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Original Article

Hepatoprotective Role of *Melaleuca leucadendra* and *Cassia surattensis* Extracts against Isoniazid and Rifampicin-Induced Hepatotoxicity in Swiss Albino Mice

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ABSTRACT

Background: Liver disorders arising from various causes, including infections, toxins, and certain medications, remain a significant global health challenge. Ongoing research focuses on the development of novel drug compounds to enhance liver protection and improve therapeutic outcomes. Thus, the present study aims to assess the hepatoprotective potential of two plant extracts, *viz. Melaleuca leucadendra* and *Cassia surattensis* in mitigating liver toxicity induced by Isoniazid (INZ) and Rifampicin (RIF) in Swiss albino mice. **Materials and Methods:** The hepatotoxicity was induced using INZ and RIF, and the protective effect of the extracts alone and in combination was assessed through a series of assays. Liver enzyme activities, Masson's Trichrome (MT staining), and histopathological analysis were performed to evaluate the extent of liver damage and recovery. Immunohistochemistry (IHC) was used to analyze the countenance of NF-κB and the extent of fibrosis. The standard drug, N-acetylcysteine, was used for comparison. **Results:** Our results indicate that the combination of plant extracts significantly reduced liver enzyme levels, attenuated inflammation, and minimized histopathological damage, showing a similar protective effect as the standard drug N-acetyl cysteine. The IHC analysis revealed a molecular mechanism through which the plant combination exerts its hepatoprotective effects via modulating NF-κB. **Conclusion:** In conclusion, the plant combination demonstrates promising hepatoprotective activity as compared to individual extracts by exerting their antioxidative, antifibrotic, and anti-inflammatory properties, potentially offering a natural therapeutic approach for liver injury caused by various chemotherapeutics.

Keywords: Antifibrotic, Anti-Inflammatory, *Cassia surattensis*, *Melaleuca leucadendra*, N-acetylcysteine.

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INTRODUCTION

Drug-Induced Hepatotoxicity (DIH) refers to liver damage caused by adverse medication reactions. Although it is relatively rare, it affects an estimated 14 to 19 individuals per 100,000 people. DIH contributes to fewer than 1% of all cases of Acute Liver Injury (ALI).[1] DIH further categories as direct, idiosyncratic, and indirect types. Direct DIH is dose-dependent, predictable, and reproducible. In contrast, idiosyncratic DIH is unpredictable, not related to dose, and varies among individuals, though it is more common with drugs taken at doses ≥50 mg/day. Some cases occur after dose escalation, suggesting a threshold effect. Indirect DIH results from the drug's pharmacological or immune-modulating actions rather than intrinsic toxicity. For example, immune checkpoint inhibitors can trigger T-cell–mediated liver injury, while monoclonal antibodies may alter immune balance, leading to hepatitis.[2] DIH occurs through multiple molecular mechanisms, influenced by genetic and individual factors. Through Phase I and II reactions, drugs are metabolized in hepatocytes, generating reactive metabolites that can bind cellular components, disrupt mitochondrial function, induce oxidative stress, and deplete antioxidants like glutathione. Depending on the extent of damage, these events can trigger inflammation, apoptosis, or necrosis. Impaired drug transport and bile acid accumulation also contribute to hepatotoxicity. Endoplasmic Reticulum (ER) stress and disrupted autophagy further exacerbate injury by impairing protein folding and the removal of damaged organelles. Immune-mediated reactions, triggered by drug-protein adducts or DAMPs, amplify hepatocyte death through cytokine release. Tumor Necrosis Factor-alpha (TNF-α), a key pro-inflammatory cytokine, promotes hepatocyte apoptosis and necrosis through

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activation of death receptors and inflammatory pathways. Additionally, gut microbiota imbalances can modulate immune responses and drug metabolism, influencing DIH susceptibility and severity.[3] Isoniazid (INZ) and Rifampicin (RIF), cornerstone drugs in tuberculosis treatment, are both implicated in DIH via distinct and overlapping molecular mechanisms.[4] The combined hepatotoxic effects of Isoniazid (INZ) and Rifampicin (RFP) are complex and vary among studies. Most findings indicate that RFP worsens INZ-induced liver injury by activating the Pregnane X Receptor (PXR), which upregulates enzymes like CYP3A4 and CYP2E1, leading to increased production of toxic INZ metabolites such as acetylhydrazine and hydrazine. This enhances oxidative stress and liver damage. RFP also Promotes Protoporphyrin IX (PPIX) accumulation by inducing ALAS1, while INZ metabolites inhibit ferrochelatase, disrupting heme formation and contributing to cholestatic injury.[5]

Synthetic drugs used to treat hepatotoxicity often present significant challenges, including limited efficacy, cost, and adverse side effects.[6] These medications may not effectively target the underlying mechanisms of liver injury, leading to suboptimal outcomes. Moreover, their use can result in additional liver damage, complicating treatment strategies. The advancement of safer and more efficacious therapeutic options remains a critical need in the management of liver diseases.[6]

Herbal medicines are promising as alternatives for managing liver disorders, particularly due to their traditional use and demonstrated hepatoprotective properties. Accumulating research data highlights the cellular-level mechanisms and potential of herbal agents in treating hepatotoxicity. Several herbs show efficacy through biochemical markers, data gaps persist, necessitating further exploration of relevant molecular targets.[7] *Melaleuca leucadendra*, a member of the Myrtaceae family, is also known as *Melaleuca cajuputi* subsp. *cajuputi*. The leaves of *Melaleuca leucadendra* are known for their antibacterial, anti-inflammatory, and analgesic properties, and have been traditionally used to treat pain, burns, respiratory infections, influenza, and digestive issues.[8] *Melaleuca leucadendra* possesses a potent gastroprotective effect responsible for its anti-inflammatory, antioxidant, and antiapoptotic effects.[9] *Cassia surattensis* is a fast-growing ornamental shrub or small tree native to South and Southeast Asia, known for its bright yellow flowers and traditional use in herbal medicine for treating skin diseases, constipation, and liver disorders.[10] *Cassia surattensis* flowers are rich in a variety of phytochemicals, including flavonoids, phenolics, tannins, and alkaloids. These bioactive constituents are known for their potent antioxidant properties, which play a crucial role in mitigating oxidative stress.[11] Preliminary findings suggest that combining these botanicals may offer greater therapeutic efficacy than individual plant extracts alone. This study supports the rationale for developing polyherbal formulations as safer, natural alternatives to conventional hepatoprotective drugs and encourages further pharmacological validation of their combined effects. Therefore, the present research establishes the potential of these medicinal plants—*Cassia surattensis* and *Melaleuca leucadendra*—as an effective strategy to combat hepatotoxicity.

MATERIALS AND METHODS

Chemicals

The major chemicals and compounds utilized in this study, such as ethanol and diethyl ether, were acquired from the Institutional chemical store.

Experimental animal

The current study used 36 healthy Swiss albino mice of body weight 30-40 g obtained from an institutional animal house. Before initiating the research, the animals were acclimated to the lab environment for 7 days. The circumstances were maintained at a temperature of 26±5ºC, air moisture 55±5%, and a light/dark cycle of 12/12 hr.

Collection and authentication of plant material

The plant materials used in this study were collected and authenticated by Scientist D from the Botanical Garden of the Indian Republic, located in Sector 38A, Gautam Buddha Nagar, Noida (PIN: 201303), Uttar Pradesh, India. Voucher specimens were prepared and deposited for future reference. This rigorous authentication process was undertaken to ensure taxonomic accuracy, which is critical for the reproducibility and scientific validity of phytochemical and pharmacological investigations.

Grouping and treatment schedule

The animals were divided into six groups, each consisting of four animals.

Group I (Normal Control) received distilled water only for 14 consecutive days.

Group II (INZ+RIF), toxic group**,** was treated with a combination of INZ (100 mg/kg) + RIF (150 mg/kg) p.o. for 14 consecutive days.

Group III (N-acetyl cysteine), Standard group, was administered 100 mg/kg N-acetylcysteine orally, one hour before induction with INZ (100 mg/kg) + RIF (150 mg/kg) combination p.o. for 14 consecutive days.

Group IV (Melaleuca leucadendra), treatment 1, received 100 mg/kg *Melaleuca leucadendra* extract orally, one hour before induction with INZ (100 mg/kg) + RIF (150 mg/kg) p.o. for 14 consecutive days.

Group V (Cassia surattensis), the treatment 2, was administered with 500 mg/kg *Cassia surattensis* plant extract orally, one hour before the INZ (100 mg/kg) + RIF (150 mg/kg) combination p.o. for 14 consecutive days.

Group VI (MLE+CSSE), treatment 3, was administered a combination of 400 mg/kg (*Melaleuca leucadendra* + *Cassia surattensis*) extracts orally, one hour before induction with INZ (100 mg/kg) + RIF (150 mg/kg), for 14 consecutive days.

Collection of blood and Serum separation

After 14 days of treatment, the mice were subjected to an overnight fast before being anesthetized using an intraperitoneal injection of ketamine (100 mg/kg) combined with xylazine (12.5 mg/kg). Blood was collected via cardiac puncture, and the animals were subsequently euthanized through cervical dislocation. Approximately 2 to 2.5 mL of blood was drawn from each mouse and transferred into serum separator tubes. The samples were left undisturbed at room temperature for 30 min to allow clotting. Following this, the samples were centrifuged at 3000 rpm for 10 min at room temperature. The resulting serum was carefully extracted using a micropipette (1 mL) and stored at −20°C until liver function markers were assessed.

Analysis of liver marker enzymes

The ALT, AST, ALP, and Total bilirubin were assessed in the serum of each animal of all the experimental Swiss albino mice using commercially obtained assay kits. The experiments were done as per the manufacturer's procedure specifications.

Estimation of NF-kB by IHC

Thin liver sections (4 µm) from Swiss albino mice were placed on special slides and prepared using xylene and alcohol. To reveal the NF-κB protein, the tissues were heated in citric acid buffer using a microwave. Natural enzyme activity in the tissues was blocked to avoid false results. The sections were then treated overnight with a specific antibody at 4°C. After staining, the liver cells were examined under a microscope at high magnification.[12]

Masson's Trichrome (MT) staining

Liver tissue sections were deparaffinized and rehydrated through graded alcohols, then stained with Weigert's iron hematoxylin for 10 min to visualize cell nuclei, followed by rinsing in water. The sections were then stained with acid fuchsin for 5 min to stain cytoplasmic elements, briefly rinsed, and differentiated in 1% phosphomolybdic acid for 5 min. After draining, methyl blue was applied as a counterstain to highlight collagen fibers, followed by dehydration, clearing in xylene, and mounting with DPX. Stained slides were observed under a light microscope, and fibrotic changes were evaluated by identifying collagen deposition, which appeared blue.[13]

Histopathological evaluation

Liver samples collected from the experimental animals were rinsed with 0.1 M ice-cold Phosphate-Buffered Saline (PBS). A portion of the tissue was preserved in 10% formalin for histological evaluation. The fixed liver sections were stained using Hematoxylin and Eosin (H&E) and examined for histopathological alterations.[14,15]

RESULTS

Effect of Melaleuca Leucadendra and Cassia surattensis extracts on the liver enzymes

Figure 1 demonstrates the significant differences in the liver enzymes, i.e., Total bilirubin, AST, ALT, and ALP. The combination treatment group (MLE+CSSE), comprising MLE and CCSE, demonstrated a notable attenuation in ALT, AST, ALP, and total bilirubin levels compared to the INZ (100 mg/kg) + RIF (150 mg/kg) treated group (toxic group). Notably, the combination therapy's effect was comparable to that of the standard N-acetylcysteine treatment group, which also exhibited substantial improvements in these liver function markers. These findings suggest that the combination of *Melaleuca leucadendra* and *Cassia surattensis* may offer a hepatoprotective alternative with efficacy on par with N-acetyl cysteine in mitigating liver damage induced by INZ and RIF (Figure).

Effect of Melaleuca Leucadendra and Cassia surattensis extracts on NF-kB using IHC

Immunohistochemical staining of liver sections was performed to assess the expression of NF-κB across various experimental groups. In the normal control group (A), minimal NF-κB immunoreactivity was observed (green arrow), indicating negative expression or baseline expression. The INZ+RIF-treated toxic group (B) showed intense nuclear localization of NF-κB (red arrow), reflecting a strong inflammatory response and significant activation of the NF-κB pathway. Treatment with N-acetylcysteine (C) resulted in a moderate reduction in NF-κB expression compared to the toxic group, confirming its partial protective effect. MLE-treated (D) and CSSE-treated (E) groups also exhibited a noticeable decrease in NF-κB staining, with MLE showing slightly greater inhibition. Notably, the MLE+CSSE combination group (F) demonstrated a considerable reduction in NF-κB expression, approaching levels similar to the normal control group. The weak immunopositivity in this group indicates a synergistic effect of the combination, resulting in a significantly better suppression of NF-κB activation compared to individual treatments and the standard antioxidant control. These findings suggest that the MLE+CSSE combination effectively attenuates inflammatory signaling in the liver, highlighting its potential as a therapeutic strategy against drug-induced hepatotoxicity (Figure 2).

Effect of *Melaleuca leucadendra* and *Cassia surattensis* extracts on fibrosis using MT staining

Masson's Trichrome staining was used to evaluate collagen deposition and fibrotic changes in liver tissues across experimental groups. The normal control group (A) showed well-preserved

hepatic architecture with minimal collagen presence around the central vein and portal triads (green arrow). In contrast, the INZ+RIF-induced toxic group (B) exhibited intense blue staining, indicating substantial collagen accumulation (red arrow) and severe liver fibrosis. The N-acetylcysteine-treated group (C) showed a moderate reduction in fibrotic areas, suggesting partial hepatoprotection. Treatment with MLE (D) and CSSE (E) individually led to noticeable decreases in collagen deposition compared to the toxic group, though fibrotic strands remained evident in periportal areas. Remarkably, the MLE+CSSE combination group (F) demonstrated a significant reduction in collagen content, with only minimal fibrous expansion around the portal regions. The architecture closely resembled that of the normal group, suggesting substantial reversal of fibrosis. Quantitative image analysis confirmed that the combination treatment resulted in the lowest collagen proportionate area among all treatment groups. These observations strongly support the synergistic antifibrotic efficacy of MLE and CSSE co-administration in mitigating drug-induced liver injury (Figure 3).

Histopathological Observations

Histological examination of liver tissues revealed distinct changes among the groups. In image A (normal control), liver sections displayed normal histological architecture with well-arranged polygonal hepatocytes, clear cytoplasm, centrally located nuclei, and no signs of fatty changes, inflammation, or vascular congestion. Image B (INZ+RIF-treated) showed extensive hepatic damage, including dense cytoplasm, significant fatty changes, cytoplasmic vacuolation, pyknosis, central vein congestion, and heavy inflammatory infiltrates, indicating severe toxicity. Image C (N-acetylcysteine-treated) revealed mild histological damage, with mostly preserved hepatic structure, minimal vacuolation, slight fatty changes, and reduced inflammation. In image D (MLE-treated), mild to moderate hepatic alterations were observed, such as moderate fatty infiltration, vacuolation, and mild pyknosis. Image E (CSSE-treated) showed moderate

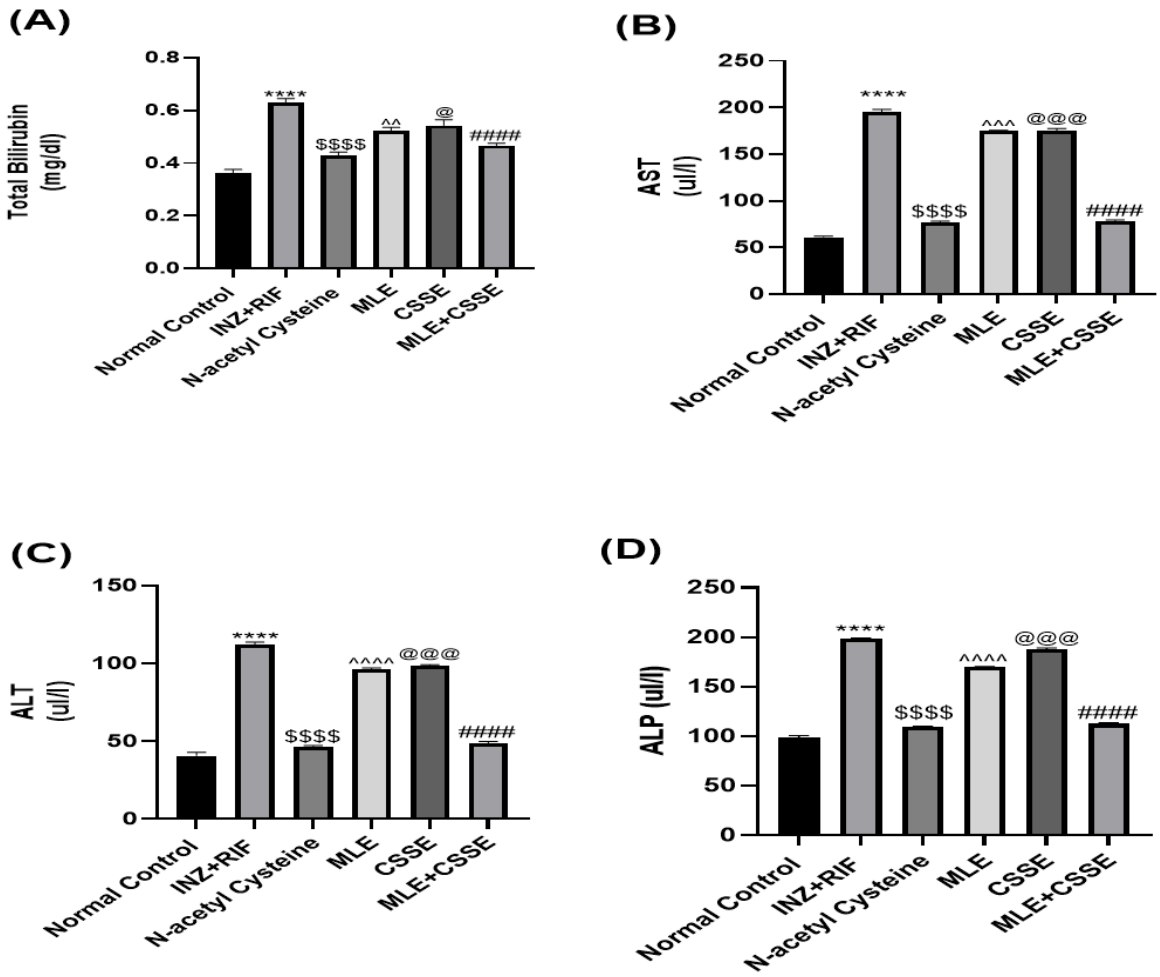

Figure 1: Effect of treatments on various liver enzymes in Swiss albino mice. (A) Total Bilirubin, (B) AST, (C) ALT, & (D) ALP in normal control, INZ + RIF treated, N-acetyl cysteine treated, MLE, CSSE & combination of both extracts i.e, MLE+CCSE. Data are expressed as mean ± SEM. $^{****}p$<0.0001(Normal control vs INZ+RIF); p<0.0001 (INZ+RIF vs N-acetyl cysteine); $^{\#\#\#\#}p$<0.0001 (INZ+RIF vs MLE+CCSE); $^{\wedge\wedge}p$<0.001 (INZ+RIF vs MLE); $^{\wedge\wedge\wedge}P$<0.0001 (INZ+RIF vs MLE); $^{\wedge\wedge\wedge\wedge}p$<0.0001 (INZ+RIF vs MLE); $^{@}p$<0.01 (INZ+RIF vs CSSE); $^{@@@}p$<0.0001(INZ+RIF vs CSSE). Data are expressed as mean ± SEM (n=6). Significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test.

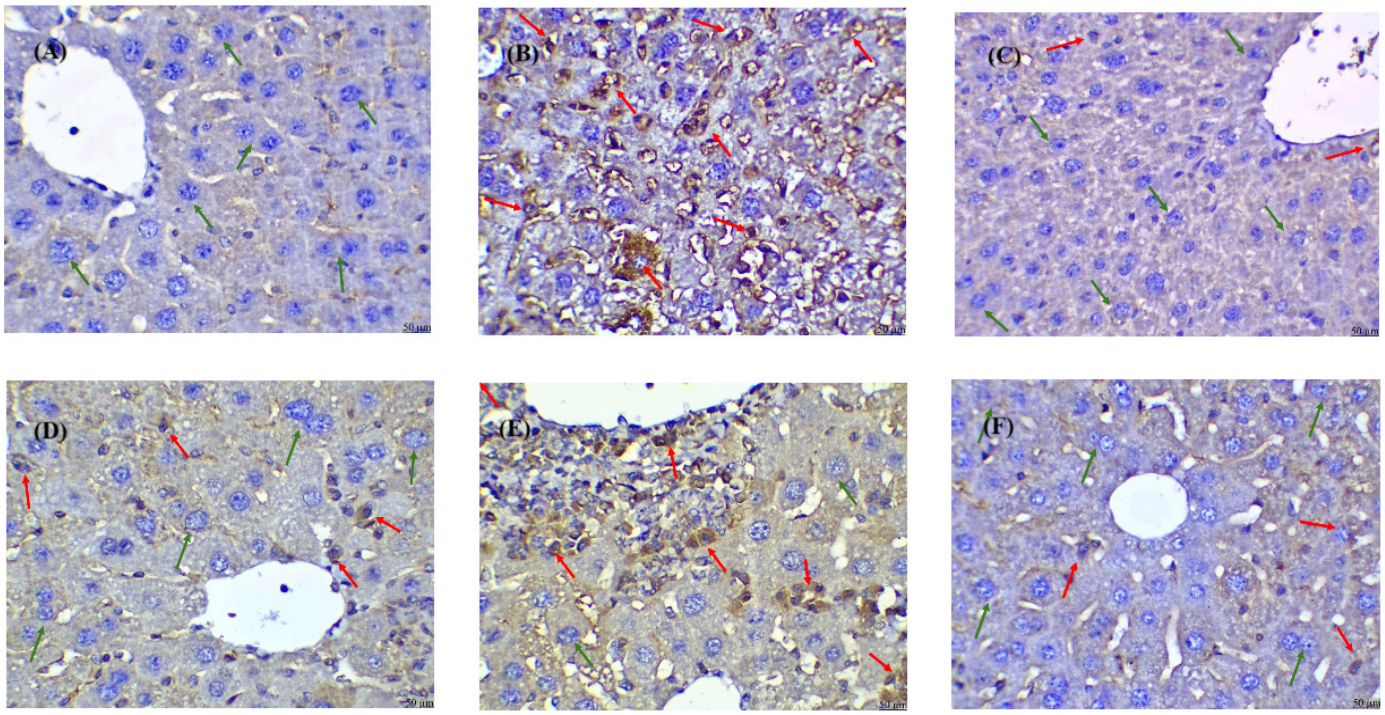

Figure 2: IHC examination of Nf-kB in different groups of Swiss albino mice. (A) Normal control; (B) INZ+RIF(Toxic); (C) N-acetyl cysteine; (D) MLE; (E) CSSE; (F) MLE+CSSE. The green arrow indicates negative expression, and the red arrow indicates very strong expression.

to severe histological disruption with persistent fatty changes, vacuolation, and inflammatory infiltrates. Image F (MLE+CSSE combination) exhibited only mild hepatocyte damage, minimal fatty changes and vacuolation, mild pyknosis, and minimal inflammatory infiltration, without central vein congestion. The histological appearance in image F was comparable to that of the standard treatment in image C, suggesting that the combination treatment provided significant protection against hepatotoxicity, equal to the effect observed with N-acetylcysteine (Figure 4).

DISCUSSION

DIH remains a significant clinical challenge, particularly in the treatment of tuberculosis, where isoniazid and rifampicin are cornerstone therapies. These agents, though effective antimicrobials, are frequently implicated in hepatic injury due to their metabolism in the liver, leading to oxidative stress, mitochondrial dysfunction, and inflammatory responses.[16]

Modern anticancer therapies have improved survival but often cause adverse effects. These include toxicity to the nervous system, kidneys, heart, and liver. Hepatotoxicity, in particular, can limit treatment use. Early detection and close collaboration between oncologists and hepatologists are essential to prevent severe liver damage.[17] Hepatotoxicity involves complex molecular mechanisms that disrupt key cellular functions, particularly in the mitochondria. Certain toxic compounds can impair the electron transport chain, leading to reduced fatty acid marked inflammation β-oxidation and resulting in microvesicular steatosis. This condition may progress to inflammation, steatohepatitis, and ultimately cirrhosis. Additionally, mitochondrial dysfunction can cause excessive production of reactive oxygen species, triggering oxidative stress.[18] NF-κB activation plays a crucial role in hepatotoxicity, particularly in DIH. When activated by stressors like toxins or inflammation, NF-κB triggers the production of pro-inflammatory cytokines, leading to oxidative stress and immune cell recruitment. This process damages liver cells, promoting apoptosis and tissue injury.

Activation of NF-κB can negatively influence Nrf2 activity, resulting in a reduced antioxidant response during inflammatory conditions. This crosstalk reduces Nrf2-driven protection, exacerbating oxidative damage and promoting liver cell injury in DIH. This shift toward a pro-inflammatory state may further aggravate hepatocellular damage during DIH.[19] Overall, NF-κB-driven inflammation and oxidative stress significantly contribute to liver dysfunction and injury, making them potential therapeutic targets for managing hepatotoxicity.[19]

In the present study, the result of the immunohistochemical analysis revealed that the INZ+RIF group showed intense NF-κB activation, indicating significant inflammation. Taking isoniazid and rifampicin together boosts liver metabolic activity, producing reactive metabolites that increase mitochondrial generation of Reactive Oxygen Species (ROS). The buildup of ROS damages mitochondrial membranes and hampers electron transport chain efficiency, causing oxidative stress and cellular damage in liver cells. This oxidative imbalance triggers the NF-κB pathway.

N-acetylcysteine reduced NF-κB expression moderately, while MLE and CSSE treatments also showed reduced activation, with MLE being slightly more effective. The MLE+CSSE combination resulted in a marked reduction in NF-κB expression, similar to the standard drug, i.e., N-acetyl cysteine, highlighting its potential as an effective strategy to reduce inflammation and manage drug-induced hepatotoxicity.

The term "liver function tests" can be misleading, as many of these tests indicate liver injury rather than actual liver function. A predominant increase in ALT and AST compared to ALP and bilirubin suggests hepatocellular damage. In contrast, a greater rise in ALP and bilirubin relative to ALT and AST points to a cholestatic pattern. When both ALP and transaminases (ALT/AST) are elevated, it reflects a mixed pattern of liver injury. Elevated bilirubin levels alone, with normal ALT, AST, and ALP, are classified as isolated hyperbilirubinemia.[20]

The results of this experiment indicate that the combination of *Melaleuca leucadendra* and *Cassia surattensis* plant extracts significantly reduced liver enzyme levels—ALT, AST, ALP, and total bilirubin—in mice exposed to INH and RIF-induced hepatotoxicity. This reduction was comparable to the standard hepatoprotective agent, N-acetylcysteine, suggesting that the combined plant extracts offer effective protection against liver injury. The findings support the potential of MLE+CSSE as a promising natural alternative for managing drug-induced liver damage.

Masson's Trichrome stain is commonly used in histology to differentiate tissue components by color. It is especially known for staining collagen fibers blue, while other elements like muscle fibers and cytoplasm appear red, and cell nuclei are stained dark. This staining technique is particularly useful for evaluating fibrosis and other structural changes in tissues.[21] Hepatic fibrosis is a consequence of prolonged liver inflammation from chronic liver diseases and may advance to cirrhosis if untreated. It results from an imbalance between the formation and breakdown of fibrous tissue. While early-stage fibrosis can be reversible if the underlying cause is addressed, continued liver damage leads to excessive collagen deposition, replacing normal liver tissue and limiting recovery. With the increasing prevalence of obesity and metabolic syndrome, Metabolic-Associated Fatty Liver Disease (MAFLD) has become the most common chronic liver condition, particularly in the U.S. The global burden is rising, making liver fibrosis and cirrhosis major public health concerns and significant contributors to mortality and healthcare costs.[22]

In the current study the results of Masson's Trichrome staining find that INZ+RIF administration caused extensive collagen deposition, indicating severe hepatic fibrosis. While MLE and CSSE treatments each reduced fibrosis to some extent or varying degrees, the combination of MLE and CSSE produced a pronounced reduction in collagen accumulation, significantly equal to N-acetyl cysteine. Liver tissue in this group closely resembled the normal control, with minimal fibrotic expansion. Quantitative analysis confirmed that the combination treatment significantly outperformed the standard treatment, highlighting its superior antifibrotic potential in mitigating drug-induced liver injury.

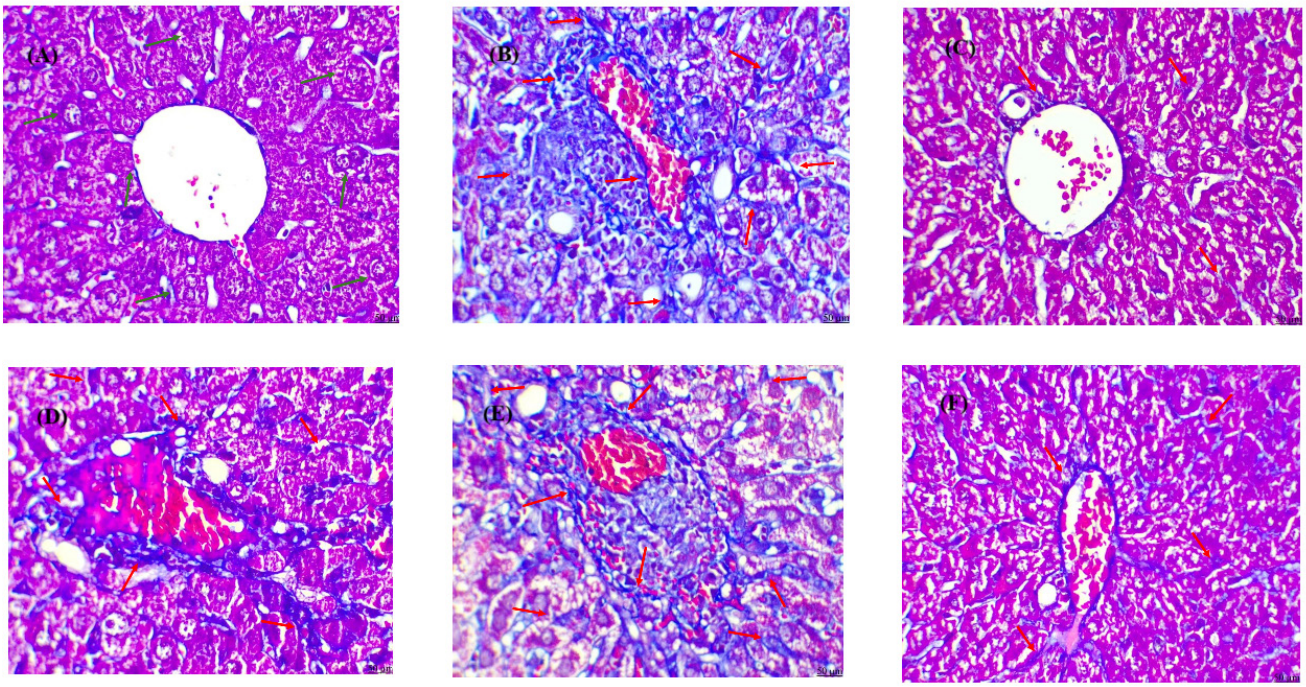

Figure 3: MT-staining of liver tissue in different groups of Swiss albino mice. (A) Normal control; (B) INZ+RIF(Toxic); (C) N-acetyl cysteine; (D) MLE; (E) CSSE; (F) MLE+CSSE. The green arrow indicates no collagen deposition, and the red arrow indicates collagen deposition.

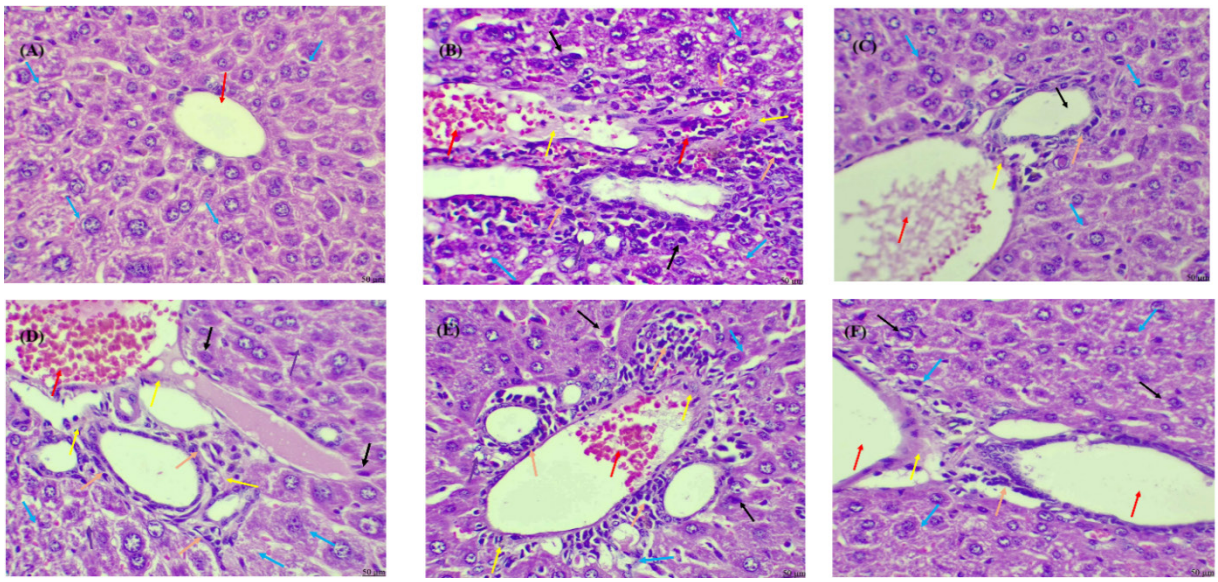

Figure 4: Histopathological examinations by H&E. staining of liver tissue of Swiss Albino mice. (A) Normal Control; (B) INZ+RIF (Toxic); (C) N-acetyl cysteine; (D) MLE; (E) CSSE; (F) MLE+CCSE. The red arrow indicates the central vein, the blue arrow indicates the hepatocyte structure, the yellow arrow indicates cytoplasm, the black arrow indicates Pyknosis, the purple arrow indicates vacuolation, and the orange arrow indicates the inflammatory infiltrates.

Drug-Induced Hepatotoxicity (DIH) is characterized by various histopathological changes that reflect the severity and type of liver injury. Common findings include hepatocyte vacuolation, often due to steatosis, and central vein congestion or pyknosis, which indicates severe cell damage or necrosis. Inflammatory infiltrates, particularly around portal areas, suggest an immune-mediated response, while cytoplasmic changes like ballooning or vacuolization are typically associated with hepatocellular injury. These histological alterations can help differentiate DIH from other liver conditions and are crucial in determining the severity and potential progression of the injury, including fibrosis or cirrhosis in chronic cases.[23]

The histological analysis of this experiment revealed that the normal control group showed intact liver architecture, while the INZ+RIF-treated group exhibited severe hepatic damage with fatty changes, vacuolation, pyknosis, and inflammation. The N-acetylcysteine-treated group showed minimal damage, with preserved structure and mild changes. The MLE-treated group had moderate alterations, while the CSSE-treated group displayed significant disruption. However, the MLE+CSSE combination showed mild damage, with minimal vacuolation and inflammation, similar to N-acetylcysteine, indicating effective hepatoprotection.

Nerolidol ameliorates ischemia-reperfusion kidney injury by preserving renal function and curbing oxidative stress, inflammation, and fibrosis.[24] In the present context, nerolidol from*Melaleuca leucadendra* likely mediates hepatoprotection through its antioxidant and anti-inflammatory actions. Similarly, Quercetin mitigates oxidative damage by lowering ROS levels, elevating antioxidant enzymes.[25] Thus, quercetin derivatives in *Cassia surattensis* probably underpin the observed liver protection by countering oxidative stress.

CONCLUSION

In conclusion, *Melaleuca leucadendra* and *Cassia surattensis* both demonstrated hepatoprotective effects, with *Melaleuca leucadendra* showing greater efficacy compared to *Cassia surattensis*. The combination of *Melaleuca leucadendra* and *Cassia surattensis* demonstrated significant hepatoprotective effects compared to N-acetyl cysteine, as evidenced by liver enzyme assays, immunohistochemistry, Masson's trichrome staining, and histopathological evaluation. This combination exhibited notable antioxidative, antifibrotic, and anti-inflammatory properties, highlighting its potential as an effective therapeutic agent for liver protection.

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ABBREVIATIONS

DIH: Drug-induced hepatotoxicity; **ALI:** Acute liver injury; **ER:** Endoplasmic reticulum; **DAMPs:** Damage-Associated Molecular Patterns; **TNF-α:** Tumor necrosis factor-alpha; **INZ:** Isoniazid; **RIF:** Rifampicin; **PXR:** Pregnane X receptor; **CYP3A4:** Cytochrome P450 3A4; **CYP2E1:** Cytochrome P450 2E1; **PPIX:**

Protoporphyrin IX; **ALSA1:** Aminolevulinic Acid Synthase 1; **PCR:** Polymer chain reaction; **ELISA:** Enzyme-Linked Immunosorbent Assay; **PBS:** Phosphate-buffered saline; **MLE:** *Melaleuca leucadendra* extract; **CSSE:** *Cassia surattensis* extract; **DPX:** Dibutyl phthalate-Polystyrene-Xylene; **AST:** Aspartate Transaminase; **ALT:** Alanine Transaminase; **ALP:** Alkaline Phosphatase; **MT:** Masson"s Trichome; **Nrf2:** Nuclear factor erythroid 2–related factor 2; **Nf-kB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **MAFLD:** Metabolic Dysfunction-Associated Fatty Liver Disease; **IAEC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), by the guidelines set by CPCSEA, Government of India. The study was designed to ensure minimal discomfort to the animals, adhering strictly to ethical principles including the 3Rs—Replacement, Reduction, and Refinement.

SUMMARY

The combination of *Melaleuca leucadendra* and *Cassia surattensis* has shown promising hepatoprotective effects, likely due to their synergistic antioxidant, anti-inflammatory, and antifibrotic properties. This herbal formulation helps protect liver tissues from damage induced by chemicals, enhances liver function markers, and supports the regeneration of hepatic cells, suggesting its potential as a natural therapeutic agent for liver disorders.

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