg/day and Rifampicin at a dosage of 100 mg/kg/day was administered orally as a single morning dose daily for a duration of 14 days.[10] After two weeks, terminal sampling was carried out.

After a week of acclimation, the rats were randomly assigned to two groups: Group-A, comprising 10 rats as the control group, and experimental group consisting of 40 rats. Group-A was provided with regular food for a period of 14 days. Subsequently, the experimental group were subdivided into Groups B, C, D, and E. Group-B rats were administered a co-administration of Isoniazid at a dosage of 50 mg/kg/day and rifampicin at a dosage of 100 mg/kg/day, given as a single morning dose orally, each day for a duration of 14 days. Group-C rats received a co-administration of isoniazid at a dosage of 50 mg/kg/day and rifampicin at a dosage of 100 mg/kg/day as a single morning dose orally, each day for 14 days. In addition, silymarin was orally administered at a dosage of 200 mg/kg/day for the same duration of 14 days. Group-D rats were given a co-administration of isoniazid at a dosage of 50 mg/kg/day and rifampicin at a dosage of 100 mg/kg/day as a single morning dose orally daily for 14 days. Additionally, walnut leaf extract was administered at a dosage of 0.2 g/kg one hour before INH, spanning a total time interval of 2 weeks. Group-E rats received a co-administration of isoniazid at a dosage of 50 mg/kg/day and rifampicin at a dosage of 100 mg/kg/day, daily as a single morning dose orally for 14 days. Additionally, silymarin was administered orally daily at a dosage of 200 mg/kg/day for 14 days. Furthermore, walnut leaf extract was given at a dosage of 0.2 g/kg one hour before INH, over a total time interval of 2 weeks. Terminal blood sampling was carried out after 15 days of treatment and samples were sent for liver function tests.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 27 software. The results were presented as mean and standard deviation. One-way ANOVA, followed by the post-hoc Tukey test, were applied to compare the quantitative parameters among the five groups. A significance level of $p<0.05$ was used to determine statistical significance.

RESULTS

Value of mean AST (U/L) of Group-B was highest (168.20±4.85) followed by Group-D (95.20±7.08), Group-C (87.80±13.34), Group-E (53.30±7.32) and Group-A was (50.40±6.96). The value of mean ALT (U/L) was highest in Group-B (233.60±29.98) and lowest for Group-E (51.80±7.21). Likewise, the value of mean ALP (U/L) was highest for Group-B (288.30±19.51) followed by Groups C, D (191.90±8.81) and then Group-E (133.70±7.42). Table-I displays the mean and standard deviations of AST, ALT and ALP levels of all study groups.

A highly significant difference in mean values of AST, ALT and ALP was found between groups A and

B, groups A and C, groups A and D, groups B and C, groups B and D, and groups D and E. Table-II shows Inter Group Comparison of mean AST, ALT and ALP of all study groups (n=10).

Table-I: Comparison of Mean Aspartate Transaminase of across Groups (n=50)

Parameters	Group-A (n=10) Mean±SD	Group-B (n=10) Mean±SD	Group-C (n=10) Mean±SD	Group-D (n=10) Mean±SD	Group-E (n=10) Mean±SD	*p*-value
AST (U/L)	50.40±6.96	168.20±4.85	87.80±13.34	95.20±7.08	53.30±7.32	<0.001
ALT (U/L)	39.80±5.20	233.60±29.98	86.90±8.33	93.70±6.94	51.80±7.21	<0.001
ALP (U/L)	131.30±4.99	288.30±19.51	191.90±8.81	191.20±7.39	133.70±7.42	<0.001

**ALT:Alanine Transaminase), ALP: Alkaline Phosphatase, AST: Aspartate Transaminase*

Table-II: Post-Hoc Tukey Test showing inter-group Comparison of Liver Function Tests across Groups (n=50)

Group Comparison	Group-A Vs. Group-B	Group-B Vs. Group-C	Group-A Vs. Group-C	Group-B Vs. Group-D	Group-A Vs. Group-D	Group-A Vs. Group-E	Group-C Vs. Group-D	Group-D Vs. Group-E
AST (U/L)	<0.001	<0.001	<0.001	<0.001	<0.001	0.938	0.298	<0.001
ALT (U/L)	<0.001	<0.001	<0.001	<0.001	<0.001	0.397	0.841	<0.001
ALP (U/L)	<0.001	<0.001	<0.001	<0.001	<0.001	0.988	0.100	<0.001

**ALT:Alanine Transaminase), ALP: Alkaline Phosphatase, AST: Aspartate Transaminase*

DISCUSSION

The liver, the body's largest solid organ, is crucial for metabolism and detoxification, making it highly vulnerable to toxins. It also plays a key role in drug metabolism, nutrient processing, and waste excretion.[11] Due to its role in metabolizing and eliminating drugs and chemicals, the liver can suffer toxicity from these substances or their metabolites. Drug-induced liver injury (DILI) can result from direct damage, where drugs convert to active metabolites that harm cellular macromolecules, or from indirect immune responses. Direct injury involves lipid peroxidation, protein malfunction, nucleic acid damage, and oxidative stress. Many medications are known to potentially cause DILI.[12]

Liver injury from anti-TB drugs is a major cause of acute liver failure (ALF), and current liver protection treatments are often ineffective. This highlights the urgent need for regenerative medicines like silymarin, which is in high demand due to its antioxidant properties and effectiveness in preventing liver damage.[10]

The current study aimed to observe the reversal of hepatotoxic effects of isoniazid and rifampicin by giving silymarin and walnut leaf extract via estimation of AST, ALT, ALP and histopathological parameters for hepatoxicity. Increased levels of serum biomarkers are important for assessing the degree of liver damage experimentally.[13]

Our results showed that INH and RIF induced hepatotoxic changes were prevented by both silymarin and walnut leaf extract, but the combined effects of both showed a better hepatoprotective effects.

We induced hepatotoxicity by co-administration of Isoniazid 50mg/kg/day and Rifampicin 100 mg/kg/day as single morning dose per oral daily for 14 days.[14] This is in accordance with the study done by Dubiwak et al. who used INH and RIF to induce hepatoxicity.[15] This study showed histo-pathological changes secondary to use of INH and RIF. These findings includes inflammation and necrosis which are consistent with the study conducted by Lian *et al.,* in 2013 who demonstrated INH-RIF induced hepatotoxicity in liver biopsy specimens.[16] The INH-RIF treated group showed the histological picture of inflammatory cell infiltration with hypertrophy of hepatocytes, vacuolar degeneration, fatty degeneration and necrosis which are all signs of acute inflammation.

The use of silymarin resulted in prevention of hepatic damage in the rats. Emphasis on silymarin was given in this present study due to its common usage as hepatoprotective agent in cases of hepatitis. There was a significant difference in the ALT, AST and ALP level between the control, Group-B (ALT= 233.60±29.98, AST= 168.20±4.85 and ALP= 288.30±19.51) and Group-C (ALT=86.90±8.33, AST= 87.80±13.34 and ALP= 191.90±8.81). This finding was consistent with Mukhtar *et al.,* who studied hepatoprotective effect of silymarin against drug induced hepatotoxicity.[9] Eminzade *et al.,* also found hepatoprotective effect of silymarin against INH-RIF induced hepatoxicity.[17]

Group-D was treated with 0.2g/Kg walnut leaf extract in which we observed well-established hepatoprotective effects, after inducing hepatotoxicity. This study a highly significant improvement in serum AST, ALT and ALP with a *p*-value <0.001, along with and prevention of liver damage in histopathology specimen. According to the results, reversal of the hepatic damage in rats was seen with the usage of both silymarin and walnut leaf extract. These findings were consistent with a study conducted by Eidi *et al.,* who studied hepatoprotective effects of walnut leaf extract in CCl4 induced liver injury in rats.[18]

This study showed that combination of Silymarin and walnut leaf extract suggest synergistic effects of these compounds when used together. Hence walnut leaf extract can be used as adjunct to Silymarin for the treatment of liver ailments.

LIMITATION OF STUDY

Due to financial constraints our study was small scale study which involved only 50 rats and measured only few parameters of liver function tests.

CONCLUSION

Silymarin and walnut leaf extract effectively and synergistically ameliorate hepatotoxicity induced by INH-RIF. Individually in comparison, silymarin is slightly superior to walnut leaf in hepatoprotection. The synergistic impact of combining walnut leaf extract and silymarin was found to be more significant in mitigating hepatotoxicity compared to the individual use of silymarin or walnut leaf extract alone. This highlights the need for further investigation into the active components of walnut leaves and their potential interactions with other medications.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MA & MK: Data acquisition, data analysis, critical review, approval of the final version to be published.

AAR & IS: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

FR & SMA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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