

Protective effect of linalool against morphine-induced renal injury: Modulation of oxidative stress and PI3K/AKT/L-FABP signaling pathway

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ABSTRACT

Objective(s): Opioid misuse and prolonged therapeutic use are increasingly linked to systemic toxicities, including renal dysfunction. Morphine has been shown to induce oxidative stress and disrupt intracellular signaling pathways implicated in kidney injury. Linalool, a naturally occurring monoterpene with antioxidant and anti-inflammatory properties, may protect against drug-induced nephrotoxicity. This study investigated the protective effects of linalool against morphine-induced renal injury in rats, focusing on oxidative stress parameters and modulation of the PI3K/AKT/L-FABP signaling pathway.

Materials and Methods: Thirty-two male Wistar rats were randomly allocated into four groups (n = 8/group): Control, Morphine (10 mg/kg, SC), Linalool (50 mg/kg, IP), and Morphine + Linalool. Treatments were administered for three consecutive days using a conditioned place preference protocol to model morphine exposure. Renal function markers, oxidative stress parameters (MDA, SOD, CAT), histopathological alterations, and renal expression of AKT, PI3K, and L-FABP proteins were evaluated.

Results: Morphine administration significantly elevated serum creatinine, urinary injury markers, and lipid peroxidation (MDA), while decreasing antioxidant enzyme activities (SOD and CAT). Morphine also down-regulated AKT expression and PI3K, and up-regulated FABP in renal tissue. Co-treatment with linalool significantly improved biochemical parameters, attenuated histopathological damage, reduced oxidative stress, suppressed FABP overexpression, and restored AKT and PI3K expression.

Conclusion: Linalool exerts renoprotective effects against morphine-induced injury by reducing oxidative stress and modulating PI3K/AKT/L-FABP signaling. These findings suggest that linalool has therapeutic potential for preventing opioid-associated renal dysfunction.

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Introduction

Addiction is a chronic, relapsing disorder marked by compulsive drug-seeking behavior despite harmful consequences (1). It is recognized as a long-term disease of the central nervous system (CNS) that imposes significant medical and socioeconomic burdens worldwide (1, 2). Koob and Volkow described addiction as a state of compulsive substance-seeking behavior, characterized by impaired control over consumption and negative emotional states during withdrawal (3).

Opioids, particularly morphine, are widely prescribed for severe pain management (4, 5) but are also commonly abused for their euphoric properties (6). Although effective

analgesics, opioids can alter neuronal and peripheral physiological signaling mechanisms associated with dependence and organ dysfunction (7), and prolonged exposure often results in tolerance, dependence, and systemic toxicity (8). Despite their clinical utility, concerns regarding opioid-induced organ injury have increased, particularly in light of the growing global prevalence of prescription and illicit opioid use (9, 10).

Opioid receptors are categorized into μ , δ , and κ subtypes, with the μ -opioid receptor playing a central role in addiction and reward pathways (11, 12). Beyond central effects, opioid receptor activation influences peripheral organ systems, including the kidneys. Renal injury associated with opioid exposure may result from hypoxia, urinary retention,

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dehydration, oxidative stress, and rhabdomyolysis (13–17).

Acute kidney injury (AKI) is characterized by a rapid decline in glomerular filtration rate (GFR) and accumulation of nitrogenous waste products (18, 19). Its pathogenesis involves ATP depletion, intracellular calcium overload, inflammatory activation, reactive oxygen species (ROS) overproduction, and apoptosis (20–23). Lipid peroxidation and oxidative stress are central contributors to renal tubular damage (21, 23, 24).

The phosphoinositide 3-kinase (PI3K)/AKT pathway is a critical intracellular signaling cascade that regulates cell survival, proliferation, metabolism, and inflammatory responses (25–27). Dysregulation of PI3K/AKT signaling has been implicated in renal fibrosis and pathological remodeling (28, 29). Additionally, liver-type fatty acid-binding protein (L-FABP), primarily expressed in proximal tubular cells, serves as an early biomarker of tubular injury and plays a protective role in intracellular lipid transport and the regulation of oxidative stress (30–33).

Linalool ($C_{10}H_{18}O$) is a naturally occurring monoterpene present in the essential oils of numerous aromatic plants (34, 35). It exhibits anti-oxidant, anti-inflammatory, analgesic, and cytoprotective properties (34–37). Previous studies have demonstrated its ability to attenuate drug-induced oxidative stress and apoptosis in renal tissue (36, 37).

Given the limited mechanistic understanding of morphine-induced renal injury, this study aimed to evaluate whether linalool could mitigate morphine-associated nephrotoxicity by modulating oxidative stress and the PI3K/AKT/L-FABP signaling pathway.

Materials and Methods

Chemicals and reagents

Morphine sulfate was obtained from a certified pharmaceutical supplier and dissolved in sterile 0.9% normal saline immediately before administration. Linalool ($\geq 98\%$ purity, Product Number: L2602, Sigma Aldrich, Merck KGaA, Darmstadt, Germany) was prepared fresh daily and dissolved in a 1:1 mixture of normal saline and polyethylene glycol (PEG-400) to ensure proper solubilization (36). Ketamine and xylazine were purchased from approved veterinary pharmaceutical sources.

Commercial diagnostic kits for serum creatinine, blood urea nitrogen (BUN), alkaline phosphatase (ALP), urinary lactate dehydrogenase (LDH), and total protein were used according to the manufacturers' instructions. Oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT), were measured using commercially available spectrophotometric assay kits. All other chemicals were of analytical grade.

Experimental animals

Thirty-two adult male Wistar rats (200–250 g) were obtained from the animal facility at Ahvaz Jundishapur University of Medical Sciences. Rats were housed in polypropylene cages (4 rats per cage) under controlled environmental conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity 50–60%, 12-hr light/dark cycle). Standard laboratory chow and water were provided ad libitum.

Animals were acclimated for one week before experimentation. All experimental procedures were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethics Committee (IR.AJUMS.AEC.1403.032).

Experimental design and treatment protocol

Animals were randomly assigned into four experimental groups ($n=8$ per group):

- 1. Control group:** Received subcutaneous injection of morphine vehicle (0.9% saline) and intraperitoneal injection of linalool vehicle (saline/PEG mixture).
- 2. Linalool group:** Received linalool (50 mg/kg, IP) once daily for three consecutive days (37).
- 3. Morphine group:** Received morphine sulfate (10 mg/kg, SC) once daily for three consecutive days (38).
- 4. Morphine + Linalool group:** Received linalool (50 mg/kg, IP) 30 min before morphine administration (10 mg/kg, SC) for three consecutive days.

The selected morphine dose (10 mg/kg) has previously been shown to induce dependence-related behavioral responses and renal alterations in rodents (38). The linalool dose (50 mg/kg) was selected based on prior studies demonstrating anti-oxidant and renoprotective effects without significant behavioral interference (37).

Randomization was performed using a random allocation table. Investigators performing biochemical and histological analyses were blinded to group assignments.

Conditioned place preference (CPP) protocol

To model morphine exposure and behavioral dependence, a conditioned place preference (CPP) paradigm was used as previously described (39).

The protocol consisted of:

- **Pre-conditioning phase (Day 1):** Animals were allowed free access to both chambers to assess baseline preference.
- **Conditioning phase (Days 2–4):** Morphine or vehicle was administered, and animals were confined to the designated compartment for 30 min.
- **Post-conditioning phase (Day 5):** Animals were allowed free access to both chambers to assess place preference.

Drug treatments were administered only during the conditioning phase.

Sample collection

Twenty-four hours after the final treatment, animals were anesthetized with ketamine (50 mg/kg, IP) and xylazine (5 mg/kg, IP). Blood samples were collected via cardiac puncture using sterile syringes.

Blood samples were allowed to clot at room temperature for 60 min and centrifuged at $600 \times g$ for 15 min to separate serum. Serum aliquots were stored at -80°C until analysis.

Urine samples were collected directly from the bladder using sterile syringes before euthanasia. Urine samples were centrifuged at 4000 rpm for 10 min and stored at -20°C .

Kidneys were immediately excised, rinsed in ice-cold saline, and divided into two portions:

- One portion was fixed in 10% neutral-buffered formalin for histopathological analysis.
- One portion was snap-frozen in liquid nitrogen and stored at -80°C for molecular analysis.

Biochemical assessment of renal function

Serum creatinine and BUN levels were measured using enzymatic colorimetric kits based on the Jaffe reaction and the urease method, respectively. Serum ALP activity was determined by a colorimetric assay that measures the conversion of p-nitrophenyl phosphate to p-nitrophenol. ALP was included as part of a non-specific tissue injury panel because elevated ALP may reflect hepatorenal damage or membrane disruption in systemic toxicity models (40).

Urinary LDH activity was measured using a kinetic UV assay based on NADH oxidation. Urinary total protein concentration was determined using the Bradford method.

Urinary Protein-to-Creatinine Ratio (UPCR) was calculated by dividing urinary total protein concentration (mg/dl) by urinary creatinine concentration (mg/dl).

Serum cystatin C levels were measured using a commercially available ELISA kit according to the manufacturer's instructions and were used as a sensitive marker of GFR.

The following indices were calculated:

Fractional excretion of sodium (FENa)

$$\text{FENa (\%)} = \left[\frac{\text{urine Na} \times \text{serum creatinine}}{\text{serum Na} \times \text{urine creatinine}} \right] \times 100$$

Fractional excretion of potassium (FEK)

$$\text{FEK (\%)} = \left[\frac{\text{urine K} \times \text{serum creatinine}}{\text{serum K} \times \text{urine creatinine}} \right] \times 100$$

Renal failure index (RFI)

$$\text{RFI} = \frac{\text{Urine Na} \times \text{Serum Creatinine}}{\text{Urine creatinine}}$$

Measurement of oxidative stress parameters

Malondialdehyde (MDA)

Lipid peroxidation was quantified using the thiobarbituric acid reactive substances (TBARS) method. Briefly, serum samples were mixed with thiobarbituric acid reagent and heated at 95 °C for 30 min. After cooling, absorbance was measured at 532 nm. MDA concentration was expressed as nmol/ml.

Superoxide dismutase (SOD)

SOD activity was determined based on inhibition of superoxide-mediated reduction reactions. Absorbance was measured at 560 nm, and results were expressed as U/mL.

Catalase (CAT)

CAT activity was measured by monitoring the decomposition of hydrogen peroxide at 240 nm. Results were expressed as U/ml.

All assays were performed in duplicate.

Histopathological examination

Formalin-fixed kidney tissues were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Sections (4–6 µm thick) were prepared with a microtome and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope by a blinded pathologist. Renal injury was evaluated qualitatively based on:

- Glomerular hypertrophy
- Tubular epithelial degeneration
- Cast formation
- Interstitial edema
- Mesangial expansion

Representative photomicrographs were captured using a digital imaging system (magnification ×400).

Western blot analysis

Protein expression levels of PI3K, AKT, and L-FABP in renal tissues were determined by western blot analysis. Briefly, frozen kidney tissues were homogenized in ice-cold RIPA lysis buffer supplemented with protease and phosphatase inhibitors. The homogenates were centrifuged at 12,000×g for 15 min at 4 °C, and the supernatants were collected. Total protein concentration was determined using the Bradford protein assay. Equal amounts of protein (30–50 µg) were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes

were blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 hr at room temperature. The membranes were incubated overnight at 4 °C with primary antibodies against PI3K, AKT, L-FABP, and GAPDH. Following washing with TBST, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hr at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL) reagents and quantified by densitometric analysis using ImageJ software. Protein expression levels were normalized to GAPDH and expressed relative to the control group.

Statistical analysis

Data were expressed as mean±standard error of the mean (SEM). Statistical analyses were conducted in SPSS. Normality was assessed with the Shapiro–Wilk test. Comparisons among groups were performed using one-way analysis of variance (ANOVA) with Tukey's *post hoc* test. A *P*-value<0.05 was considered statistically significant.

Results

Linalool attenuated morphine-induced renal dysfunction

One-way ANOVA revealed significant differences among experimental groups in serum creatinine levels (*P*<0.001). Morphine administration significantly increased creatine kinase levels compared with the control group (*P*<0.01), indicating impaired renal filtration. Co-administration of linalool significantly reduced creatine kinase levels compared with the morphine-only group (*P*<0.01), restoring values toward control levels. Linalool alone did not significantly differ from control animals (*P*>0.05) (Figure 1A). Similarly, the urinary myoglobin-to-creatine kinase ratio was significantly elevated in the morphine group compared with controls (*P*<0.01), reflecting renal stress

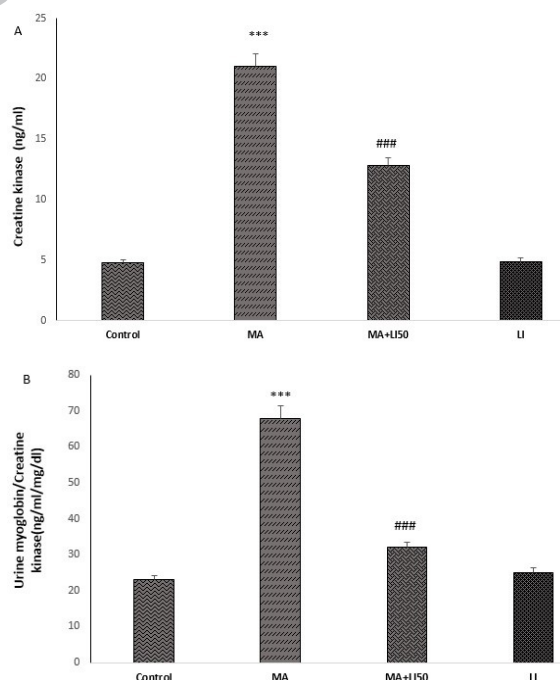


Figure 1. Effect of linalool on rat renal function markers (A) Creatine kinase levels and (B) Urinary myoglobin-to-creatine kinase ratio in experimental groups: Control, Morphine (MA), Linalool (LI), and Morphine + Linalool (MA+LI). Data are presented as mean±SEM (n=8 per group). Statistical analysis: One-way ANOVA followed by Tukey's *post hoc* test. *P*-values: ****P*<0.001, *P*<0.01 versus Control group; ###*P*<0.001, ##*P*<0.01 versus Morphine group. Linalool co-administration significantly attenuated morphine-induced increases in both parameters.

and possible muscle-related nephrotoxicity. Treatment with linalool significantly reduced this ratio compared with morphine-treated animals ($P<0.01$) (Figure 1B).

Linalool reduced enzymatic markers of tissue injury

Statistical analysis demonstrated significant intergroup differences in urinary LDH-to-creatinine ratio and serum ALP activity (ANOVA, $P<0.001$ for both). Morphine significantly increased urinary LDH levels compared with the control group ($P<0.001$), indicating tubular cellular injury. Co-treatment with linalool significantly attenuated this elevation ($P<0.01$ vs morphine group), though values remained slightly above control levels.

Serum ALP activity was also significantly elevated following morphine administration ($P<0.01$ vs control), while linalool significantly reduced ALP levels compared with morphine alone ($P<0.05$). Although ALP is primarily a liver enzyme, its elevation may reflect concurrent hepatorenal injury or release from tubular cell membranes due to systemic morphine toxicity. Nevertheless, linalool partially restored this parameter toward control levels (Figure 2).

Linalool improved renal functional indices

Significant differences were observed among groups for FENa, FEK, RFI, UPCR, and cystatin C (ANOVA, $P<0.001$ for all parameters).

Fractional excretion indices

Morphine significantly reduced FENa and FEK compared with control animals ($P<0.001$), indicating impaired tubular handling of electrolytes. Linalool co-administration significantly increased FENa and FEK relative to the morphine group ($P<0.01$ and $P<0.001$, respectively), indicating improved tubular function.

Renal failure index (RFI)

Morphine significantly decreased RFI compared with control ($P<0.01$). Linalool significantly restored RFI toward control levels ($P<0.01$ vs morphine).

Urinary protein-to-creatinine ratio (UPCR)

Morphine significantly increased UPCR compared with control animals ($P<0.001$), indicating glomerular dysfunction. Linalool significantly reduced UPCR compared with the morphine group ($P<0.01$), suggesting improvement in glomerular integrity.

Cystatin C

Serum cystatin C levels were significantly elevated in morphine-treated animals compared with controls ($P<0.001$), confirming renal impairment. Linalool significantly decreased cystatin C levels compared with

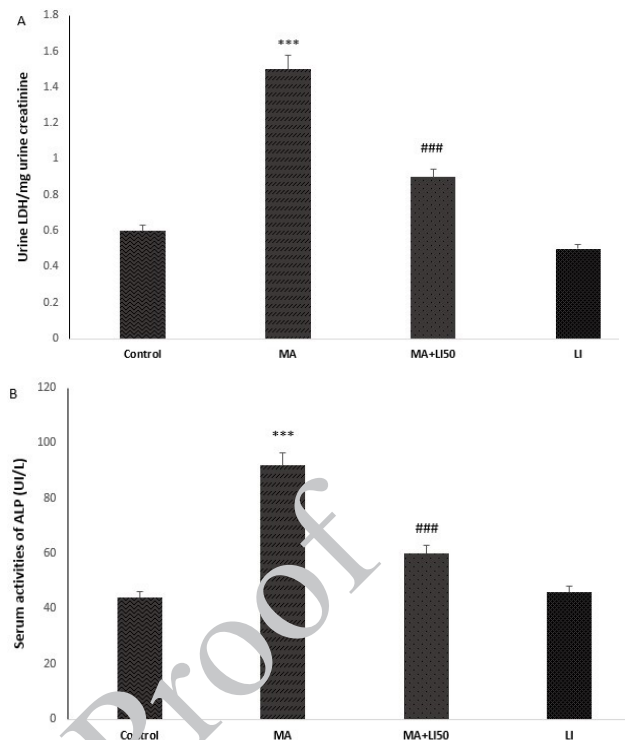


Figure 2. Effect of linalool on rat tissue injury markers (A) Urinary LDH-to-creatinine ratio and (B) Serum alkaline phosphatase (ALP) activity in all groups. Data are mean $\pm$ SEM (n=8). Statistical analysis: One-way ANOVA with Tukey's test. P-values: *** $P<0.001$, $P<0.01$ versus Control group; ### $P<0.01$, ## $P<0.01$, # $P<0.05$ versus Morphine group. Linalool significantly reduced the elevation of these enzymes induced by morphine.

morphine ($P<0.001$), indicating functional recovery (Table 1).

Linalool mitigated morphine-induced oxidative stress

Significant intergroup differences were detected in oxidative stress markers (ANOVA, $P<0.001$).

Lipid peroxidation (MDA)

Morphine treatment resulted in a marked increase in serum MDA levels compared with controls ($P<0.001$), indicating enhanced lipid peroxidation. Linalool significantly reduced MDA levels compared with morphine alone ($P<0.001$), restoring values close to baseline (Figure 3A).

Anti-oxidant enzymes

SOD activity was significantly reduced in morphine-treated rats compared with controls ($P<0.001$). Linalool significantly restored SOD activity compared with morphine-treated rats ($P<0.01$). Similarly, CAT activity was

Table 1. Renal functional parameters including Fractional Excretion of Sodium (FENa), Fractional Excretion of Potassium (FEK), Renal Failure Index (RFI), Urinary Total Protein-to-Creatinine Ratio (UPCR), and Serum Cystatin C levels across rat experimental groups (Control, Morphine [MA], Linalool [LI], and Morphine + Linalool [MA+LI])

Parameters	Control	MA	LI	MA+LI150
FENa%	1.08 $\pm$ 0.04	0.5 $\pm$ 0.01***	1.006 $\pm$ 0.01	0.67 $\pm$ 0.05**
FEK%	143.31 $\pm$ 7.69	37.75 $\pm$ 2.57***	144.58 $\pm$ 5.4	94.78 $\pm$ 4.52###
RFI	0.51 $\pm$ 0.09	0.31 $\pm$ 0.02**	0.49 $\pm$ 0.06	0.52 $\pm$ 0.01**
UTP/Creatinine Ratio	2.05 $\pm$ 0.39	3.63 $\pm$ 0.37***	1.91 $\pm$ 0.07	2.23 $\pm$ 0.25**
Cystatin C	0.64 $\pm$ 0.04	1.2 $\pm$ 0.1***	0.65 $\pm$ 0.04	0.78 $\pm$ 0.015###

Data are presented as mean $\pm$ SEM (n=8 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. P-values: ** $P<0.01$ and *** $P<0.001$, versus Control group; ### $P<0.001$ and ## $P<0.01$ versus Morphine group.

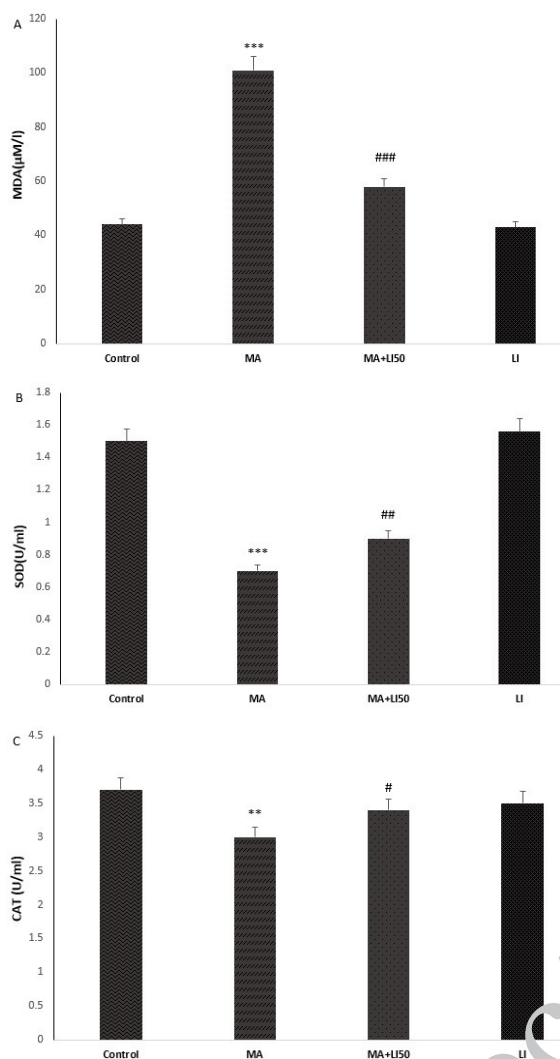


Figure 3. Effect of linalool on rat oxidative stress biomarkers in rat experimental groups (Control, Morphine [MA], Linalool [LI], and Morphine + Linalool [MA+LI]) (A) Serum malondialdehyde (MDA) levels, (B) Superoxide dismutase (SOD), and (C) Catalase (CAT) activities. Data are mean±SEM (n=8). Statistical analysis: One-way ANOVA followed by Tukey's test. *P*-values: **P*<0.001 versus Control group; ****P*<0.001, ##*P*<0.01 versus Morphine group. Morphine significantly increased lipid peroxidation and decreased anti-oxidant enzyme activities, while linalool reversed these effects.

significantly decreased in the morphine group ($P<0.001$ vs control). Linalool significantly increased CAT activity compared with morphine alone ($P<0.01$), although levels remained slightly lower than controls (Figure 3B–C).

These findings indicate that linalool effectively counteracts morphine-induced oxidative imbalance.

Histopathological findings

Histological examination revealed normal renal architecture in control and linalool-only groups. In contrast, kidneys from morphine-treated rats showed:

- Glomerular hypertrophy
- Tubular epithelial degeneration
- Luminal cast formation
- Interstitial cellular infiltration

These alterations indicate glomerular and tubular injury. Co-administration of linalool markedly attenuated these pathological changes, preserving glomerular structure and reducing tubular degeneration. Histological improvements correlated with biochemical and molecular findings (Figure 4).

Linalool modulated PI3K/AKT/L-FABP protein expression

Western blot analysis revealed significant intergroup differences in AKT, PI3K, and L-FABP protein expression (ANOVA, $P<0.001$ and $P<0.01$).

AKT expression

Morphine significantly up-regulated AKT expression compared with control animals ($P<0.001$), suggesting activation of stress-related signaling pathways. Linalool significantly reduced AKT expression compared with morphine alone ($P<0.01$), restoring levels toward baseline (Figure 5A).

L-FABP expression

L-FABP expression was significantly reduced in morphine-treated rats ($P<0.001$ vs control), indicating tubular stress and impaired lipid handling. Linalool significantly increased L-FABP expression compared with morphine alone ($P<0.01$), supporting improved tubular protection (Figure 5B).

PI3K expression

Morphine significantly down-regulated PI3K expression

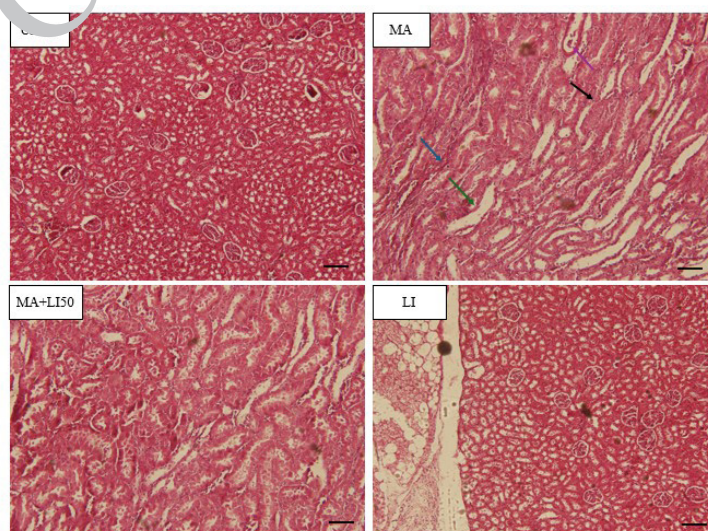


Figure 4. Representative photomicrographs of rat kidney tissues stained with Hematoxylin and Eosin (H&E) (×200 magnification, scale bar=100 μm). The Control and Linalool (LI) groups show normal renal architecture. The Morphine (MA) group shows interstitial fibrosis (blue arrow), tubular atrophy (black arrow), infiltration (violet arrow), and tubular dilatation (green arrows). The Morphine + Linalool (MA+LI) group shows marked histological improvement.

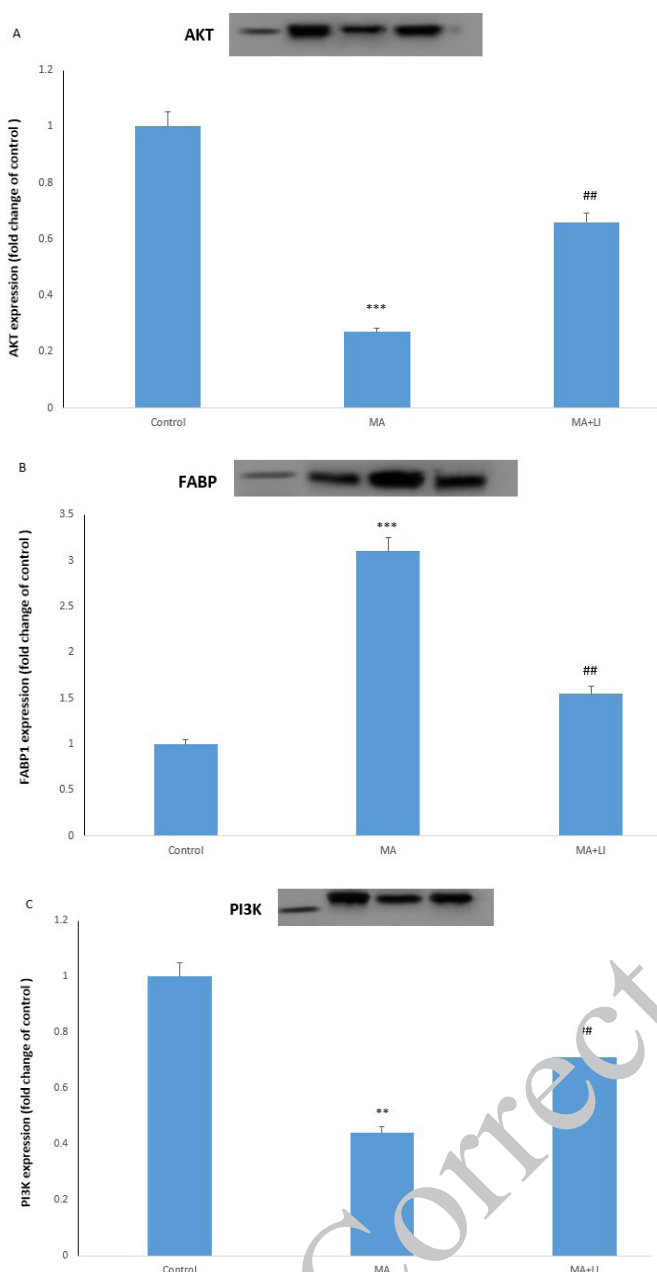


Figure 5. Effect of linalool on protein expression of signaling molecules in renal tissue (A) AKT, (B) L-FABP, and (C) PI3K. Protein expression was normalized to GAPDH. Data are mean $\pm$ SEM (n=8). Statistical analysis: One-way ANOVA with Tukey's test. P-values: *** P <0.001, ** P <0.01 versus Control group; ### P <0.001, ## P <0.01 versus Morphine group.

relative to controls (P <0.01). Linalool significantly increased PI3K expression compared with morphine-treated animals (P <0.01), suggesting restoration of upstream regulatory signaling (Figure 5C).

Discussion

The present study demonstrates that short-term morphine exposure induces significant renal dysfunction, characterized by an imbalance in oxidative stress, biochemical alterations, histopathological injury, and dysregulation of the PI3K/AKT/L-FABP signaling pathway. Importantly, linalool administration effectively attenuated these changes, suggesting a multifactorial renoprotective mechanism (36, 37).

Morphine administration significantly increased serum creatinine, cystatin C, urinary LDH, and the protein/creatinine ratio, indicating impairment of both glomerular filtration and tubular integrity. These findings align with previous reports of morphine-associated renal dysfunction and elevated biochemical markers of nephrotoxicity (38, 41). The observed increase in serum ALP after morphine administration may reflect renal tubular membrane damage or concurrent hepatorenal involvement (40).

Morphine-induced renal injury is thought to result from several interconnected mechanisms, including direct oxidative stress-mediated tubular toxicity (16, 17). A key finding of this study is a marked increase in lipid peroxidation (MDA) and a reduction in anti-oxidant enzyme activities (SOD and CAT) after morphine exposure. These results support the hypothesis that oxidative stress is a central mediator of opioid-induced nephrotoxicity (21-24).

Our findings demonstrate that morphine significantly down-regulated both AKT and PI3K expression. This pattern suggests an imbalance within the signaling axis, potentially reflecting stress-induced maladaptive activation rather than coordinated survival signaling. The PI3K/AKT pathway plays a crucial role in regulating cell survival, inflammation, and cellular stress responses (25-29). Therefore, modulating this pathway represents a promising therapeutic target for renal injury and oxidative stress-related disorders (28, 29).

Liver-type fatty acid binding protein (L-FABP) plays a crucial role in intracellular fatty acid trafficking and protection against lipid-induced oxidative damage (30-33). In this study, morphine significantly increased L-FABP expression, indicating compromised tubular metabolic resilience. Up-regulation of L-FABP may exacerbate lipid peroxidation and oxidative stress, amplifying tubular injury. Previous studies have suggested that urinary L-FABP serves as a sensitive mechanistic biomarker for AKI (30,31).

Linalool is a naturally occurring monoterpene with well-documented anti-oxidant and anti-inflammatory properties (34, 35). In this study, linalool significantly reduced MDA levels and restored SOD and CAT activities, confirming its ability to counteract ROS overproduction. These findings align with previous reports demonstrating the anti-oxidant and nephroprotective effects of linalool (36, 37).

The renoprotective effects observed here likely result from several interrelated mechanisms, including direct ROS scavenging, restoration of anti-oxidant enzyme activity, modulation of intracellular signaling pathways, and preservation of tubular metabolic homeostasis. Linalool increased morphine-induced down-regulation of AKT and PI3K, suggesting re-establishment of physiological signaling balance (25-29). Decreases in L-FABP expression may further contribute to protection against lipid-mediated oxidative injury (30-33).

Previous studies have reported similar renoprotective effects of linalool in experimental models of gentamicin- and rifampicin-induced nephrotoxicity (36, 37), supporting the biological plausibility of the present findings.

Taken together, the data suggest that morphine induces oxidative stress-driven renal injury, accompanied by dysregulation of survival signaling pathways and suppression of tubular protective mechanisms. Linalool mitigates these effects by reducing ROS-mediated membrane damage, restoring anti-oxidant defenses, normalizing PI3K/AKT signaling (25-29), and preserving L-FABP expression to maintain tubular resilience (30-33). This coordinated action

results in biochemical, molecular, and histological recovery.

Given the widespread clinical use of opioids, identification of adjunctive strategies to mitigate organ toxicity is of considerable importance. Although this study was conducted in an animal model, the findings suggest that anti-oxidant-based interventions such as linalool may provide protective benefits against opioid-associated renal injury. Further studies are warranted to determine long-term protective efficacy, investigate protein-level confirmation of signaling changes, evaluate dose-response relationships, and assess clinical applicability in chronic opioid exposure models (36, 37).

Some limitations should be acknowledged. The study duration was relatively short. Inflammatory cytokines were not measured. Only male animals were included. Future investigations incorporating inflammatory profiling and chronic exposure models would strengthen mechanistic understanding (25–31).

Conclusion

This study provides evidence that morphine induces renal injury via oxidative stress and disruption of PI3K/AKT/L-FABP signaling. Linalool effectively attenuates these changes, restoring biochemical, structural, and molecular homeostasis. These findings highlight linalool's potential as a renoprotective adjunct for opioid-associated nephrotoxicity.

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Authors' Contributions

S S, M R, and K H designed the study and developed the research concept. S S, M R, SP N, M KP, and P MC conducted the experiments and collected and processed the data. S S, M R, and K H analyzed and interpreted the data. S S and M R drafted the manuscript and prepared the figures and visualizations. K H and SP N critically revised and edited the manuscript for important intellectual content. K H supervised, directed, and managed the study and was responsible for funding acquisition. S S, M R, K H, SP N, M KP, and P MC approved the final version of the manuscript for publication and agreed to be accountable for all aspects of the work in accordance with ICMJE and COPE guidelines.

Conflicts of Interest

No conflicts of interest were disclosed.

Declaration

All data generated or analyzed in this study are included

in the published article.

References

1. Volkow ND, Blanco C. The changing opioid crisis: development, challenges and opportunities. *Mol Psychiatry* 2021 Jan;26:218-233.
2. Degenhardt L, Charlson F, Ferrari A, Santomauro D, Erskine H, Mantilla-Herrera A, *et al.* The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Psychiatry* 2018;5:987-1012.
3. Morrill JA, Axelrath S. Neurobiology of addiction: A disorder of choice. In: *Treating opioid addiction* 2019; Cham: Springer International Publishing. pp. 49-78.
4. Yaksh TL, Wallace MS. Opioids, analgesia, and pain management. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*. New York: McGraw-Hill Medical 2011; 481-526.
5. Nafziger AN, Barkin RL. Opioid therapy in acute and chronic pain. *J Clin Pharmacol* 2018 Sep;58:1111-1122.
6. Compton WM, Jones CM, Baldwin GT. Relationship between nonmedical prescription-opioid use and heroin use. *N Engl J Med* 2016;374:154-163.
7. Kosten TR, George TP. The neurobiology of opioid dependence: Implications for treatment. *Sci Pract Perspect* 2002;1:13-20.
8. Baldini A, Von Korff M, Lin EH. A review of potential adverse effects of long-term opioid therapy: A practitioner's guide. *Prim Care Companion CNS Disord* 2012;14:PCC.11m01326.
9. Obal D, Notarianni A, Smith L, Gross ER. Pro-con debate: Opioids and perioperative organ injury—friend or foe?. *Anesth Analg* 2026;142:832-842.
10. Peacock A, Leung J, Laney S, Colledge S, Hickman M, Rehm J, *et al.* Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. *Addiction* 2018;113:1905-1926.
11. Paulson H, Williams J. Basic opioid pharmacology: An update. *Br J Pain* 2012;6:11-16.
12. Pasternak TW. Molecular insights into μ opioid pharmacology: From the clinic to the bench. *Clin J Pain* 2010;26:S3-S9.
13. Mahappallil M, Sabu J, Friedman EA, Salifu M. What do we know about opioids and the kidney?. *Int J Mol Sci* 2017;18:223.
14. Didik S, Golosova D, Xu B, Staruschenko A. Opioids and the kidney: A compendium. *Kidney* 2023;4:1816-1823.
15. Atici S, Cinel I, Cinel L, Doruk N, Eskandari G, Oral U. Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model. *J Biosci* 2005;30:245-252.
16. Bellini L, Vadori M, De Benedictis GM, Busetto R. Effects of opioids on proximal renal tubular cells undergoing ATP depletion. *J Pharmacol Sci* 2016;131:288-291.
17. Lan X, Rai P, Chandel N, Cheng K, Lederman R, Saleem MA, *et al.* Morphine induces albuminuria by compromising podocyte integrity. *PLoS One* 2013;8:e55748.
18. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. *Lancet* 2019;394:1949-1964.
19. Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. *Compr Physiol* 2012;2:1303-1353.
20. Padanilam BJ. Cell death induced by acute renal injury: A perspective on the contributions of apoptosis and necrosis. *Am J Physiol Renal Physiol* 2003;284:F608-F627.
21. Tomsa AM, Alexa AL, Junie ML, Rachisan AL, Ciumarnean L. Oxidative stress as a potential target in acute kidney injury. *Peer J* 2019;7:e8046.
22. Wu MY, Yang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, *et al.* Current mechanistic concepts in ischemia and reperfusion injury. *Cell Physiol Biochem* 2018;46:1650-1667.
23. Ratliff BB, Abdulmahdi W, Pawar R, Wolin MS. Oxidant mechanisms in renal injury and disease. *Antioxid Redox Signal* 2016;25:119-146.
24. Gyurászová M, Gurecká R, Bábíčková J, Tóthová L. Oxidative stress in the pathophysiology of kidney disease: implications for noninvasive monitoring and identification of biomarkers. *Oxid Med Cell Longev* 2020;2020:5478708.
25. Manning BD, Toker A. AKT/PKB signaling: Navigating the

- network. *Cell* 2017;169:381-405.
26. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. *Cell* 2017;170:605-635.
 27. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002;296:1655-1657.
 28. Li R, Shi C, Wei C, Wang C, Du H, Liu R, et al. Fufang Shenhua tablet inhibits renal fibrosis by inhibiting PI3K/AKT. *Phytomedicine* 2023;116:154873.
 29. Wang H, Gao L, Zhao C, Fang F, Liu J, Wang Z, et al. The role of PI3K/Akt signaling pathway in chronic kidney disease. *Int Urol Nephrol* 2024;56:2623-2633.
 30. Xu Y, Xie Y, Shao X, Ni Z, Mou S. L-FABP: A novel biomarker of kidney disease. *Clin Chim Acta* 2015;445:85-90.
 31. Ferguson MA, Vaidya VS, Waikar SS, Collings FB, Sunderland KE, Gioules CJ, Bonventre JV. Urinary liver-type fatty acid-binding protein predicts adverse outcomes in acute kidney injury. *Kidney Int* 2010;77:708-714.
 32. Matsui K, Kamijo-Ikemori A, Sugaya T, Yasuda T, Kimura K. Renal liver-type fatty acid binding protein (L-FABP) attenuates acute kidney injury in aristolochic acid nephrotoxicity. *Am J Pathol* 2011;178:1021-1032.
 33. Negishi K, Noiri E, Maeda R, Portilla D, Sugaya T, Fujita T. Renal L-type fatty acid-binding protein mediates the bezafibrate reduction of cisplatin-induced acute kidney injury. *Kidney Int* 2008;73:1374-1384.
 34. Pereira I, Severino P, Santos AC, Silva AM, Souto EB. Linalool bioactive properties and potential applicability in drug delivery systems. *Colloids Surf B Biointerfaces* 2018;171:566-578.
 35. Aprotosoiaie AC, Hăncianu M, Costache II, Miron A. Linalool: A review on a key odorant molecule with valuable biological properties. *Flavour Fragrance J* 2014;29:193-219.
 36. Babaenezhad E, Dezfoulian O, Moradi Sarabi M, Ahmadvand H. Monoterpene linalool restrains gentamicin-mediated acute kidney injury in rats by subsiding oxidative stress, apoptosis, and the NF- κ B/iNOS/TNF- α /IL-1 β pathway and regulating TGF- β . *Naunyn Schmiedebergs Arch Pharmacol* 2024; 397: 5701-5714.
 37. Azirak S, Özgöçmen M. Linalool prevents kidney damage by inhibiting rifampicin-induced oxidative stress and apoptosis. *Tissue Cell* 2023;82:102097.
 38. Jalili C, Rashidi I, Roshankhah S, Jalili F, Salahshoor MR. Protective effect of genistein on the morphine-induced kidney disorders in male mice. *Electron J Gen Med* 2020;17:1.
 39. Wu HE, Schwasinger ET, Terashvili M, Tseng LF. Dextromorphine attenuates the morphine-produced conditioned place preference via the sigma1 receptor activation in the rat. *Eur J Pharmacol* 2007;562:221-226.
 40. Atici S, Cinel I, Cinel L, Demek N, Eskandari G, Oral U. Liver and kidney toxicity in chronic use of opioids: an experimental long term treatment model. *J Biosci* 2005;30:245-252.
 41. Lan X, Rai P, Chandel N, Cheng K, Lederman R, Saleem MA, et al. Morphine induced albuminuria by compromising podocyte integrity. *PLoS One* 2013;8:e55748.