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Original Research Article

Hepatoprotective and Antioxidant Effects of Osajin in Acetaminophen-induced Hepatotoxicity in Rats

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Qiaolin Wang[1] and Jingjing Cheng[1]

Abstract

Background: Osajin (OSJ), a prenylated isoflavone from *Maclura pomifera*, has specific ethnobotanical or traditional medicinal uses. While its role in oxidative stress modulation is established, its efficacy in preventing acetaminophen (APAP)-induced hepatotoxicity remains unexplored. This study evaluates the hepatoprotective effects of OSJ against APAP-induced liver injury in a rat model.

Objectives: To investigate whether Osajin (OSJ), a prenylated isoflavone from *Maclura pomifera,* confers hepatoprotective effects against acetaminophen (APAP)-induced liver injury in rats, and to assess its impact on oxidative stress–related pathways underlying APAP hepatotoxicity.

Materials and Methods: Twenty-four rats were divided, with six rats in each group, comprising four groups: control (distilled water), APAP control (single APAP dose on day 5), APAP + silymarin (100 mg/kg for a week), and APAP + OSJ (30 mg/kg for a week). Serum biomarkers and hematological parameters were assessed. Lipid profile markers, total cholesterol (TC), and triglycerides (TGs) were evaluated. Oxidative stress was analyzed by measuring protein carbonyl (PCO) levels and the ferric-reducing ability, while antioxidant activity was assessed using superoxide dismutase and catalase assays. Histopathological examination was conducted to assess liver tissue damage.

Results: OSJ significantly reduced alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels, restored hematological parameters and lipid homeostasis, and alleviated oxidative stress by enhancing antioxidant enzyme activity. Although slightly less effective than silymarin (SM), OSJ provided substantial hepatoprotection. Histopathological analysis confirmed preserved hepatic architecture with reduced inflammation and necrosis, further supporting its protective role.

Conclusion: In this study, nursing care plays a critical role in translating scientific findings into clinical practice, particularly through the monitoring and management of adverse effects and effective collaboration with physicians. OSJ demonstrated notable hepatoprotective and antioxidant properties, indicating its potential utility as an agent for mitigating acetaminophen-induced hepatotoxicity.

Keywords

Osajin, hepatotoxicity, acetaminophen, oxidative stress, antioxidants, histopathology

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Introduction

Acetaminophen (APAP) (paracetamol) prevails to be a widely used analgesic and anti-pyretic agent, easily available as an over-the-counter drug, with billions of doses consumed annually worldwide (Bessems & Vermeulen, 2001). Its widespread availability and well-established safety profile at therapeutic doses have made it a first-line option for managing mild to moderate pain and fever. However, APAP overdose, whether accidental or intentional, remains a leading cause of drug-induced liver injury (DILI) globally, contributing to nearly 50% of acute liver failure (ALF) cases in the USA (Yoon et al., 2016). The hepatotoxicity of APAP is attributed primarily to its metabolic activation by CYP2E1, which leads to the formation of a highly reactive metabolite (Jaeschke & Ramachandran, 2024). Under normal physiological conditions, *N*-acetyl-para-benzoquinone imine (NAPQI) is efficiently detoxified through conjugation with reduced

[1]Hepatobiliary and Pancreatic Surgery, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Third Hospital of Shanxi Medical University, Tongji Shanxi Hospital, Taiyuan, Shanxi, China

Corresponding author:
Jingjing Cheng, Hepatobiliary and Pancreatic Surgery, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Third Hospital of Shanxi Medical University, Tongji Shanxi Hospital, Taiyuan, Shanxi 030032, China.
E-mail: chengjingjing@sxbqeh.com.cn

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glutathione (GSH), rendering it harmless. However, excessive APAP consumption depletes hepatic GSH stores, resulting in the accumulation of NAPQI and subsequent oxidative stress, mitochondrial dysfunction, and hepatocellular necrosis (Hinson et al., 2010). Secondary inflammatory responses exacerbate this process, further amplifying liver injury. Given the limited treatment options available, primarily *N*-acetylcysteine (NAC) as an antidote, it is critical to explore novel hepatoprotective agents, particularly from natural sources, to mitigate APAP-induced hepatotoxicity (Akakpo et al., 2022). APAP toxicity is caused by the majority of APAP being glucuronidated and sulfated, resulting in the creation of inert, water-soluble conjugates that are eliminated in urine. However, cytochrome P450 enzymes (primarily CYP2E1, CYP3A4, and CYP1A2) convert 5%–10% of the dosage to NAPQI. In case of overdose, GSH reserves become rapidly decreased, leaving excess NAPQI free to covalently bind to cellular macromolecules, leading to oxidative stress, protein dysfunction, and toxicity. Oxidative stress plays a crucial role in APAP-induced liver toxicity, as evidenced by increased levels of reactive oxygen and markers of lipid and protein oxidation in affected tissues (Hinson et al., 1998).

Additionally, inflammation exacerbates APAP-induced liver damage. Activation of Kupffer cells, the resident macrophages of the liver, leads to the secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) (Ramachandran & Jaeschke, 2017). The nuclear factor-kappa B (NF-κB) signaling pathway plays a crucial role in this process, further promoting prolonged inflammatory response and hepatocellular injury (Liu et al., 2017). NAC is the mainstay treatment for APAP toxicity since it serves as a precursor of GSH and helps restore GSH levels, thus facilitating detoxification of the reactive metabolite NAPQI (Ershad et al., 2024). NAC therapy has been proven to work best when administered immediately after an overdose, and its effectiveness is reduced following a delayed initiation of treatment (Smilkstein et al., 2004). In addition, some patients with hepatotoxicity develop despite NAC treatment during the therapeutic window, which warrants alternative therapeutic strategies.

Due to these restrictions, there is increased interest in the identification of new hepatoprotective agents, especially the use of natural agents that can diminish liver damage induced by APAP. A lot of phytochemicals, derived from plants, demonstrated significant protective effects against the liver through attenuation of oxidative stress and inflammation in experimental models. Due to these properties, phytochemicals have received much attention for their pharmacognostical properties that offer therapeutic benefits (Subramanya et al., 2018). Different plant derivatives' bioactive compounds, including flavonoids, phenols, glycosides, steroids, polyphenols, and alkaloids, have been shown to counter the damage to the liver against compounds like APAP. These compounds have hepatoprotective actions due to their ability to reduce oxidative stress, modulate inflammatory pathways, and preserve mitochondrial function (Ganesan et al., 2018). Due to their multi-functional properties, natural compounds are a good candidate for liver protection and can be used as alternatives to conventional therapies (Guo et al., 2017).

Osajin (OSJ) is a flavonoid structurally isolated from *Maclura pomifera* (Osage orange), which has recently become a candidate for hepatoprotection owing to its pharmacological effects. Moreover, OSJ has a good pharmacokinetic profile with high bioavailability and low toxicity in preclinical models. Although these results are promising, the hepatoprotective potential of OSJ for APAP-induced liver injury in rats has not been investigated yet. OSJ, due to its proven pharmacological characteristics, is expected to be an effective and novel natural therapy agent for the prevention of APAP-induced hepatotoxicity. Physicians play an essential role in filling the gap between research and patient care, and in ensuring that cancer treatment is modified to improve individual pain. Physicians and nursing care professionals play essential roles in bridging the gap between research and patient care, ensuring that treatment strategies are appropriately modified to improve individual patient outcomes. The current study aims to investigate the hepatoprotective potential of OSJ in an in vivo rat model of APAP-induced liver toxicity.

Materials and Methods

Chemicals and Reagents

APAP (CAS no. 103-90-2) and OSJ (CAS no. SMB00092), a natural product derived from a plant source (≥95% purity, LC/MS-ELSD), along with other analytical-grade reagents, were obtained from Sigma–Aldrich (USA) and used in this study.

Experimental Animals

Male Wistar rats, weighing between 150 and 200 g, were obtained and kept in a controlled environment with a 12-h light/dark cycle, a temperature of 25°C ± 2°C, and 60% relative humidity. They had unrestricted access to a standard diet and water. The study was conducted in compliance with ethical guidelines approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Design

The rats were allocated into four groups, each consisting of six animals (n = 6 per group). Group I served as the normal control and received only the vehicle. Group II was administered APAP (750 mg/kg, orally). Group III received APAP along with SM (100 mg/kg). Group IV was treated with APAP and a dose of OSJ (30 mg/kg, orally) (Alhilal et al., 2023).

Biochemical Analysis

Alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities in serum

samples were determined using commercial kits. Lipid profile markers, including TC and TGs, were analyzed enzymatically.

Hematological Parameters

Hematological indices (HGB, RBC count, and HCT) were measured using an automated hematology analyzer.

Oxidative Stress Markers

Ferric reducing antioxidant power (FRAP) and protein carbonyl (PCO) assay were determined in the liver tissue homogenate using commercial kits from Sigma–Aldrich, USA.

Antioxidant Enzyme Activity

According to the instructions, superoxide dismutase (SOD) and catalase (CAT) activity were measured in the liver tissue homogenate using commercial colorimetric assay kits (Zell Bio GmbH, Ulm, Germany).

Histopathological Analysis

Liver samples were fixed in formalin 10% solution. After preparing the paraffin-embedded block by an automatic tissue processor, the tissue was cut into sections of 4–6 μm thickness by a rotary microtome. The samples were dyed with hematoxylin and eosin (H&E) and photographed with the camera under a microscope for histopathological examination.

In addition to qualitative assessment, a semi-quantitative histopathological scoring system (Ishak or Knodell scores) was employed to enhance the robustness and reproducibility of the findings. This grading system evaluated key parameters of liver injury, including hepatocellular necrosis, inflammatory infiltration, vacuolar degeneration, sinusoidal congestion, and architectural disruption. Each parameter was scored on a scale from 0 to 3 as follows: 0 = no abnormality; 1 = mild cellular changes or localized alterations; 2 = moderate necrosis/inflammation or regionally distributed lesions; 3 = severe necrosis, extensive inflammation, or significant architectural disruption.

Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons among different groups were performed using one-way analysis of variance (ANOVA) to assess the overall significance of treatment effects. When a significant difference was detected by ANOVA, Tukey's *post hoc* test was applied for multiple comparisons between individual groups. A p value less than .05 ($p < .05$) was considered to indicate statistical significance. Graphical and statistical analyses were conducted using GraphPad Prism (version 10, GraphPad Software, USA).

Results

Effects of OSJ on Liver Function Markers in APAP-induced Hepatotoxicity

As illustrated in Figure 1a–1c, the levels of ALT, AST, and LDH were elevated in the APAP-treated group, indicating liver damage. However, co-administration of SM (100 mg/kg) and OSJ (30 mg/kg) significantly reduced these enzyme levels compared to the APAP group. These findings suggest that OSJ possesses hepatoprotective properties against APAP-induced liver toxicity, as demonstrated by the notable decrease in ALT, AST, and LDH levels. Although its efficacy was slightly lower than that of SM, OSJ still provided considerable liver protection, emphasizing its potential as a natural hepatoprotective agent.

Effects of OSJ on Hematological Parameters in APAP-intoxicated SD Rats

The effects of OSJ on hematological parameters in APAP-intoxicated rats were evaluated by measuring HGB, RBC, and HCT levels (Figure 2). As shown in Figure 2a–2c, APAP administration significantly reduced HGB, RBC, and HCT levels. However, treatment with SM and OSJ (30 mg/kg) significantly increased hemoglobin concentration when compared to the APAP group. These findings indicate that OSJ treatment effectively mitigates hematological alterations caused by APAP intoxication.

Effects of Osajin on Lipid Profile in APAP-intoxicated SD Rats

The impact of OSJ on the lipid profile of APAP-intoxicated rats was assessed by measuring TC and TGs (Figure 3). As Figure 3a and 3b depicts, APAP administration led to an increase in TC and TG levels. On the other hand, SM and OSJ treatment reduced TC and TGs, suggesting that OSJ exerts a lipid-lowering effect. Overall, these findings indicate that OSJ effectively restores lipid homeostasis in APAP-intoxicated rats, demonstrating a protective role comparable to that of SM.

Effects of OSJ on Tissue Oxidative Stress Markers in APAP-intoxicated Rats

As Figure 4a and 4b depicts, APAP treatment increased FRAP and PCO levels compared to the control group, indicating heightened oxidative stress. However, treatment with both SM and OSJ (30 mg/kg) inhibited FRAP and PCO activity. These findings suggest that OSJ exhibits antioxidant properties by reducing plasma oxidative stress.

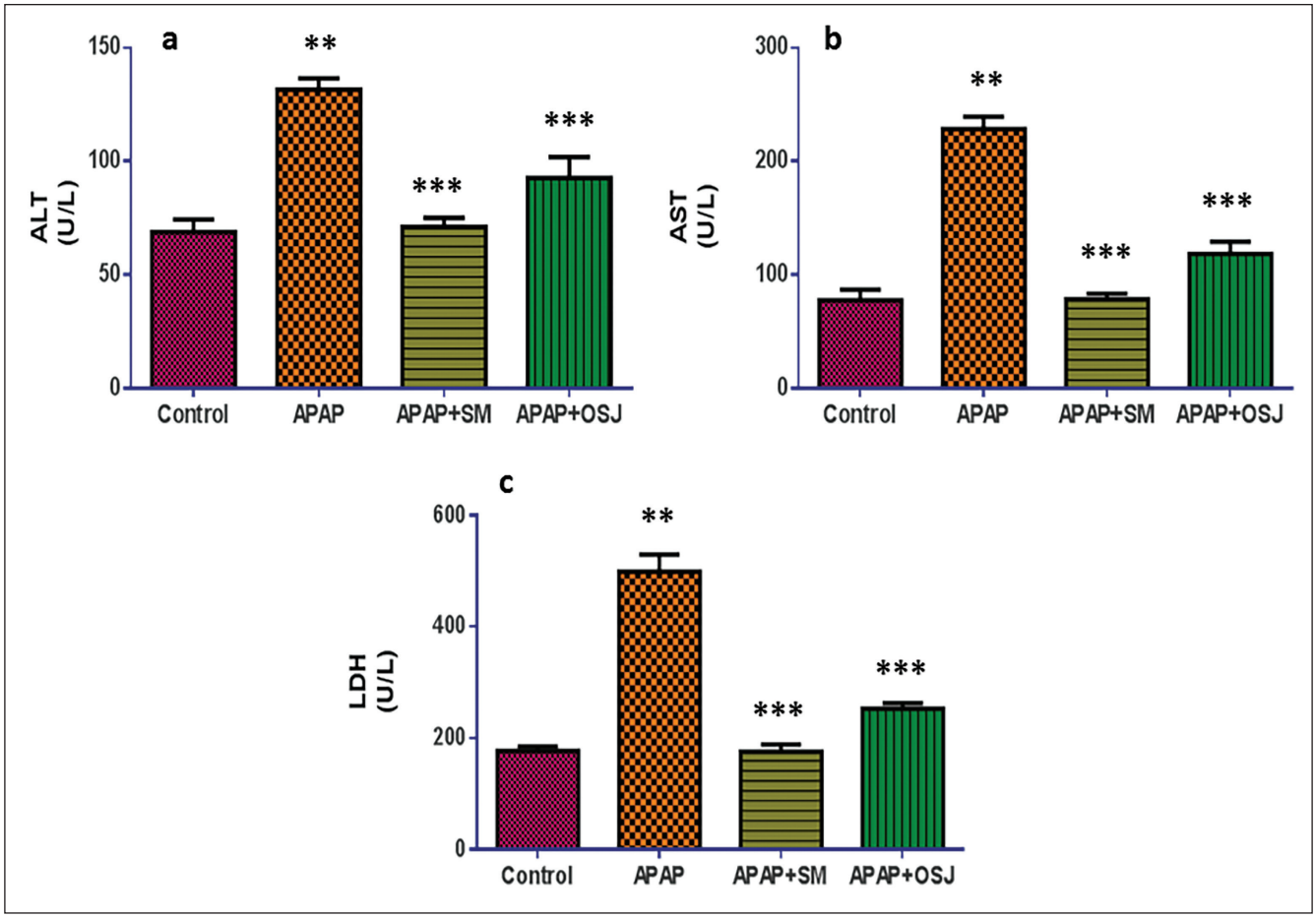


Figure 1. Impact of Osajin (OSJ) on Liver Function Markers in the Serum of Acetaminophen (APAP)-intoxicated Sprague-Dawley (SD) rats. (a) Alanine Transaminase (ALT, U/L), (b) Aspartate Transaminase (AST, U/L), and (c) Lactate Dehydrogenase (LDH, U/L) Levels are Presented. Data are Expressed as Mean ± SD (n = 6). A p Value of Less Than .05 was Considered Statistically Significant. **p < .05 Versus Control; ***p < .05 Versus APAP.

Effects of Osajin on Antioxidant Enzyme Activity in APAP-intoxicated Rats

As shown in Figure 5a and 5b, APAP administration significantly reduced SOD and CAT activity compared to the control group, indicating impaired antioxidant defense. However, treatment with both SM and OSJ (30 mg/kg) significantly restored SOD and CAT activity, suggesting that OSJ enhances enzymatic antioxidant defense mechanisms. Overall, these findings suggest that OSJ effectively mitigates APAP-induced oxidative stress by enhancing the activity of crucial antioxidant enzymes, similar to the effects observed with SM treatment.

Histopathological Analysis of Liver Sections in Different Experimental Groups

Figure 6, Group 1, the normal control group exhibits a well-preserved architecture, showing normal hepatocytes arranged in a uniform pattern with intact sinusoids and central veins (Figure 6a). There is no evidence of inflammation, necrosis, or fibrosis, indicating normal liver function. Group II severe hepatic damage is evident in the APAP-intoxicated group. The liver section shows pronounced architectural disruption with extensive hepatocyte degeneration, inflammation, and necrosis. Marked congestion and infiltration of inflammatory cells around the central vein and portal areas suggest significant oxidative stress and hepatotoxicity (Figure 6b). Group III SM administration demonstrates protective effects, as shown by a reduction in hepatic damage. The liver architecture is partially restored, with fewer necrotic areas, reduced inflammatory infiltration, and improved hepatocyte integrity compared to the APAP-treated group (Figure 6c). Similar to the SM group, the liver section of OSJ-treated rats (Group IV) showed considerable preservation of hepatic structure. The hepatocytes appear more intact, with reduced inflammation and necrosis compared to the APAP group. This indicates that OSJ effectively protects against APAP-induced

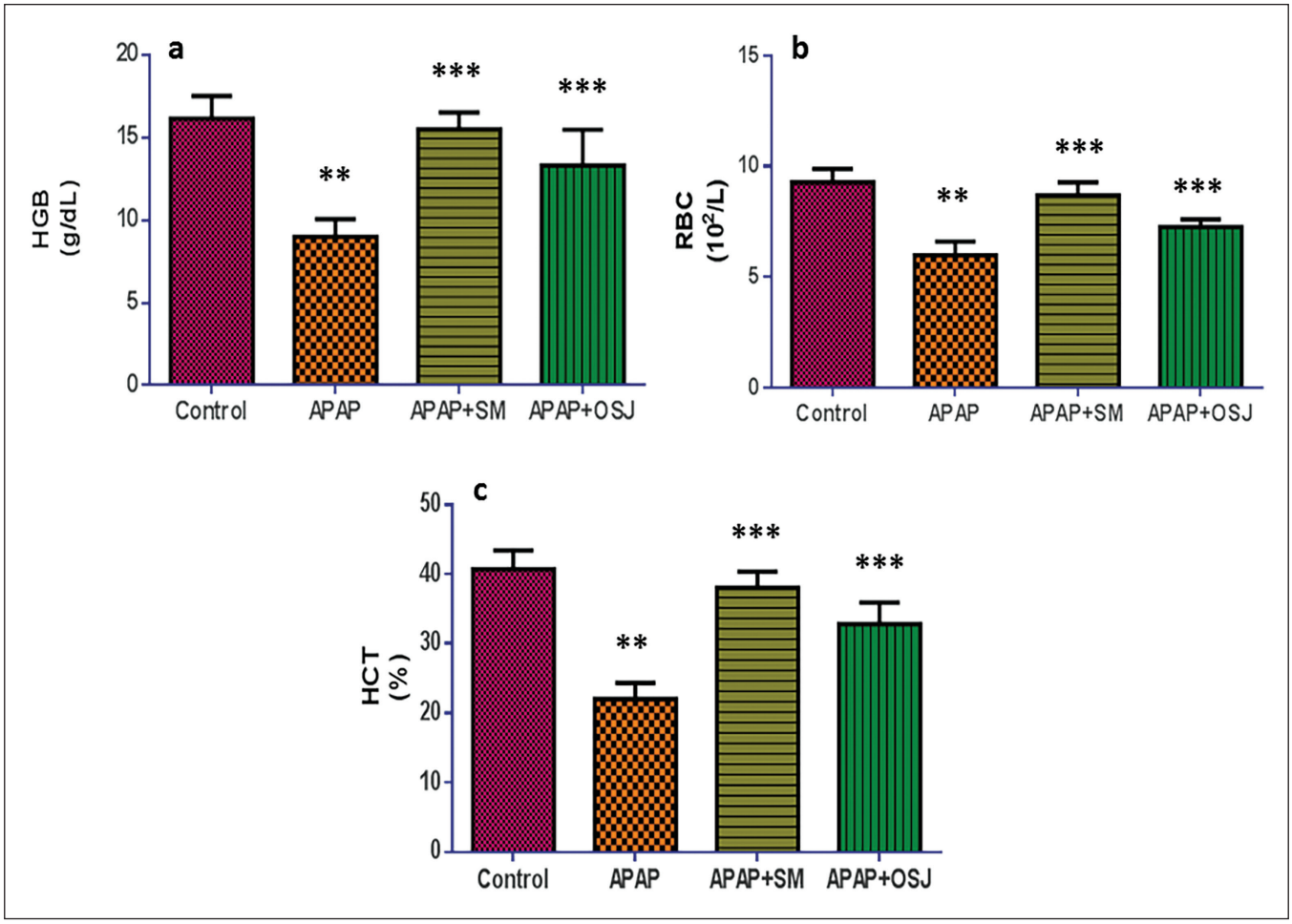


Figure 2. Effects of Osajin (OSJ) on Hematological Parameters in Acetaminophen (APAP)-intoxicated Sprague-Dawley (SD) Rats. (a) Hemoglobin Concentration (HGB, g/dL), (b) Total Red Blood Cell Count (RBC, 10^2/L), and (c) Hematocrit (HCT, %). Data are Presented as Mean ± SD (n = 6). A p Value of Less Than .05 was Considered Statistically Significant. $^{**}p < .05$ Versus Control; $^{***}p < .05$ Versus APAP.

hepatotoxicity, supporting its potential as a therapeutic agent for liver damage (Figure 6d).

Histopathological examination of liver specimens from all experimental cohorts revealed differentiated patterns of hepatic damage and rescue, with each parameter being semi-quantitatively ranked on a 0–15 scale that included necrosis, inflammation, vacuolar degeneration, sinusoidal congestion, and overall architectural integrity. The control cohort (Panel a) displayed normal hepatocyte structure and a completely intact liver framework, lacking any necrosis or inflammatory cell infiltration, and registered a cumulative injury score of 0–1, indicative of intact liver parenchyma. Conversely, the APAP control cohort (Panel b) exhibited pronounced hepatic injury typified by centrilobular necrosis, moderate to extensive inflammatory infiltrates, marked vacuolar degeneration, and sinusoidal congestion, earning the maximal injury score of 9–12 that underscores severe hepatic damage. The APAP + SM cohort (Panel c) exhibited only slight vacuolization, accompanied by scant necrosis or inflammatory cell infiltration, and nearly complete preservation of lobular structure, resulting in a substantially lower injury score of 2–4, consistent with hepatoprotection. The APAP + OSJ cohort (Panel d) displayed diminished necrosis and inflammatory cell infiltration compared to the APAP control, and a moderate preservation of hepatic architecture, yielding an intermediate injury score of 4–6, further endorsing the hepatoprotective capacity of OSJ.

Discussion

The hepatoprotective effects in a rat model of APAP-induced liver damage are strongly supported by the current research. Along with enhancements in oxidative stress markers and histopathological traits, reduced levels of serum ALT, AST, and LDH suggest that OSJ therapy greatly lessened liver damage. Hallmarks of liver damage, increased serum ALT, AST, and LDH, reflect the leaked activity of these enzymes

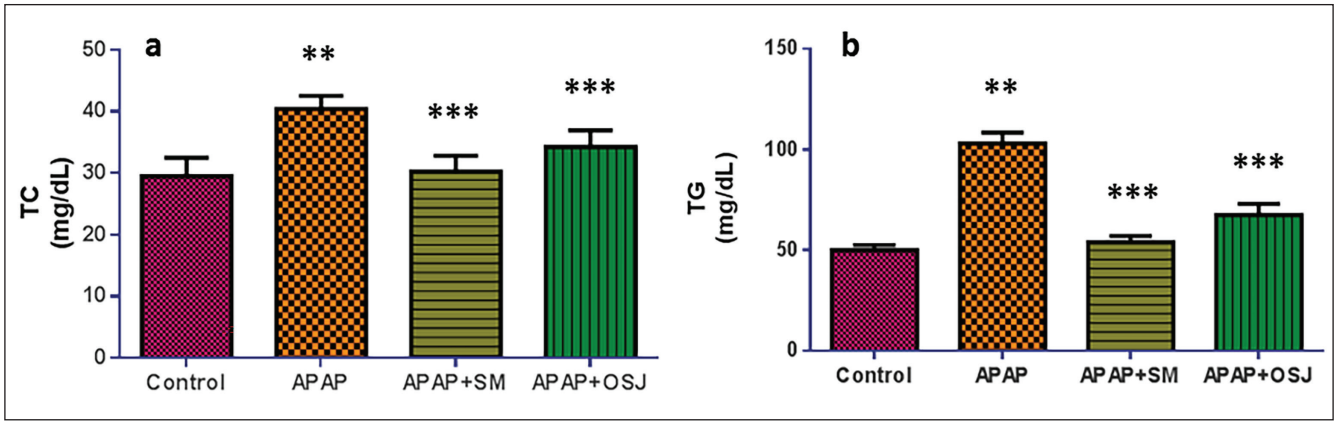


Figure 3. Effects of Osajin (OSJ) on the Lipid Profile of Acetaminophen (APAP)-intoxicated Sprague-Dawley (SD) Rats. (a) Total Cholesterol (TC, mg/dL) and (b) Triglycerides (TGs, mg/dL). Data are Expressed as Mean ± SD (n = 6). A p Value of Less Than .05 was Considered Statistically Significant. **p < .05 Versus Control; ***p < .05 Versus APAP.

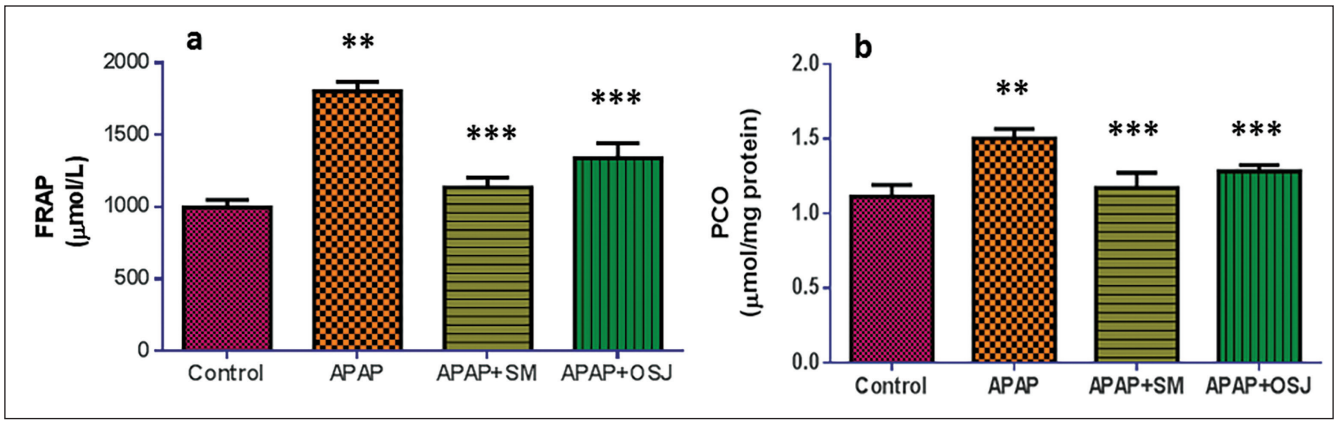


Figure 4. Effects of Osajin (OSJ) on Tissue Oxidative Stress Markers in Acetaminophen (APAP)-intoxicated Sprague-Dawley (SD) rats. (a) Ferric Reducing Ability of Plasma (FRAP, µmol/L) and (b) Protein Carbonyl (PCO, µmol/mg Protein). Data are Presented as Mean ± SD (n = 6). A p Value of Less Than .05 was Considered Statistically Significant. **p < .05 Versus Control; ***p < .05 Versus APAP.

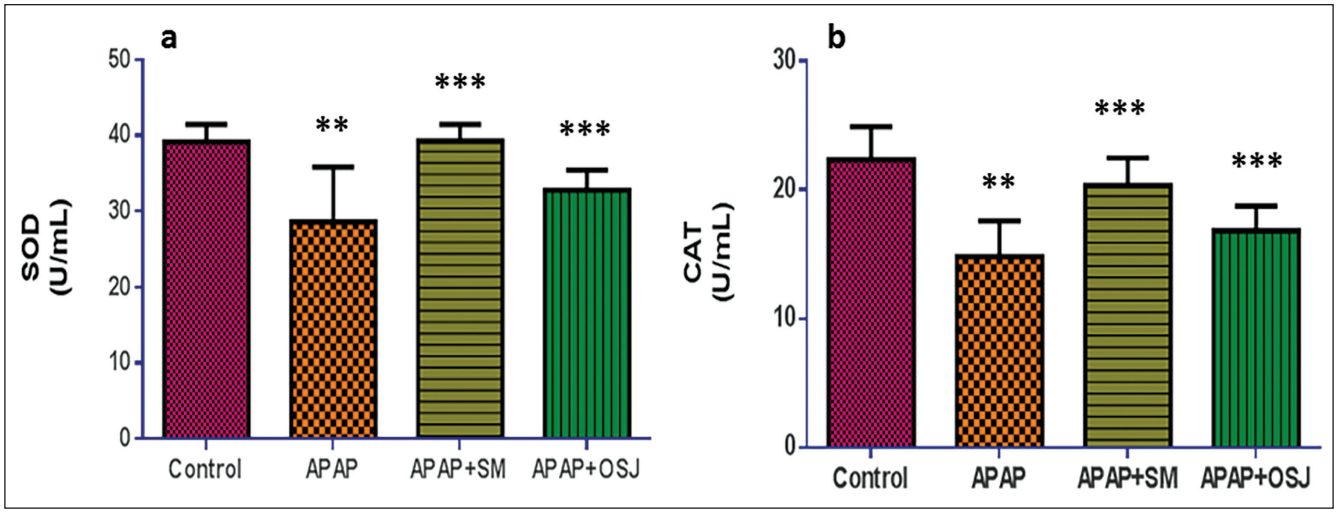


Figure 5. Effects of Osajin (OSJ) on Antioxidant Enzyme Activity in Acetaminophen (APAP)-intoxicated Sprague-Dawley (SD) rats. (a) Superoxide Dismutase (in SOD, U/mL) and (b) Catalase (CAT, U/mL). Data are Expressed as Mean ± SD (n = 6). A p Value of Less Than .05 was Considered Statistically Significant. **p < .05 Versus Control; ***p < .05 Versus APAP.

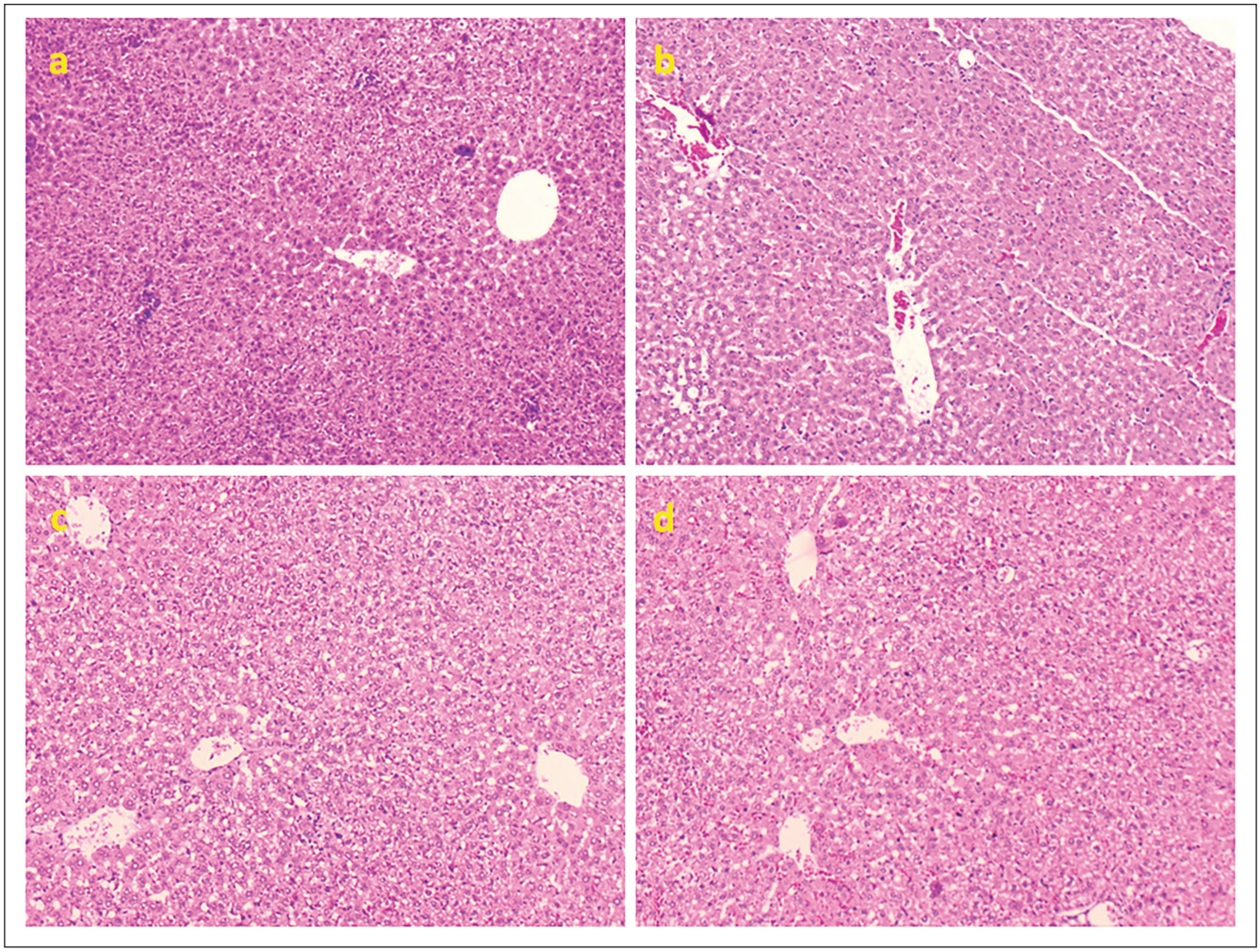


Figure 6. Microscopic Images of Liver Sections from Various Experimental Groups. (a) Normal Control Group, (b) Negative Control Group Receiving APAP Alone, (c) Silymarin-treated Group (100 mg/kg), and (d) Osajin (OSJ)-treated Group (30 mg/kg).

from injured hepatocytes into the circulation. The marked decrease in these enzyme levels in OSJ-treated groups highlights its capacity to maintain hepatocyte membrane integrity. The pronounced attenuation of these enzymatic markers in the OSJ-treated groups underscores its efficacy in preserving hepatocellular membrane integrity. Comparable decreases in liver enzyme activities have been documented in APAP-induced hepatotoxicity models (Ghobadi et al., 2019), wherein other natural antioxidants, including *Allium tripedale and Ficus exasperata extracts,* have similarly exhibited significant hepatoprotective properties.

By lowering PCO levels and increasing FRAP values, OSJ effectively negated oxidative stress, indicating its strong ROS-scavenging capability. A marker of oxidative damage to proteins, protein carbonylation is therefore reduced to show better redox homeostasis. Moreover, the recovery of antioxidant enzyme activities, including SOD and CAT, sheds light on OSJ's participation in strengthening endogenous defense systems. These results match with research on other prenylated isoflavones, which in experimental models of oxidative stress (Hwang et al., 2021) have shown comparable antioxidant properties. Inflammation is essential in increasing liver damage after an oxidative attack. Often, in APAP-induced hepatotoxicity, NF-κB signaling—a major mediator of inflammatory responses—is activated (Ramachandran et al., 2018). Even if this study did not directly measure NF-κB activity, the noted histological changes showed reduced necrosis and inflammatory cell infiltration, which suggests that OSJ could have anti-inflammatory properties. These results fit with earlier studies of natural substances, including *Cordia africana* and *Vernonia amygdalina*, which change inflammatory signaling pathways to guard the liver from injury (Geresu et al., 2022).

In this context, the normalization of hematological indices and lipid profiles observed following OSJ treatment indicates a broader protective modulation of systemic responses. By restoring RBC count, Hb levels, and WBC balance, OSJ may be mitigating the inflammatory cascade and oxidative stress

associated with APAP toxicity. Likewise, the correction of lipid abnormalities suggests that OSJ preserves or restores hepatic metabolic function, possibly by maintaining membrane integrity, enhancing mitochondrial efficiency, or modulating lipid-regulating enzymes. These findings support the notion that OSJ's hepatoprotective effects extend beyond direct cellular protection to include systemic anti-inflammatory and metabolic regulatory actions (Liao et al., 2023; McGill & Jaeschke, 2014; Yan et al., 2018).

Histopathological investigation showed great hepatoprotection in OSJ-treated groups along with near-normal liver architecture and little evidence of necrosis or inflammation. This architectural conservation is important because it highlights OSJ's power to preserve the regenerative ability of functional liver tissue and thus act as a guard. OSJ offers the benefit of dual antioxidant and anti-inflammatory activity over traditional APAP toxicity therapies such as NAC. Although NAC mainly restores GSH levels, which could offer improved protection against inflammatory and oxidative damage (Tenório et al., 2021).

OSJ demonstrated promising hepatoprotective effects in this study; its potential for therapeutic translation requires a more critical evaluation of its pharmacokinetic profile and bioavailability. As a prenylated isoflavone, OSJ possesses a lipophilic structure that may influence its absorption, distribution, and metabolism. However, data on its oral bioavailability remain limited. Previous studies on structurally related isoflavones have indicated poor aqueous solubility and low oral bioavailability, often necessitating the use of delivery systems to enhance systemic exposure (Zhang et al., 2009). OSJ is also likely subject to extensive first-pass metabolism in the liver, which could further limit its therapeutic efficacy unless appropriately formulated. Moreover, there is insufficient information on its half-life, tissue distribution, or clearance mechanisms *in vivo*, making it difficult to predict its pharmacodynamic window or potential toxicity at higher doses. These limitations highlight the need for comprehensive pharmacokinetic and bioavailability studies before considering OSJ for clinical application in liver diseases.

While the study demonstrates promising hepatoprotective effects of OSJ in an APAP-induced liver injury model, several limitations should be acknowledged. First, the findings are based solely on an acute rat model, and the translation of these results to humans requires further validation. Second, only a single dose and treatment duration of OSJ were tested, limiting the understanding of dose-response relationships and long-term effects. Third, mechanistic insights into the molecular pathways involved in OSJ's protective effects were not explored in detail. Additionally, the study did not assess the potential toxicity or pharmacokinetics of OSJ, which are crucial for therapeutic development. Future studies involving multiple dosing regimens, chronic models of liver injury, and detailed molecular analyses are needed to comprehensively evaluate OSJ's hepatoprotective potential.

Previous studies have explored the hepatoprotective potential of various plant-derived compounds against APAP-induced liver injury. For instance, *F. exasperata* leaf extract demonstrated hepatoprotection through modulation of oxidative stress and suppression of pro-inflammatory cytokines such as TNF-α and IL-6, along with restoration of antioxidant enzyme activity (Adetuyi et al., 2022). Similarly, *A. tripedale* extract exerted liver-protective effects by enhancing catalase and superoxide dismutase activities, reducing lipid peroxidation, and downregulating NF-κB signaling (Ghobadi et al., 2019). While OSJ also restored antioxidant defense (SOD, CAT) and improved biochemical and histological parameters in this study, direct anti-inflammatory evidence is lacking, as inflammatory mediators were not measured. However, based on its chemical structure as a prenylated isoflavone, OSJ likely shares similar mechanisms involving redox modulation and inhibition of inflammatory signaling. A side-by-side comparison of OSJ with other natural antioxidants may better contextualize its therapeutic promise.

Previous studies have reported that hepatocellular carcinoma (HCC) remains one of the most aggressive and lethal forms of liver cancer, often driven by complex molecular mechanisms and progressing to metastatic stages if not detected early (Prajapati et al., 2025). In earlier research for novel therapeutic strategies, endophytic fungi have emerged as promising renewable sources of pharmaceutically relevant compounds, offering new avenues for anti-cancer drug development. Among the molecular targets of interest in HCC, the c-MYC oncogene has gained attention due to its critical role in tumorigenesis, and recent advances have identified small molecules that selectively bind to and stabilize c-MYC G-quadruplex (G4) structures, thereby suppressing c-MYC signaling and inhibiting tumor growth (Thumpati et al., 2025). Supporting these efforts, earlier studies showed that a novel apoptotic protein, Pierisin-6, demonstrated significant cytotoxicity against HepG2 liver cancer cells by inducing apoptosis through mitochondrial pathways, specifically via caspase activation (Sarathbabu et al., 2019). Complementing this, a green nanotechnology-based approach was also successfully used to synthesize Fe_3O_4 magnetic nanoparticles using *C. guianensis* fruit extract (CGFE), which also exhibited dose-dependent cytotoxic effects against HepG2 cells (Sathishkumar et al., 2018). Such natural and nanotechnology-based therapeutics offer dual benefits-targeted cytotoxicity and biocompatibility-which are crucial in managing liver cancer. In RNA-seq, given that HCC may metastasize and contribute to the development of secondary tumors such as glioblastoma multiforme (GBM), identifying prognostic biomarkers and therapeutic agents that can suppress both primary and metastatic pathways remains a vital area of research (Mishra et al., 2023).

While the current study suggests that OSJ exerts dual antioxidant and anti-inflammatory effects in the context of APAP-induced hepatotoxicity, this conclusion is primarily based on

indirect observations such as improved oxidative stress markers, histopathological restoration, and normalization of hematological parameters. However, no direct measurements of pro-inflammatory cytokines (e.g., TNF-α, IL-6) or key transcriptional regulators like NF-κB were performed. These mediators play pivotal roles in hepatic inflammation and immune responses during APAP-induced liver injury. Therefore, the absence of such data limits the mechanistic understanding of OSJ's anti-inflammatory action. Future studies incorporating ELISA, Western blotting, or qPCR analyses of these markers are necessary to substantiate their role in modulating inflammatory signaling pathways.

Even if the results are encouraging, the anti-oxidative properties of OSJ should be investigated further in terms of its molecular pathways. Studies investigating its impact on mitochondrial function, apoptotic pathways, and inflammatory cytokine profiles could provide deeper insights. APAP (paracetamol) is a widely used analgesic and anti-pyretic agent; however, its overdose remains a leading cause of DILI and hepatic failure worldwide. While NAC is the standard treatment, there is growing interest in natural hepatoprotective agents as alternative or adjunctive therapies.

Conclusion

OSJ exhibits significant hepatoprotective and antioxidant effects against APAP-induced hepatotoxicity. Its ability to restore biochemical, hematological, and histological parameters underscores its potential as a therapeutic agent for liver diseases. Further studies are warranted to explore its clinical applicability.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

This study is approved by the Ethics Committee of Shanxi Bethune Hospital (Approval Number: YXLL-2024-272).

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ORCID iD

Jingjing Cheng https://orcid.org/0009-0007-3229-0478

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