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Original Article

Evaluation of Lemon Peel Extract as Hepatoprotective Agent against Paracetamol-Induced Liver Toxicity: Insights from Biochemical Studies

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ABSTRACT

Background: The liver serves as a vital organ in metabolic processes and detoxification, making it susceptible to damage induced by xenobiotics such as drugs. Paracetamol overdose is a common cause of hepatotoxicity. Traditional medicine has long explored botanical sources for hepatoprotective agents, highlighting the potential of natural compounds in mitigating liver damage. Lemon peel, rich in flavonoids with antioxidant properties, presents a promising candidate for hepatoprotection. **Materials and Methods:** This study aimed to investigate the hepatoprotective effects of lemon peel extract on paracetamol-induced liver toxicity in rats. The extract was prepared through standardized extraction procedures and its dose was selected based on previous studies. Wistar rats were divided into five groups and subjected to various treatments, including paracetamol overdose with or without lemon peel extract supplementation. Biochemical parameters indicative of liver function, such as serum levels of AST, ALT, ALP and bilirubin, were measured following treatment protocols. **Results:** Administration of paracetamol alone induced significant elevation in serum levels of AST, ALT, ALP and bilirubin, reflecting hepatocellular damage. However, co-administration of lemon peel extract with paracetamol resulted in reduced levels of these biochemical markers compared to the paracetamol-only group. The decrease in enzyme levels suggests potential hepatoprotective effects of lemon peel extract. **Conclusion:** Lemon peel extract demonstrated hepatoprotective effects against paracetamol-induced liver toxicity in rats, as evidenced by the attenuation of biochemical markers indicative of liver damage. These findings highlight the therapeutic potential of lemon peel extract as a natural remedy for protecting liver health and mitigating the adverse effects of hepatotoxic agents.

Keywords: Hepatoprotective, Lemon Peel Extract, Paracetamol-Induced Liver Toxicity, Biochemical Studies.

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INTRODUCTION

The liver serves as a metabolic powerhouse, playing a crucial role in processing xenobiotics, or foreign substances, through various metabolic pathways. These pathways involve Phase I reactions, such as oxidation, reduction and hydrolysis, followed by Phase II conjugation reactions, where molecules are made more water-soluble for excretion.[1] Additionally, the liver regulates the expression of drug-metabolizing enzymes, such as cytochrome P450s, ensuring efficient detoxification. Liver damage induced by drugs can occur through various mechanisms, including direct toxicity, immune-mediated reactions and idiosyncratic responses.[2] Drugs such as acetaminophen can cause hepatotoxicity by depleting glutathione and generating reactive metabolites, leading to oxidative stress and hepatocellular damage.[3] Chronic use of alcohol can also result in liver injury by inducing inflammation, steatosis and eventually progressing to fibrosis and cirrhosis, disrupting normal liver function and compromising overall health.[4] Paracetamol-induced toxicity primarily occurs due to the formation of a toxic metabolite called N-Acetyl-p-Benzoquinone Imine (NAPQI) during its metabolism. NAPQI depletes glutathione stores in the liver, leading to oxidative stress and hepatocellular damage.[5] This can result in liver necrosis and failure if not promptly treated. However, in modern medicine, there is currently no specific treatment available for liver damage induced by isoniazid and rifampicin, highlighting the need for close monitoring and management of potential side effects.

Throughout human history, plants have served as invaluable sources of medicine, offering remedies for a multitude of ailments. Indigenous cultures across the globe have long relied on botanical knowledge passed down through generations to treat illnesses

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and promote well-being. From the ancient civilizations of Egypt and China to traditional healing practices like Ayurveda and Traditional Chinese Medicine, plants have played a central role in healthcare. Many modern drugs owe their origins to compounds originally discovered in plants used in traditional medicine.[6] The pharmaceutical industry continues to explore botanical sources for drug development, recognizing the potential of natural compounds to provide effective and often less toxic treatments.[7] Examples include the discovery of aspirin from willow bark and quinine from cinchona bark.[8] The integration of plant-derived molecules into modern medicine highlights the enduring significance of plants as a vital resource for human health.

Lemons are important sources of phytochemicals, with their peel particularly rich in flavonoids, potent antioxidants known for their health benefits. Flavonoids found in lemon peel, such as hesperidin and naringin, have been associated with various health-promoting properties.[9] They exhibit anti-inflammatory and antioxidant effects, which may help reduce the risk of chronic diseases like heart disease and cancer.[10] Additionally, these compounds have shown potential in promoting cardiovascular health by improving blood vessel function and lowering blood pressure.[11] Furthermore, the flavonoids in lemon peel may support digestive health by stimulating the production of digestive enzymes and promoting gut motility.[12] Consuming lemon peel, whether grated, zested, or incorporated into dishes, can be a flavourful and nutritious way to harness the beneficial phytochemicals present in this citrus fruit, contributing to overall health and well-being.

The aim of the current study is to investigate the hepatoprotective effects of lemon peel extract on paracetamol-induced liver toxicity in rats. This research seeks to assess whether administering lemon peel extract can mitigate the adverse effects of paracetamol overdose on the liver.

MATERIALS AND METHODS

Animals

Wistar rats (180-200 g) of either sex were housed in polypropylene cages, maintained under standardized conditions (12 hr light/dark cycles, 28±2ºC) were used in the study. Animals were provided with standard pellet food and had free access to drinking water. All the animal study protocols were duly approved by the Institutional Animal Ethics Committee.

Chemicals

All the chemicals used in the study were of analytical grade.

Extraction and Phytochemical screening

The process began by squeezing lemons to extract their juice, followed by rinsing the leftover lemon peels with water and drying them under shade. The dried lemon peels were then ground into a powder, which was used for the extraction procedure. Initially, petroleum ether was used to extract fat components from the lemon peel powder. Subsequently, ethyl acetate was employed for further extraction of bioactive compounds. The resulting extract was dried and carefully stored in a cool, dry place until needed for analysis or experimentation. The phytochemical screening was performed by standard reported methods.[13]

Selection of dose

As per studies performed by Zou *et al.*,[14] extract in the dose of 150 and 300 mg/kg of body weight was used in present study.

Induction of Hepatotoxicity

Paracetamol in the dose of 3000 mg/ kg was used to induce hepatotoxicity in rats.[15]

Animal grouping

Animals were divided into five groups with six animals in each;

Group I: Normal Control

Group II: Paracetamol (3000 mg/kg i.p.).

Group III: Paracetamol (3000 mg/kg i.p.) = Silymarin (10 mg/kg, p.o.)

Group IV: Paracetamol (3000 mg/kg i.p.)+Extract (100 mg/kg, p.o.).

Group V: Paracetamol (3000 mg/kg i.p.)+Extract (200 mg/kg, p.o.).

On the 21th day that is, after 6 h of pharmacological treatments, blood was withdrawn by tail vein for the estimation of biochemical parameters.

Biochemical analysis

Blood samples were collected into the epindrop tubes and centrifuged for 10 min at 7000 rpm using micro-centrifuge to separate the serum. The levels of Serum Glutamic Oxaloacetic Transaminase (SGOT/AST), Serum Glutamic-Pyruvic Transaminase (SGPT/ALT) Serum Alkaline Phosphatase (SALP) were estimated using commercial kits (Span Diagnostics, India).

Determination of total bilirubin

The determination of total bilirubin was performed according to the standard principles and procedures of the kit manufacturer manual (Tulip Diagnostics, India).

Statistical analysis

The results were expressed as mean±SEM. Statistical analysis was carried out by using One way ANOVA followed by Dunnett's test and $p<0.05$, $p<0.01$, $p<0.001$ was considered significant.

RESULTS

Extraction and phytochemical studies

The obtained extract was yellow-brown in colour. The extract was found to be rich in lycosides, alkaloids, terpenes and carbohydrates.

Effect of Lemon peel extract administration on AST levels

The AST levels across the groups reflect variations in liver health. Group II, exposed solely to paracetamol, exhibits a notable increase compared to the normal control (Group I), indicating paracetamol-induced liver injury. However, Groups III, IV and V, which received paracetamol along with either silymarin or extract, show comparatively lower AST levels than Group II (Figure 1). This suggests potential protective effects of silymarin and the extract against paracetamol-induced liver damage, with Group V possibly showing a dose-dependent response. Top of Form

Effect of Lemon peel extract administration on ALT levels

The ALT levels across the different groups suggest variations in liver function. Group II, which received only paracetamol, shows a significant increase compared to the normal control (Group I), indicating liver damage induced by paracetamol. Group III, which received paracetamol along with silymarin, shows a lower ALT level compared to Group II, suggesting a potential protective effect of silymarin on liver function. Groups IV and V, receiving paracetamol along with different doses of extract, also show lower ALT levels compared to Group II, implying a potential protective effect of the extract against paracetamol-induced liver damage, with the higher dose possibly providing greater protection (Figure 2).

Effect of Lemon peel extract administration on ALP levels

The ALP levels across the groups depict variations in liver function. Group II, administered only paracetamol, exhibits a significant increase compared to the normal control (Group I), suggesting paracetamol-induced liver damage. Conversely, Groups III, IV and V, which received paracetamol along with either silymarin or extract, show lower ALT levels than Group II. This indicates potential protective effects of silymarin and the extract against paracetamol-induced liver injury. Notably, Group V shows comparable ALT levels to Groups III and IV, indicating a potential dose-dependent response to the extract (Figure 3).

Effect of Lemon peel extract administration on bilirubin levels

The bilirubin levels across the groups reflect variations in liver health. Group II, exposed solely to paracetamol, exhibits a notable increase compared to the normal control (Group I), indicating paracetamol-induced liver injury. However, Groups III, IV and V, which received paracetamol along with either silymarin or extract, show comparatively lower bilirubin levels than Group II (Figure 4). This suggests potential protective effects of silymarin and the extract against paracetamol-induced liver damage, with Group V possibly showing a dose-dependent response.

DISCUSSION

Paracetamol is indeed a standard antipyretic agent commonly used to manage fever.[16] However, prolonged and high doses of paracetamol can lead to hepatocellular damage. The active metabolite of paracetamol, known as N-acetyl-p-Benzoquinone Imine (NAPQI), is primarily responsible for this hepatotoxicity. NAPQI is formed through the metabolism of paracetamol by the enzyme cytochrome P450, particularly the isoform CYP2E1, in the liver.[17] Normally, NAPQI is detoxified by conjugation with

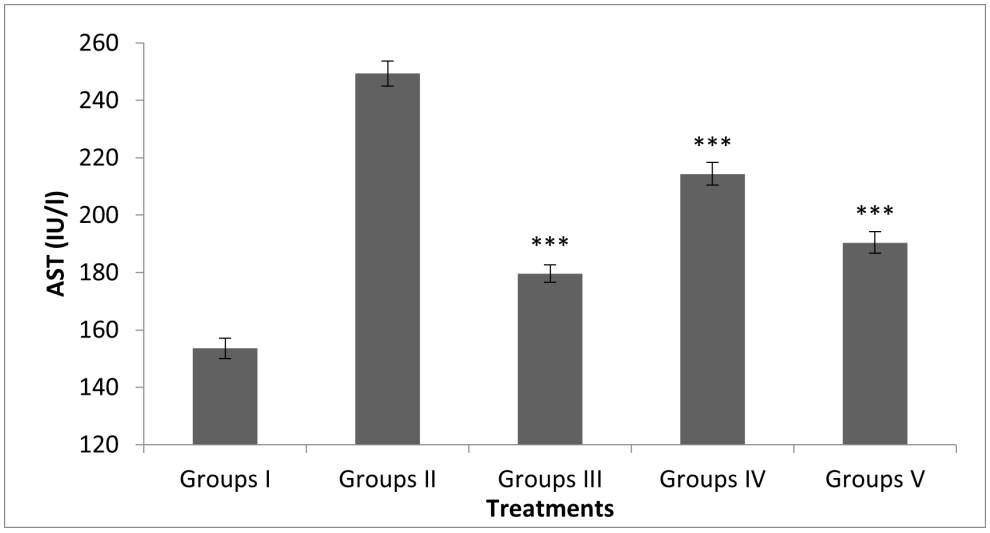

Figure 1: Effect of Lemon peel extract administration on AST levels in isoniazid-rifampicin treated rats. Results are given as mean±SEM of six animals in each group.

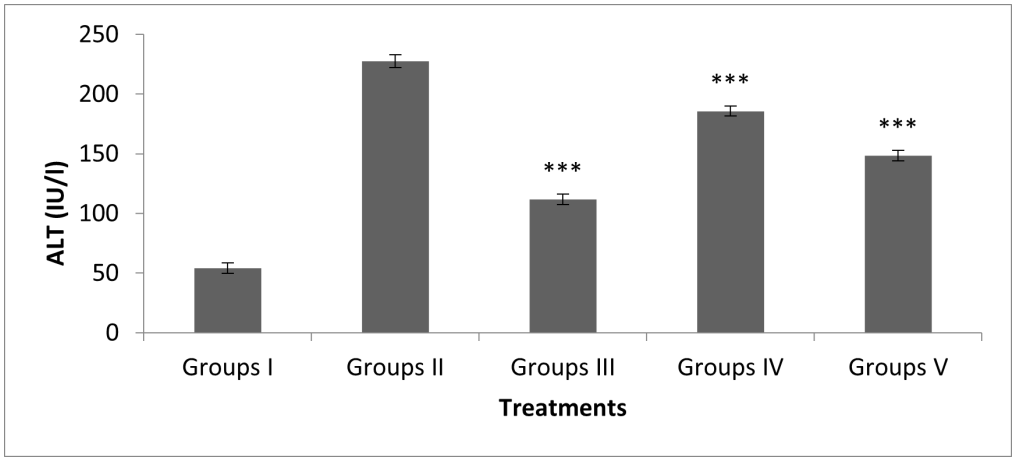

Figure 2: Effect of Lemon peel extract administration on ALT levels in isoniazid-rifampicin treated rats. Results are given as mean±SEM of six animals in each group.

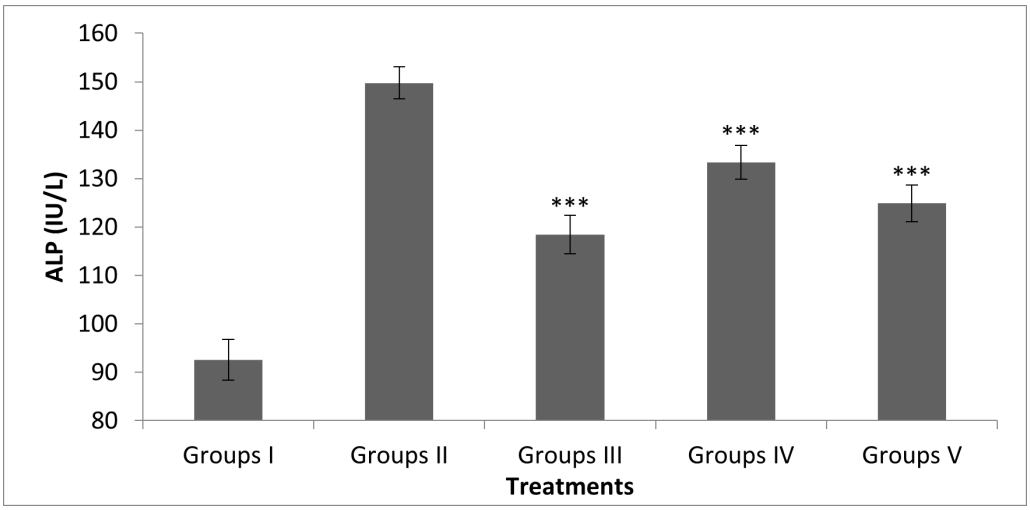

Figure 3: Effect of Lemon peel extract administration on ALP levels in isoniazid-rifampicin treated rats. Results are given as mean±SEM of six animals in each group.

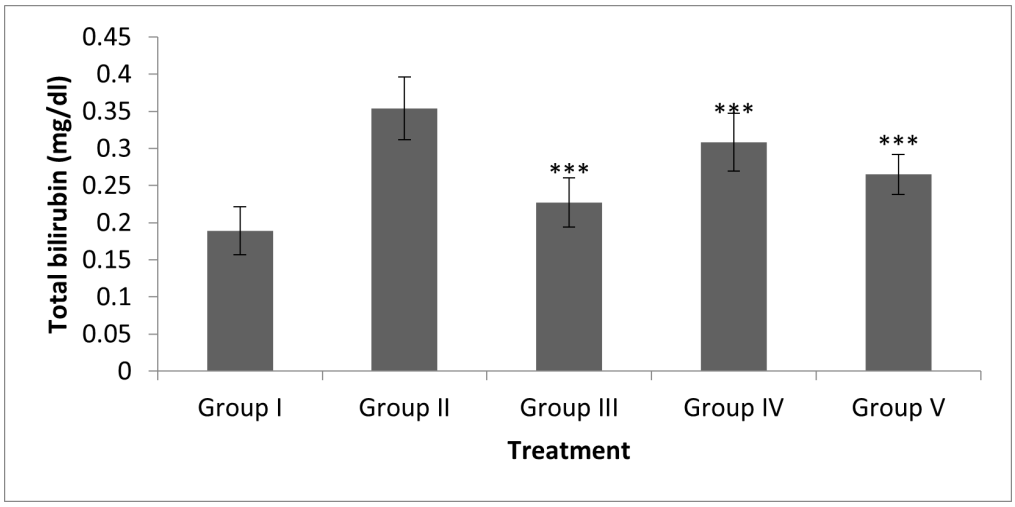

Figure 4: Effect of Lemon peel extract administration on total bilirubin in isoniazid-rifampicin treated rats. Results are given as mean±SEM of six animals in each group.

glutathione, a process mediated by the enzyme Glutathione S-Transferase (GST). However, when large doses of paracetamol overwhelm the capacity of the liver to conjugate NAPQI with glutathione, NAPQI accumulates and reacts with cellular macromolecules, such as proteins and lipids, leading to oxidative stress, mitochondrial dysfunction and ultimately hepatocellular damage.[18] This cascade of events can culminate in liver cell necrosis and liver failure if not promptly treated. Treatment strategies for managing and reversing hepatotoxicity frequently involve the use of radical scavengers and antioxidants.[19]

Antioxidants, often natural compounds derived from plants or synthesized, possess the ability to neutralize Reactive Oxygen Species (ROS) and free radicals generated during oxidative stress. By donating electrons or hydrogen atoms, antioxidants can effectively stabilize these highly reactive molecules, preventing them from causing cellular damage.[20] Moreover, antioxidants can also modulate signaling pathways involved in inflammation and cell death. For instance, Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of antioxidant enzymes and phase II detoxifying enzymes. The present study investigated the potential ameliorative effects of Lemon peel extract on hepatocellular damage induced by Paracetamol in rats.[21] oxicity, offering potential avenues for therapeutic interventions in liver health.

AST is an enzyme found primarily in the liver, heart, skeletal muscle, kidneys and brain. Its physiological role involves catalyzing the reversible transfer of an amino group between aspartate and α-ketoglutarate to form oxaloacetate and glutamate.[22] AST plays a crucial role in amino acid metabolism, particularly in the urea cycle and the synthesis of amino acids.[23] In the context of paracetamol-induced toxicity, elevated AST levels serve as a biomarker of liver damage. Paracetamol metabolite NAPQI depletes cellular glutathione stores and leads to oxidative stress and hepatocellular injury. As liver cells undergo damage and necrosis, AST is released into the bloodstream, resulting in elevated serum levels.[24] Therefore, in paracetamol-induced toxicity, increased AST levels reflect the extent of hepatocellular damage and serve as an indicator of liver injury. The findings of the present study suggest that treatment with the extract resulted in a decrease in AST levels compared to the control group, indicating a potential prevention of hepatocellular damage. This observation implies that the extract may possess hepatoprotective properties, as evidenced by the reduction in AST levels, which serve as a marker of liver injury. The decrease in AST levels suggests that the extract could mitigate the detrimental effects of the toxic insult, potentially by reducing oxidative stress, enhancing cellular antioxidant defences, or modulating inflammatory pathways.

ALT is an enzyme primarily found in the liver, although it is also present in smaller amounts in the kidneys, heart and skeletal muscle. Its physiological role involves catalysing the reversible transfer of an amino group between alanine and α-ketoglutarate to form pyruvate and glutamate.[25] ALT plays a crucial role in amino acid metabolism, particularly in gluconeogenesis, where it helps convert alanine into pyruvate for energy production or storage as glucose. As liver cells undergo damage and necrosis, ALT is released into the bloodstream, resulting in elevated serum levels.[26] Therefore, increased ALT levels in paracetamol-induced toxicity reflect the extent of hepatocellular damage and serve as an indicator of liver injury. The present study observed a reduction in ALT levels in the group treated with the extract, indicating a potential prevention of hepatocellular damage. This suggests that the extract may possess hepatoprotective properties, as evidenced by the decrease in ALT levels. The findings imply that the extract could mitigate the harmful effects of factors such as paracetamol-induced toxicity, potentially through mechanisms such as reducing oxidative stress or enhancing cellular antioxidant defences.

ALP is a crucial enzyme found in various tissues throughout the body, notably in the liver.[27] Its levels in the bloodstream are indicative of liver health, as elevated ALP levels often signify liver damage or dysfunction.[28] In our study, we observed the effects of different treatments on ALP levels in rats subjected to paracetamol-induced hepatotoxicity. Interestingly, Group II, which was administered only paracetamol, exhibited significantly elevated ALP levels compared to the normal control group, indicating liver damage due to paracetamol overdose. However, Groups III, IV and V, which received additional treatments of silymarin or extracts, showed varying degrees of reduction in ALP levels compared to Group II. Particularly noteworthy is Group III, where the combination of paracetamol and silymarin led to a notable decrease in ALP levels, suggesting a protective effect of silymarin against paracetamol-induced liver damage. The groups receiving different doses of the extract also demonstrated varying degrees of protection against liver damage, as evidenced by their ALP levels.

Bilirubin, a yellowish pigment produced during the breakdown of red blood cells, serves as a crucial indicator of liver function.[29] Elevated levels of bilirubin in the bloodstream often signify liver dysfunction, with jaundice being a common symptom.[30] Group II, administered solely with paracetamol, exhibited markedly higher bilirubin levels compared to the normal control group, indicative of liver damage caused by paracetamol overdose. However, intriguingly, Groups III, IV and V, which received additional treatments of silymarin or extracts, displayed varying degrees of reduction in bilirubin levels compared to Group II. Notably, Group III, receiving the combination of paracetamol and silymarin, demonstrated a notable decrease in bilirubin levels, suggesting a protective effect of silymarin against paracetamol-induced liver injury.

CONCLUSION

The study investigates the hepatoprotective effects of Lemon peel extract against paracetamol-induced liver damage in rats. Paracetamol overdose can lead to hepatocellular damage due to the accumulation of its toxic metabolite, NAPQI, causing oxidative stress and liver injury. Elevated levels of liver enzymes AST, ALT and ALP, along with bilirubin, serve as markers of liver damage. Treatment with Lemon peel extract resulted in reduced levels of AST, ALT and ALP compared to the control group, indicating potential mitigation of hepatocellular damage. This suggests that the extract may possess hepatoprotective properties, possibly by reducing oxidative stress and enhancing cellular antioxidant defenses. Moreover, the decrease in bilirubin levels further supports the protective effect of the extract against paracetamol-induced liver injury. These findings underscore the therapeutic potential of Lemon peel extract in safeguarding liver health and mitigating the detrimental effects of hepatotoxic agents like paracetamol.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AST: Aspartate Aminotransferase; **ALT:** Alanine Aminotransferase; **ALP:** Alkaline Phosphatase; **GST:** Glutathione S-transferase; **i.p.:** Intraperitoneal; **Nrf2:** Nuclear Factor Erythroid 2-related Factor 2; **NAPQI:** N-acetyl-p-benzoquinone imine; **ROS:** Reactive Oxygen Species; **SGOT:** Serum Glutamic Oxaloacetic Transaminase; **SGPT:** Serum Glutamic-Pyruvic Transaminase; **SEM:** Standard Error of the Mean.

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