ORIGINAL ARTICLE DOI: https://doi.org/10.35845/abms.2026.1.522 Advances in Basic Medical Sciences

Hepatoprotective Effect of Aqueous Azadirachta indica Leaf Extract Against Erythromycin-Induced Changes in Albino Wistar Rats

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ABSTRACT

OBJECTIVE: To evaluate the hepatoprotective activity of aqueous neem leaf extract on erythromycin-induced loss in hepatocyte count of albino wistar rats.

METHODOLOGY: Eighty male albino Wistar rats were included in the experiment and divided into four groups. Group A was an untreated control. Group B received Erythromycin alone, while Group C received both Erythromycin and aqueous neem leaf extract. Group D was treated only with neem extract. Body weight was recorded before and after treatment. After sacrifice, livers were processed for H&E staining. Hepatocyte count was assessed in ten random microscopic fields per liver section at 100X magnification using a calibrated ocular reticule. Data were analyzed by one-way ANOVA with Tukey's post hoc test (SPSS v23); $P \leq 0.05$ was considered significant.

RESULTS: Group A maintained a normal hepatocyte count ($16.13 \pm 0.44 \mu m^2$), consistent with intact liver tissue. A noticeable reduction was observed in Group B ($8.94 \pm 0.43 \mu m^2$), indicating significant hepatocellular loss following liver injury. In Group C, hepatocyte count increased to $11.63 \pm 0.46 \mu m^2$, suggesting partial restoration of hepatic tissue. Group D showed a hepatocyte count of $15.76 \pm 0.35 \mu m^2$, which was comparable to the control group and may indicate a protective effect with preservation of normal liver morphology. For Hepatocyte count using Kruskal wallis test and $p<0.05$..

CONCLUSION: The aqueous neem leaf extract obtained from Azadirachta indica showed a notable protective effect by improving hepatocyte count and preserving liver architecture. This beneficial effect can be attributed to the strong antioxidant properties of neem, which help in reducing oxidative stress and limiting cellular injury.

Keywords: Erythromycin, Hepatotoxicity, Hepatocytes, Hemorrhages

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Date of submission: 07-05-2026

Date of acceptance: 18-06-2026

Date of publication: 30-06-2026

INTRODUCTION

The liver is a unique organ with self-regenerative capacity, serving a dual function of excretion and secretion. Its primary function is to detoxify endogenous substances. As a result, the liver is exposed to various toxic agents such as xenobiotics, environmental pollutants, alcohol consumption, and viral infections. Structurally, the liver is a soft, highly vascular organ with a pinkish-brown color and smooth surface. It is composed mainly of parenchyma, which contains the functional liver cells known as hepatocytes.[1]

Microscopically, each liver lobe is made up of hepatic lobules that form the basic structural and functional units of the liver. Within these lobules, hepatocyte plates are arranged in a radiating pattern around the central vein, allowing blood flow and metabolic exchange. Blood from the hepatic artery and portal vein passes through sinusoids, where detoxification, metabolism, and nutrient processing occur.[2] The liver also plays a crucial role in bile production, which aids digestion and absorption of fats. In addition, it stores essential nutrients such as glycogen, vitamins, and minerals for use by the body. The liver is responsible for synthesizing plasma proteins, including albumin and clotting factors. Because of these metabolic, storage, and detoxifying functions, the liver is considered one of the most important organs for maintaining physiological health.[3]

Erythromycin is a macrolide antibiotic that inhibits bacterial growth by interfering with protein synthesis. It acts by attaching to the 50S subunit of the bacterial ribosome, blocking formation of proteins necessary for bacterial growth and reproduction. Due to its effectiveness and safe therapeutic profile, erythromycin is widely used in clinical practice. It is commonly

This Article may be cited as: Jamshed N,Rasheed A,Raza I,Tabassum A,Khan A,Rashid S.Hepatoprotective Effect of Aqueous Azadirachta indica Leaf Extract Against Erythromycin-Induced Changes in Albino Wistar Rats.Adv Basic Med Sci.2026;10(1): 40 - 47. DOI: https://doi.org/10.35845/abms.2026.1.522

prescribed for infections such as tonsillitis, urinary tract infections, bronchopneumonia, mastitis, arthritis, and lymphadenitis. Moreover, it is active against susceptible microorganisms such as Chlamydia, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Pasteurella, and Mycoplasma. Erythromycin is effective against gram-positive bacteria and some gram-negative organisms. It is often used as an alternative treatment for patients allergic to penicillin antibiotics. The drug is widely prescribed for respiratory tract infections such as pneumonia and bronchitis. It is also useful in treating skin and soft tissue infections.[4] It has the ability to penetrate body tissues, increasing its therapeutic effectiveness. It is available in tablets, capsules, suspensions, and topical preparations. Due to its broad spectrum of antibacterial activity, erythromycin remains an important drug in the treatment of bacterial infections. The drug also plays an important role in managing sexually transmitted infections caused by Chlamydia.[5]

However, when erythromycin stearate was given orally to Wistar rats for 14 days, a daily dose of 100 mg/kg body weight resulted in marked liver injury and raised ALT and alkaline phosphatase enzymes, and hepatotoxicity. This exposure caused structural alterations in hepatic tissue, reflecting dose-dependent cellular damage and impaired liver function over the treatment period.[6]

In pharmacognosy, Azadirachta indica (Neem) is widely acknowledged as a medicinal plant with significant therapeutic potential, often referred to as the King of the Therapeutic Plant Kingdom due to its bioactive constituents. Phytochemical analyses of neem leaves have identified triterpenoids, sesquiterpene lactones such as azadirachtin, limonoids, nimbinin, quercetin, and β-sitosterol, all contributing to its pharmacological efficacy. Limonoids are among the most significant bioactive compounds, exhibiting antineoplastic, antimicrobial, and insecticidal properties.[7]

Neem possesses many medicinal and biological properties. It has antioxidant effects and is known for its anti-cancer potential. Neem also shows antibacterial and antiviral activities, helping the body fight harmful microorganisms.[8] It also shows protective effects that may help reduce inflammation in the body. Traditional use suggests it can support overall immune health and resistance against minor infections. Some studies also indicate its role in promoting general well-being when used in appropriate amounts. Because of these properties, it is valued in herbal medicine for multiple therapeutic benefits.[9]

Moreover, neem has anti-inflammatory properties. It also helps purify the blood and strengthen the immune system. Because of its antifungal and skin-healing benefits, neem is often used to treat acne, rashes, and other skin infections. Neem extract is known for its antioxidative activity, which helps protect the liver from damage.[10]

METHODOLOGY

A randomized, controlled experimental trial was conducted in Baqai Medical University Karachi, from 1-Jan- 2022 to 15-March-2022, after approval by the institutional ethical committee BMU-EC/2021-05. In this study, we used 80 male Wistar rats weighing 180 to 200 g, ranging in age from 13 to 14 weeks, that were purchased from the Animal House of Baqai Medical University. Male Wistar rats were housed in a controlled environment with a temperature of 30°C and a cycle of 14 days and 10 nights and were placed in plastic cages with 5 rats per cage.

The rats were provided adequate nutrition and drinking water, and they were acclimatized for approximately 10 days before beginning the study. Erythrocin R (Erythromycin 500 mg tablet), produced was purchased from a pharmacy in Malir Cantt.

Preparation of Aqueous Neem Leaf Extract: At the Pakistan Council of Scientific and Industrial Research (PCSIR), Fresh neem leaves were washed thoroughly with distilled water to remove dust and contaminants. The cleaned leaves were dried and ground into a fine powder. About 100 g of the powder was mixed with distilled water in a 1:10 ratio and heated on a hot plate to extract the active compounds. After cooling, the mixture was filtered through Whatman No. 1 filter paper to obtain a clear extract. The prepared extract was then used for the experiment.

Young and healthy rats were selected for the experiment. Weak and older rats, as well as female rats, were excluded from the study. 80 young, healthy male rats were chosen for the study. The sample size was calculated using Open EPI for comparison of two means; however, the sample size was increased to account for reliable and valid results, biological variability, and meaningful statistical analysis.[12] Randomization: In total, 80 male rats were randomly divided into four groups; each group consists of 20 rats.

Group A was designated as the Control group and did not receive treatment; instead, the animals were fed their regular diets.

On the other hand, Group B was administered Erythromycin at a dosage of 100 mg/kg of body weight daily for 14 days through the use of gastric gavage. Group C was administered erythromycin at a dosage of 100 mg/kg of body weight (as this dose is hepatotoxic[6]) and the aqueous Neem extract at a dosage of 500 mg/kg of body weight (as this dose is hepatoprotective[12]) via gastric gavage. Group D only received aqueous Neem extract, in the hopes of achieving a dosage of 500 mg/kg of body weight via gastric gavage.

All animals were weighed utilizing an electronic weighing scale before the start of the study and again every 3 days. The animals were kept in plastic cages.

Following the administration of the last dose of treatment, all of the animals were weighed and then anesthetized. After anesthetizing the animals, a midline incision was created from the manubrium to the lower abdomen in order to expose the internal organs.

The data were analyzed using SPSS version 23. The results are shown as mean ± standard error (SE). ANOVA with Tukey's post hoc test was used to identify significant differences between groups, with a P-value ≤ 0.05 indicating statistical significance.

Micrometric measurements were used to assess the morphometric variations. An ocular micrometer was mounted in the right eyepiece, and a stage micrometer was mounted on the microscope stage. A calibration of the ocular scale was performed by means of the stage micrometer, which has typical spacing of its divisions at 0.01 mm (10 µm). The ocular micrometer was mounted into the right eyepiece, and the stage micrometer onto the stage. Low magnification (4 X objective with 10 X eyepiece) was used so that the scale divisions could be focused for precise measurements. The ocular and stage micrometer scales were made parallel, and the number of ocular divisions that exactly fit the stage micrometer divisions was counted. For this particular instance, 2 ocular divisions equated to 5 stage micrometer divisions. The least count, or calibration factor, of the ocular micrometer was therefore calculated.

The least count of the ocular micrometer or calibration factor can be determined by: (1) 2 ocular divisions equaled 5 divisions (5 x 10 µm = 5 µm) of the stage micrometer. Then (1) ocular division = 50µm ÷ 2 divisions = 25 µm. Therefore, (1) division of ocular = 0.025mm or 25 µm. At 10X objective with a 10X eyepiece, the stage micrometer was aligned with the ocular micrometer. In this configuration, 10 ocular divisions matched 10 stage micrometer divisions, thus each ocular division was equal to 0.01 mm (10 µm).

At the higher magnification (40X objective, 10X eyepiece), 60 ocular divisions corresponded to 15 stage micrometer divisions. Hence, the value of 0.0025 mm (2.5 µm) per ocular division.

At the highest magnification (100X objective, 10X eyepiece), 50 ocular divisions coincided with 5 stage micrometer divisions. Therefore, 1 ocular division equaled 0.001 mm (1 µm).

Liver cells (hepatocytes) and their nuclei were measured at this magnification. Cells having clearly visible nuclei were selected from four random areas of the liver sections. Size was then computed. The diameter of the cell occupied 5 divisions of the ocular. Cell diameter would be 6 division x1um =6um. The ocular reticule, consisting of a 10 x 10 grid (100 small squares), was located in the left eyepiece and was calibrated at 100X magnification. Four reticule squares matched one stage micrometer division in both axes. Therefore, 1 square = 0.0025 mm (2.5 µm). Length of 10 squares = 25 µm, area of 100 squares = 625 µm² (0.000625 mm²). Ten random microscopic fields were taken in each liver section; hepatocytes were counted in each field within the 100-square grid.

RESULTS

The mean value of the hepatocyte count per microscopic field in group A animals was recorded as 16.13 ± 0.44 µm². While in group B, erythromycin-treated rats, their mean values of hepatocyte count per field in animals were 8.94±0.43µm². There was a significant reduction ($p<0.01$) in the number of hepatocytes recognized in group B animals, when the difference in mean compared to the control group A animals, as shown in the given table-1. The results of erythromycin plus neem-treated rats of group C, the mean values of hepatocyte count per field were 11.63±0.46µm². There was a marked decrease ($p<0.05$) in the mean cell count of animals in group C in comparison with the control group A animals, but markedly increase in the mean hepatocyte counts ($p<0.01$) in group C animals as this group was given erythromycin and aqueous neem leave extract which showed improvement in hepatocytes count, compared to group B hepatocyte count where hepatocyte count is significantly decrease this group only given erythromycin , as shown in the given table-1. Furthermore, the Mean values of group D positive control hepatocyte count per field were 15.76±0.35µm² as mentioned in the given table-1. The mean hepatocyte count in group D showed a slight decrease when compared with the control group A, but this reduction was statistically insignificant ($p>0.05$). However, when group D was compared with group B and group C, a significant increase in the average hepatocyte count was observed. These findings are presented in Tables 1 and 2.

Overall, the results show clear differences among the experimental groups. The erythromycin-treated group showed a strong decrease in hepatocyte count, indicating clear liver damage. In contrast, the group treated with both erythromycin and neem extract showed improvement in hepatocyte count, suggesting partial recovery of liver structure. The positive control group showed values close to the normal control group, indicating minimal changes. These findings suggest that neem leaf extract may help protect liver cells against erythromycin-induced damage. The gradual improvement from group B to group C highlights the protective effect of neem in restoring hepatocyte health.

GROUP A (control Group)-400X

Histologically, the liver appears to be structurally normal. Liver cells (hepatocytes) form radially around the central veins. Sinusoids appear thin and defined, both structures that indicate a normal structural status. Nuclei in the hepatic cords appear centralized and are of a large size. There is no evidence of inflammation, atrophy as shown in Figure a.

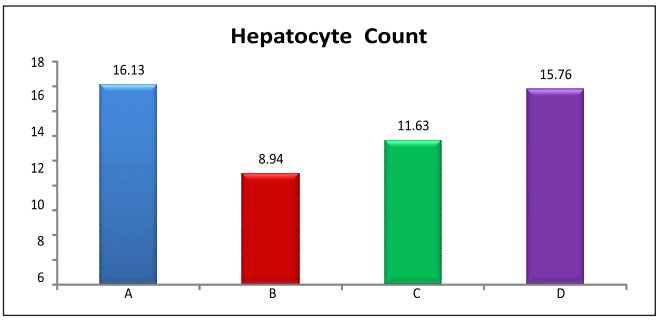
Table 1: Mean Comparison of Hepatocyte Count µm² of Animals

Group	Treatment	Hepatocyte Count µm² Mean ±SD
A (n=20)	Control	16.13±0.44
B (n=20)	Treated	8.94±0.43
C (n=20)	Protected	11.63±0.46
D (n=20)	Positive Control	15.76±0.35
*p<0.05 was obtained using Kruskal wallis test	*p<0.05 was obtained using Kruskal wallis test	*p<0.05 was obtained using Kruskal wallis test

Table 2: Statistical Analysis of Differences in Hepatic Cell Count per Reticule µm²of Rats between Different Study Groups

Comparison	Statistical Comparison	Difference of Means	*p*-value
A vs B	Control vs Treated	7.19	<0.01*
A vs C	Control vs Protected	4.5	<0.01*
A vs D	Control vs Positive Control	0.37	0.06
B vs C	Treated vs Protected	-2.69	<0.01*
B vs D	Treated vs Positive Control	-6.82	<0.01*
C vs D	Protected vs Positive Control	-4.13	<0.01*

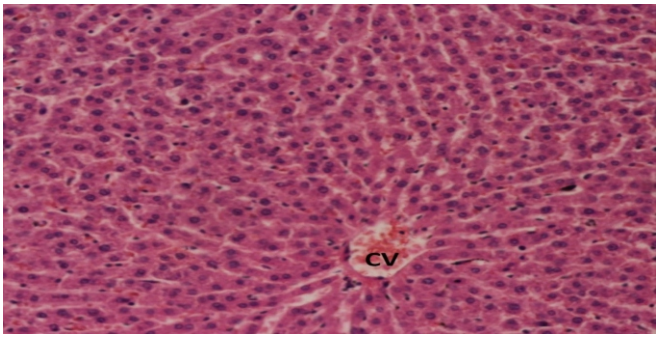
Figure 1: Hepatocyte Count



Group A(control group)400X

Histologically, the liver appears to be structurally normal. Liver cells (hepatocytes) form radially around the central veins. Sinusoids appear thin and defined, both structures that indicate a normal structural status. Nuclei in the hepatic cords appear centralized and are of a large size. There is no evidence of inflammation, atrophy, or degeneration as shown in Figure 2.

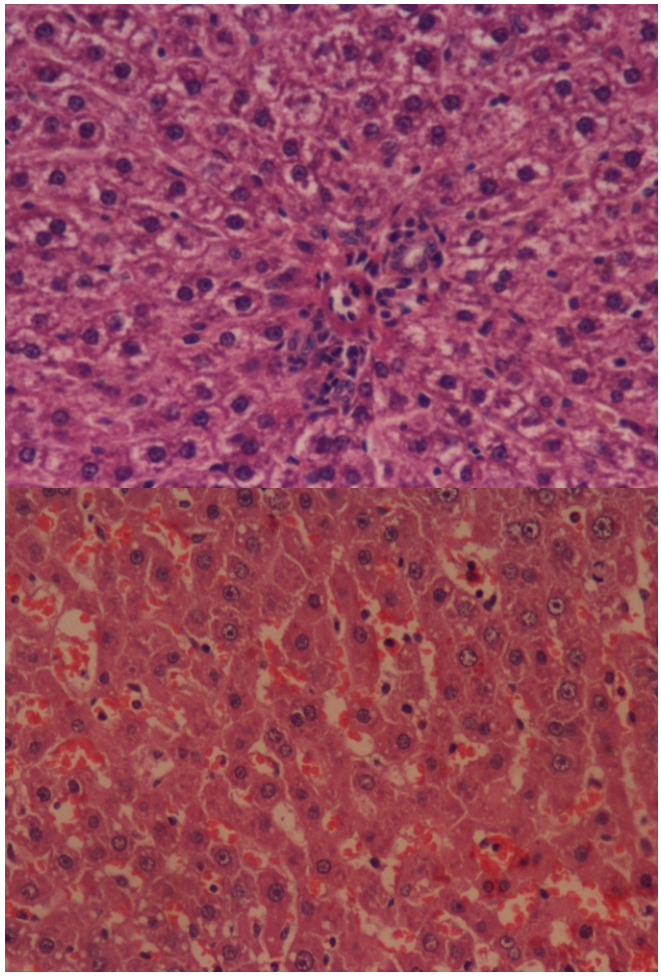
Figure 2: Group A (control Group) - 400X



GROUP B (ERYTHROMYCIN TREATED RATS)-400X

The Histopathology image clearly shows that Erythromycin causes structural damage to the Liver. The liver has been damaged, as evidenced by hepatocyte pyknosis and visible fat degeneration inside the cells. The cell nucleus appears to be at the edge of the cytoplasm in a few instances, and this indicates cellular stress. Additionally, it can be seen that there is less than normal amount of liver cells and therefore there is some degree of damage to the liver. All of these findings show that there was a significant disruption of normal liver structure and function due to the toxic effects of drugs.

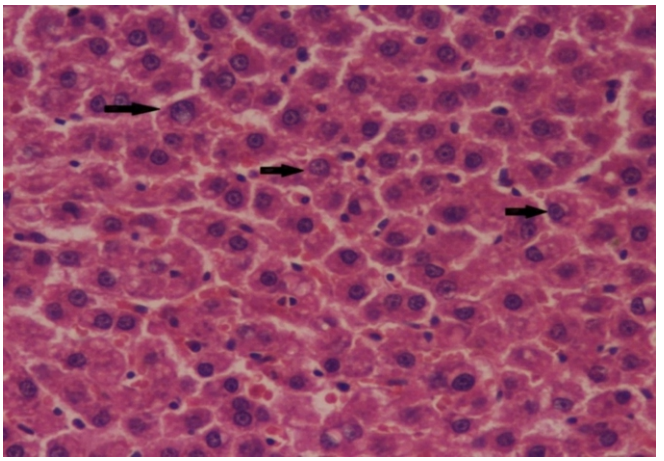
Figure 3 (a)(b): Group B (Erythromycin Treated Rats) - 400X



GROUP C (ERYTHROMYCIN AND NEEM TREATED RATS)-400X (H&E)

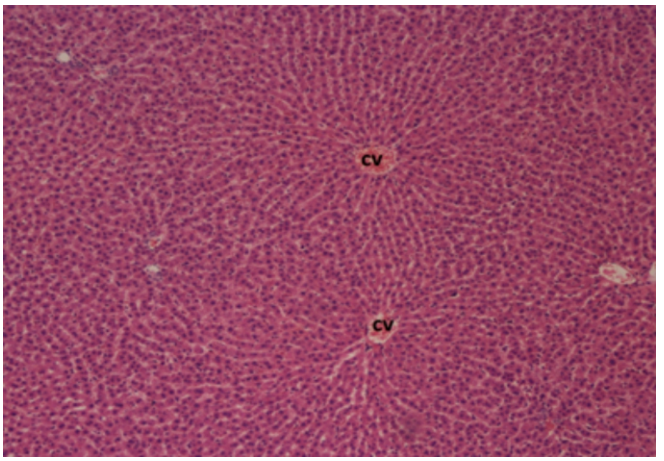
The histopathological image shows mild alterations in liver tissue structure. Hepatocytes appear slightly increased in size, and a mild congestion is present within the tissue. Overall, the liver architecture is largely preserved, with only minimal changes suggesting a mild degree of tissue response without significant damage as shown in Figure 4.

Figure 4: Group C (Erythromycin and NEEM Treated Rats) 400X (H&E)



The histopathology images of GROUP D (positive control) at 100x H&E show normal liver architecture. Hepatocytes are arranged in a branching pattern around the central vein and portal triads. The tissue appears well preserved, with no signs of congestion or inflammatory cell infiltration. Overall, the liver structure remains intact, indicating healthy and stable hepatic morphology. These findings suggest normal cellular organization and proper liver function, with no evidence of toxic damage or pathological alterations as shown in Figure 5.

Figure 5: Group D (positive control) at 100x H&E of histopathological image of normal liver



DISCUSSION

Hepatic injury, caused by various agents including toxic chemicals, medicines, alcohol, and viral hepatitis, often shares a unifying mechanism of cellular damage. A central mechanism in hepatocyte damage is the disruption of cellular redox homeostasis, resulting in oxidative stress.[11]

Erythromycin, a macrolide antibiotic, is a recognized bacteriostatic agent used to treat infections caused by gram-positive bacteria. Though considered a rare clinical occurrence, hepatotoxicity is a recognized adverse effect of erythromycin. Notably, with prolonged treatment, it emerges as a significant agent of drug-induced liver injury (DILI), with its incidence ranking among the more frequent causes of hepatic damage.[12]

Bioactive compounds, including nimbidine found within the leaves of a neem tree (Azadirachta indica) have exhibited a considerable degree of anti-inflammatory properties. The natural components that constitute neem assist in minimizing the inflammation response through modulating the body's immunological and cellular reaction to an insult or irritant. Due to these therapeutic effects, neem has been utilized as a medicinal agent in traditional forms of medical treatment to support wound healing, diminish acute inflammatory conditions, and provide protective functions to tissues as well as restore balance in the physiological balance.[13] In addition to providing this anti-inflammatory benefit, neem extracts can be beneficial in numerous ways. Neem extract is rich in antioxidants that protect cells from oxidative stress and damage. Additionally, neem extract supports the liver by promoting detoxification and protects the skin by inhibiting bacteria thereby enhancing skin health. Overall, neem supports the body's ability to naturally detoxify itself and maintain a state of general wellness.[14]

Recent investigations have elucidated their mechanism, which involves the potent inhibition of key pro-inflammatory signalling pathways, including NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) and COX-2 (Cyclooxygenase-2), thereby reducing the synthesis of inflammatory mediators.[15]

The hepatoprotective potential of Azadirachta indica (neem) leaf extract is corroborated by the concordant findings of *Alanazi AZ et al*. In their investigation, they demonstrated that a specific dosage of aqueous neem extract effectively restored disturbed hepatic architecture and improved hepatic cell count, indicating a significant recovery of liver structure and function. The authors proposed that this therapeutic effect is likely mediated by the flavonoid quercetin, a key constituent of neem, which exerts potent antioxidant activity.[16]

These findings are in close agreement with the work of A*slam et al*., who also observed significant hepatoprotective effects of neem leaf extract in their study. The similarity in results strengthens the evidence supporting neem's protective role in liver injury. Such consistent outcomes across studies suggest that neem may be a dependable and practical natural option for managing hepatic damage and supporting liver recovery in therapeutic applications and research settings.[17]

In our study, albino rats given Erythromycin for two weeks

showed increasing liver cell damage and a major loss of the liver's normal structure. These findings are consistent with the work of *Leise et al*, who confirmed that erythromycin causes central necrosis and liver cell degeneration, leading to a reduced hepatocyte count, particularly at high doses or with long-term use.[18] The mechanism behind this damage, as explained by Singh P, is likely the generation of free radicals and subsequent oxidative stress. Our observations confirmed this with evidence of hepatocellular necrosis and significant inflammation.[19] Prolonged exposure to erythromycin can induce significant stress in liver cells, ultimately leading to hepatocyte injury. This cellular damage results in a noticeable reduction in the overall number of hepatocytes. Such effects highlight the potential hepatotoxic nature of long-term erythromycin use, where continuous exposure disrupts normal liver structure and impairs cellular integrity and function over time.[20]

The liver damage caused by Erythromycin is a complex process that leads to a reduced number of liver cells. Research by *Kwon Y, et al.* explains that erythromycin disrupts the energy centers (mitochondria) in liver cells, causing fat to build up (steatosis). This cellular damage contributes to the death of liver cells, leading to reduced hepatocyte count.[21] This finding is consistent with the work of *Zafrani et al.*, who observed that animals treated with Erythromycin developed cholestasis, hepatitis, and liver cell injury, all of which resulted in a decreased hepatocyte count.[22]

Rats were given aqueous neem leaf extract in addition to erythromycin, and liver damage was much reduced in the *Dubois RN* study, which was demonstrated by a considerable decrease in necrotic foci, pyknotic nuclei, and other degenerative lesions as compared to the group that received erythromycin alone.[23] As *Guru Siddappa* demonstrates, Neem's strong antioxidant qualities, mainly because of its high flavonoid concentration, are responsible for this hepatoprotective activity, as it blocks lipid peroxidation, a vital process of cellular damage, which leads to a decrease in hepatocyte count. These substances are known to prevent hepatotoxicity and bring back the normal hepatic architecture with a normal hepatocyte count.[24]

MacDonald et al. reported comparable hepatoprotective effects, demonstrating the ability of natural extracts to preserve liver architecture under toxic effects. Their findings further support the protective role of plant-based compounds in maintaining hepatocyte integrity and reducing cellular damage. This evidence strengthens the idea that natural extracts may offer a safe and effective approach to minimizing toxin-induced liver injury and promoting recovery of normal hepatic structure and function.[25] This observation aligns closely with the findings of *Jamshed N et al.*, who further validated neem's protective potential. Their study also highlighted its role in reducing hepatic damage and supporting cellular regeneration. These consistent results across research strengthen the evidence that neem extract may effectively help maintain liver structure, limit toxin-induced injury, and promote overall hepatic recovery and functional stability in experimental model.[26] Their study showed that neem extract effectively maintained normal hepatocyte architecture and hepatocyte count following paracetamol-induced injury. This is in accordance with Hussain YA's study, in which he observed preservation of hepatocyte count and tissue damage highlights neem's broad protective capabilities.[27]

Together, these studies reinforce the role of neem extract as a potent hepatoprotective agent across different models of liver toxicity. This consistent protective effect highlights its therapeutic potential in reducing oxidative stress, preserving cellular integrity, and supporting overall liver health under various toxic conditions. This study was initially planned to include a larger number of animals, but due to certain limitations, only a small sample size could be used, which may influence the accuracy of the results. Also, the study mainly focused on tissue-level observations and did not include detailed biochemical tests.

CONCLUSION

The aqueous neem leaf extract obtained from Azadirachta indica showed a notable protective effect by improving hepatocyte count and preserving liver architecture. This beneficial effect can be attributed to the strong antioxidant properties of neem, which help in reducing oxidative stress and limiting cellular injury.

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CONFLICT OF INTEREST

Author declared no conflict of interest

GRANT SUPPORT & FINANCIAL DISCLOSURE

Author declared no specific grant for this research from any funding agency in the public, commercial or non-profit sectors

AUTHORS CONTRIBUTIONS

FK: Conception, Design of the work, Data collection, and Drafting, Reviewed, Final approval, Agreement to be accountable.

AR: Conception, Design of the work, Acquistion, Data Analysis, and Drafting, Reviewed, Final approval, Agreement to be accountable.

IR: Conception, Design of the work, Interpretation of data for the work, and Drafting, Reviewed, Final approval, Agreement to be accountable.

AT: Conception, Design of the work, Data collection, and Drafting, Reviewed, Final approval, Agreement to be accountable.

AK: Conception, Design of the work, Interpretation of data for the work, Data Collection and Drafting, Reviewed, Final approval, Agreement to be accountable.

SR: Conception, Design of the work, Interpretation of data for the work, Data Collection, Data Analysis and Drafting, Reviewed, Final approval, Agreement to be accountable.

DATA SHARING POLICY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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