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Determination of the levels of certain immunological and physiological markers in rats with paracetamol-induced nephrotoxicity treated with luteolin

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Abstract

Objective

This study was conducted to investigate the protective role of luteolin in reducing paracetamol-induced nephrotoxicity through the evaluation of certain immunological and biochemical parameters in albino rats.

Materials and methods

A total of 32 adult male laboratory rats (three months and 145-165 g) were used in this study. The experiment was carried out in the animal house of the Department of Biology, College of Science, University of Al-Qadisiyah. The animals were randomly divided into four groups, with eight rats in each group. Animals in the control group were administered normal water throughout the experimental period (30 days). The first treatment group (T1), in which animals were injected with paracetamol at a dose of 470 mg/kg body weight. Animals in the second treatment group (T2) were administered luteolin at a dose of 100 mg/kg body weight. The third treatment group (T3), in which animals were injected with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight. Paracetamol and luteolin were administered once daily for 30 consecutive days.

Results

The results of the current study demonstrated a significant increase ($P<0.05$) in kidney function indicators (urea, creatinine, and uric acid), malondialdehyde (MDA) levels, and inflammatory markers (TNF-α and IL-6). These indicators accompanied by a significant decrease ($P<0.05$) in body weight gain and antioxidant parameters (GSH and CAT) in the T1 group compared with the control group. In contrast, no significant differences were observed in the T2 group compared with the control group. Meanwhile, the T3 group showed marked improvement, represented by a significant reduction ($P<0.05$) in kidney function indicators, MDA levels, and inflammatory

markers (TNF-α and IL-6). These improvements were along with a significant increase ($P<0.05$) in body weight gain and antioxidant parameters (GSH and CAT) compared with the T1 group.

Conclusion

The results of this study showed that paracetamol administration caused significant nephrotoxicity. This damage was associated with impaired renal function, increased oxidative stress, and increased inflammatory markers in rats. Luteolin treatment significantly ameliorated these changes. It does this through its antioxidant and anti-inflammatory properties, leading to improved renal function indices and restoration of antioxidant defense mechanisms. Therefore, it can be concluded that luteolin may be a promising protective agent against paracetamol-induced renal injury.

Keywords: antioxidants, luteolin compound, nephrotoxicity, oxidative stress, paracetamol

Paper type: Research paper.

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Introduction

Nephrotoxicity refers to the deterioration of kidney function resulting from the toxic effects of drugs and chemical substances. Drug-induced nephrotoxicity is considered one of the major causes of kidney diseases, including acute kidney injury (AKI) and chronic kidney disease (CKD) (Kour et al., 2023). The severity of nephrotoxicity lies in the kidneys' high susceptibility to toxic agents, as they receive approximately 25% of the cardiac output due to their rich vascular supply. In addition, renal tubules play a crucial role in the reabsorption of many compounds, while the kidneys possess high metabolic activity, making them particularly vulnerable to various forms of toxic injury (Perazella, 2010; Mody et al., 2020). Paracetamol, also known as acetaminophen, is a widely used analgesic and antipyretic drug (Kanno et al., 2016). Although it is considered safe when administered within therapeutic doses, excessive intake can result in hepatic and renal toxicity (Ahmad et al., 2012). While high doses of paracetamol are primarily conjugated with glucuronic acid and sulfate, a substantial portion is metabolized by cytochrome P450 (CYP450) enzymes, leading to the formation of toxic reactive metabolites such as N-acetyl-p-benzoquinone imine (NAPQI). This metabolite reacts with sulfhydryl groups in glutathione (GSH), causing depletion of intracellular glutathione reserves and increased production of reactive oxygen species

(ROS), which subsequently induces oxidative stress and renal cellular damage (Gamal El-Din et al., 2003; Abdel-Zaher et al., 2008). Furthermore, oxidative stress resulting from paracetamol toxicity contributes to the activation of inflammatory responses and the increased release of pro-inflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), thereby exacerbating renal injury (Ibrahim et al., 2024). Luteolin is a natural flavonoid widely distributed in vegetables and fruits such as carrots, cabbage, olive oil, celery, peppers, and apples. It exhibits a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, and anticancer effects (Fan et al., 2024). Several edible herbs rich in luteolin have been utilized to restore the balance between oxidative and antioxidative systems due to their potent ability to scavenge reactive oxygen species (ROS). A recent study demonstrated the protective effects of luteolin on the kidneys and liver of rats through the regulation of inflammatory cytokine secretion, acting as both an anti-inflammatory and antioxidant agent. In addition, luteolin was shown to enhance the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD), while reducing malondialdehyde (MDA) concentrations (Vongthip et al., 2024). The present study aimed to investigate the protective role of luteolin against paracetamol-induced nephrotoxicity in rats through the evaluation of certain biochemical, oxidative stress, antioxidant, and inflammatory markers.

Materials and methods

Luteolin compound: In this study, luteolin (Nutricost, USA) was used in capsule form. The capsules were opened and the powder containing the active ingredient was dissolved prior to administration. The administered dose was 100 mg/kg body weight. The capsules were opened and the powder containing the active ingredient was extracted. The luteolin dose was selected based on a previous study that demonstrated that a dose of 100 mg/kg body weight exerted effective biological effects.

Experimental animals: Adult male albino laboratory rats were obtained from the College of Veterinary Medicine, University of Kufa. The animals were three months old and weighed between 145-165 g. The rats were maintained for two weeks for acclimatization and health monitoring before the initiation of the experiment. The study was then completed in the animal house of the Department of Biology, College of Science, University of Al-Qadisiyah.

Studied parameters: The evaluated parameters included body weight gain rate, kidney function indicators including urea, creatinine, and uric acid, antioxidant parameters including glutathione (GSH), malondialdehyde (MDA), and catalase (CAT), as well as inflammatory markers including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).

Experimental design: A total of 32 male albino rats were used in this experiment. The animals were weighed and then randomly divided into four groups, with eight rats in each group. Control *g*roup (C) included normal rats supplied with a standard diet and normal drinking water for one month. *In the f*irst *treatment group* (T1)*, r*ats were administered paracetamol orally by gastric gavage at a dose of 470 mg/kg body weight once daily for 30 days. *In the second treatment group* (T2)*, r*ats administered luteolin at a dose of 100 mg/kg body weight for one month. Rats *of*

*the t*hird *treatment group* (T3) injected with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight for one month.

Serum urea, creatinine, uric acid, GSH, CAT, and MDA levels were measured using commercial biochemical assay kits according to the manufacturer's instructions. Serum TNF-α and IL-6 concentrations were determined using ELISA kits.

Statistical Analysis: Data were expressed as mean ± standard error (Mean ± SE). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) post hoc test to determine significant differences among groups. A value of $P < 0.05$ was considered statistically significant.

Ethical Approval: All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee of the University of Al-Qadisiyah.

Results

Weight gain rate: The results presented in Table 1 showed a significant decrease ($P<0.05$) in body weight gain rate in the T1 group with paracetamol-induced nephrotoxicity compared with the control group. The results also demonstrated a significant increase ($P<0.05$) in the T2 and T3 groups when compared with the T1 group. However, a significant decrease ($P<0.05$) was observed in the T3 group compared with both the T2 and control groups. No significant differences were recorded between the T2 group and the control group.

Table 1. Effect of paracetamol and luteolin on body weight gain rate

Group	Before Experiment (g)	After Experiment (g)	Weight Change (g)
T1	151.40 ± 1.98^{a}	133.20 ± 3.76^{b}	-18.20 ± 2.08^{c}
T2	150.80 ± 3.73^{a}	159.40 ± 4.00^{a}	8.60 ± 0.87^{a}
T3	149.00 ± 2.68^{a}	143.80 ± 6.16^{b}	-5.20 ± 4.68^{b}
C	148.60 ± 2.08^{a}	157.40 ± 2.97^{a}	8.80 ± 1.06^{a}
LSD	8.14	13.16	7.96

The numbers represent the mean ± standard error (Mean ± SE). Different letters indicate significant differences ($P<0.05$) among the groups. C: Control group. T1: First treatment group injected with paracetamol at a dose of 470 mg/kg body weight for 30 days. T2: Second treatment group administered luteolin at a dose of 100 mg/kg body weight for 30 days. T3: Third treatment group injected with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight by oral gavage for 30 days.

Kidney function indicators (serum concentrations of urea, creatinine, and uric acid): The results showed a significant increase ($P<0.05$) in serum levels of urea, creatinine, and uric acid in the T1 group with paracetamol-induced nephrotoxicity compared with the control group. The findings also demonstrated a significant decrease ($P<0.05$) in the T2 and T3 groups compared with the T1 group. However, a significant increase ($P<0.05$) was observed in the T3 group compared with both the T2 and control groups. While, no significant differences were recorded between the T2 group and the control group (Table 2).

Table 2. Effect of paracetamol and luteolin on kidney function indicators

Group	UR (mg/dl)	CR (mg/dl)	UA (mg/dl)
T1	63.59 ± 2.11^{a}	3.79 ± 0.24^{a}	5.82 ± 0.72^{a}
T2	35.17 ± 2.25^{c}	1.15 ± 0.20^{c}	2.02 ± 0.30^{c}
T3	47.31 ± 5.92^{b}	1.89 ± 0.20^{b}	3.94 ± 0.36^{b}
C	33.44 ± 2.44^{c}	1.21 ± 0.08^{c}	1.97 ± 0.17^{c}
LSD	6.66	0.579	1.32

The numbers represent the mean ± standard error (Mean ± SE). Different letters indicate significant differences ($P<0.05$) among the groups. C: Control group. T1: First treatment group injected with paracetamol at a dose of 470 mg/kg body weight for 30 days. T2: Second treatment group administered luteolin at a dose of 100 mg/kg body weight for 30 days. T3: Third treatment group injected with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight by oral gavage for 30 days.

Oxidative stress and antioxidant markers: The results demonstrated a significant decrease ($P<0.05$) in the levels of the antioxidant markers catalase (CAT) and glutathione (GSH) in the T1 group with paracetamol-induced nephrotoxicity compared with the control group (Table 3). In contrast, a significant increase ($P<0.05$) in CAT and GSH levels was observed in the T2 and T3 groups compared with the T1 group. However, the T3 group showed a significant decrease ($P<0.05$) compared with both the T2 and control groups, while no significant differences were recorded between the T2 group and the control group. Regarding malondialdehyde (MDA), the results showed a significant increase ($P<0.05$) in the T1 group with paracetamol-induced nephrotoxicity compared with the control group. Conversely, a significant decrease ($P<0.05$) was observed in the T2 and T3 groups compared with the T1 group. Nevertheless, the T3 group exhibited a significant increase ($P<0.05$) compared with both the T2 and control groups, whereas no significant differences were found between the T2 group and the control group.

Table 3. Effect of paracetamol and luteolin on oxidative stress and antioxidant markers

Group	CAT	GSH	MDA
T1	4.89 ± 0.90^{c}	924.96 ± 12.58^{c}	7.26 ± 0.34^{a}
T2	10.63 ± 0.53^{a}	2385 ± 15.02^{a}	3.20 ± 0.59^{c}
T3	8.52 ± 1.02^{b}	1630 ± 12.04^{b}	5.40 ± 0.16^{b}
C	10.66 ± 0.30^{a}	2342 ± 53.39^{a}	3.49 ± 0.67^{c}
LSD	2.03	87.10	1.46

The numbers represent the mean ± standard error (Mean ± SE). Different letters indicate significant differences ($P<0.05$) among the groups. C: Control group. T1: First treatment group injected with paracetamol at a dose of 470 mg/kg body weight for 30 days. T2: Second treatment group administered luteolin at a dose of 100 mg/kg body weight for 30 days. T3: Third treatment group injected with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight by oral gavage for 30 days.

Inflammatory markers: The results of these markers showed a significant increase ($P<0.05$) in the inflammatory markers TNF-α and IL-6 in the T1 group with paracetamol-induced nephrotoxicity compared with the control group (Table 4). In contrast, the results demonstrated a significant decrease ($P<0.05$) in the T2 and T3 groups compared with the T1 group. However, a significant increase ($P<0.05$) was observed in the T3 group compared with both the T2 and control

groups, while no significant differences were recorded between the T2 group and the control group.

Table 4. Effect of paracetamol and luteolin on inflammatory markers

Group	IL-6 (ng/L)	TNF-α (ng/L)
T1	2.87 ± 0.04^{a}	63.84 ± 1.40^{a}
T2	1.34 ± 0.10^{c}	29.48 ± 1.12^{c}
T3	2.04 ± 0.03^{b}	45.03 ± 1.52^{b}
C	1.25 ± 0.05^{c}	30.65 ± 3.11^{c}
LSD	0.204	5.85

The numbers represent the mean ± standard error (Mean ± SE). Different letters indicate significant differences ($P<0.05$) among the groups. C: Control group. T1: First treatment group injected with paracetamol at a dose of 470 mg/kg body weight for 30 days. T2: Second treatment group administered luteolin at a dose of 100 mg/kg body weight for 30 days. T3: Third treatment group injected with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight by oral gavage for 30 days.

Discussion

Body weight: The current results illustrated a significant decrease ($P<0.05$) in body weight gain rate in the T1 group treated with paracetamol at a dose of 470 mg/kg body weight for one month compared with the control group. These findings are consistent with results of other researchers, who indicated that paracetamol administration caused a significant reduction in body weight. This decrease may be attributed to loss of appetite resulting from the effect of paracetamol on the hypothalamic centers responsible for appetite regulation and digestion, leading to reduced food and water intake and consequently decreased body weight (Assi et al., 2024). The significant reduction in body weight gain may also be explained by disturbances in metabolic processes caused by the toxic effects of paracetamol (Jaeschke et al., 2024). Administration of high doses of paracetamol results in the formation of toxic metabolites such as N-acetyl-p-benzoquinone imine (NAPQI), which depletes glutathione reserves and induces oxidative stress (Liao et al., 2023). The oxidative stress generated by paracetamol toxicity contributes to mitochondrial dysfunction by disrupting cellular respiration and reducing intracellular energy production (Hu et al., 2024; Li et al., 2025). Consequently, the body increases catabolic processes to compensate for the energy deficit through the consumption of stored fats and proteins, thereby contributing to body weight loss. This reduction may also be associated with dehydration resulting from the toxic effects of paracetamol on renal tubules (Latif et al., 2021). Oxidative stress-induced damage to the epithelial cells lining the renal tubules impairs the efficiency of water and electrolyte reabsorption, leading to excessive fluid loss and subsequent body weight reduction (Pakravan et al., 2007). This explanation revealed necrosis and dilation of renal tubules accompanied by flattening and degeneration of the tubular epithelial cells, as well as the presence of cellular debris and proteinaceous casts within the tubular lumina. These alterations indicate marked impairment in renal tubular function and a reduced capacity to maintain fluid balance. Furthermore, toxic doses of paracetamol may have caused deterioration in the general physiological condition of the animals, leading to lethargy and reduced physical activity, as observed during the experimental

period. Such changes may reduce food consumption and consequently contribute to body weight loss. Regarding the T2 group treated with luteolin at a dose of 100 mg/kg body weight for one month, the results demonstrated no significant differences in body weight gain rate compared with the control group. These findings are in agreement with those reported by (Liu et al., 2021), who observed no significant differences in body weight gain in animals treated with luteolin at a dose of 100 mg/kg body weight for four weeks, confirming its relative safety at this dose. This may be explained by the fact that luteolin does not induce disturbances in physiological functions such as digestion, absorption, and metabolism (Wan et al., 2025), thereby maintaining normal feeding behavior and physiological stability in the animals. In addition, the antioxidant and anti-inflammatory properties of luteolin appear to exert greater effects under conditions of oxidative stress or cellular injury, whereas its role remains limited under normal physiological conditions (Lin et al., 2015). Finally, regarding the T3 group treated with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight for one month, the results demonstrated a significant increase ($P<0.05$) in body weight gain rate compared with the T1 group treated with paracetamol alone. These findings are consistent with those reported by (Owumi et al., 2020), who observed improvement in body weight gain following luteolin administration. This improvement may be attributed to the protective role of luteolin against paracetamol-induced toxicity (Tai et al., 2015). Luteolin is a flavonoid compound possessing potent antioxidant activity, which is mainly related to its ability to scavenge free radicals, inhibit lipid peroxidation, and enhance intracellular antioxidant defense systems. These actions contribute to the attenuation of oxidative stress and preservation of cellular integrity, thereby maintaining mitochondrial function and improving energy production. Consequently, the overall physiological condition of the animals improves, leading to increased food intake and enhanced body weight gain (Gu et al., 2024). Moreover, this improvement may also be attributed to the protective effects of luteolin on renal tubules through reducing oxidative stress and limiting tubular cellular damage. This explanation showed improvement in the histological architecture of the renal tubules, thereby enhancing the efficiency of water and electrolyte reabsorption and reducing fluid loss, ultimately leading to improved body weight gain (Hong et al., 2017).

Kidney function indicators: The results of the current study revealed a significant increase ($P<0.05$) in the levels of urea, creatinine, and uric acid in the T1 group treated with paracetamol at a dose of 470 mg/kg body weight for one month compared with the control group. These findings are consistent with those reported by (Fadda et al., 2019; Parameshappa et al., 2012). This increase may be attributed to the reduction in glomerular filtration rate (GFR) resulting from damage to the proximal renal tubules caused by oxidative stress induced by the toxic effects of paracetamol. When paracetamol is administered at high doses, it is metabolized into the toxic reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). This metabolite depletes antioxidant enzymes, particularly glutathione, thereby promoting oxidative stress within the cells (Yan et al., 2018). The proximal renal tubules are especially susceptible to this injury because of their high metabolic activity, abundance of mitochondria, and active role in reabsorption, making them more vulnerable to toxic substances (Lorz et al., 2004). Consequently, mitochondrial damage and tubular cell dysfunction occur, leading to apoptosis and necrosis of renal cells. In

addition, the accumulation of proteinaceous materials and cellular debris contributes to the formation of renal casts within the tubules, causing partial obstruction of the tubular lumen and impaired glomerular filtrate flow, which ultimately reduces glomerular filtration efficiency (Basile et al., 2012; Hanif et al., 2025). As a result, the kidney's ability to excrete nitrogenous waste products declines, leading to the accumulation of urea, creatinine, and uric acid and the elevation of their serum levels (Roy et al., 2015; Abdel-Hafez et al., 2017). The significant elevation in kidney function markers may also be attributed to the inflammatory response associated with paracetamol intoxication. The present study demonstrated a significant increase in the inflammatory cytokines TNF-α and IL-6. These cytokines exacerbate renal injury through enhancement of tubular damage, stimulation of oxidative stress, induction of mitochondrial dysfunction, increased cellular membrane permeability, and activation of cell death pathways (Sánchez-González et al., 2011). Consequently, tubular cell injury and impairment of renal functional efficiency occur, resulting in increased accumulation of nitrogenous waste products in the blood. Regarding the T2 group treated with luteolin at a dose of 100 mg/kg body weight for one month, the results showed no significant differences ($P<0.05$) in the levels of urea, creatinine, and uric acid compared with the control group. These findings are consistent with those reported by (Abubakr et al., 2025). This may be explained by the fact that luteolin does not exert harmful effects on renal tissue and does not disturb glomerular filtration rate or renal efficiency in excreting nitrogenous wastes under normal physiological conditions (Albarakati et al., 2020). Histological examination further supported this interpretation by revealing normal renal histoarchitecture without pathological alterations, confirming the relative safety of luteolin at the administered dose. This may also be attributed to the role of luteolin as an antioxidant and regulator of oxidative stress, which becomes more evident under pathological conditions associated with excessive free radical production (Lin et al., 2008). Under normal physiological conditions, where oxidative stress levels remain within normal limits, luteolin does not alter normal physiological values but rather contributes to maintaining cellular balance and functional stability. Concerning the T3 group treated with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight for one month, the results demonstrated a significant decrease ($P<0.05$) in the levels of urea, creatinine, and uric acid compared with the T1 group treated with paracetamol alone, with these values approaching those of the control group. This finding indicates the protective role of luteolin against paracetamol-induced nephrotoxicity. The present findings are consistent with several previous studies (Domitrović et al., 2013; Dar et al., 2021), which demonstrated that luteolin significantly reduced kidney function markers and improved renal functional efficiency. This protective effect may be attributed to the role of luteolin in enhancing antioxidant defenses and reducing paracetamol-induced oxidative stress. Luteolin decreases free radical generation and inhibits lipid peroxidation, thereby reducing renal cellular damage, preserving the integrity of tubular cells, and improving their functional efficiency, which ultimately reduces the accumulation of nitrogenous waste products in the blood (Mahwish et al., 2025). The absence of necrosis, reduction in renal cast formation, and preservation of open tubular lumina in most sections further confirm the protective role of luteolin in attenuating tissue injury and improving renal histological structure.

The improvement in kidney function indicators may also be related to the anti-inflammatory effects of luteolin (Chen et al., 2023). The currect study demonstrated a marked reduction in the levels of inflammatory cytokines TNF-α and IL-6 in this group compared with the T1 group, indicating the ability of luteolin to suppress the inflammatory response associated with nephrotoxicity. This suppression contributes to reducing renal tissue injury, particularly within the renal tubules. Consequently, glomerular filtration rate improves, leading to enhanced excretion of nitrogenous waste products such as urea, creatinine, and uric acid, and thereby causing a marked reduction in their serum concentrations.

Oxidative stress and antioxidant indicators: The current findings showed a significant increase ($P<0.05$) in malondialdehyde (MDA) levels, accompanied by a significant decrease ($P<0.05$) in glutathione (GSH) and catalase (CAT) levels in the serum of animals in the T1 group treated with paracetamol at a dose of 470 mg/kg body weight for one month compared with the control group. These findings are consistent with those reported by (Kandemir et al., 2017; Baponwa et al., 2022). The first study indicated that administration of paracetamol at a dose of 500 mg/kg body weight for six consecutive days caused an elevation in MDA levels and a reduction in antioxidant markers, including GSH and CAT, compared with the control group. This may be attributed to oxidative stress induced by the toxic effects of paracetamol when administered at high doses. Accumulation of its toxic reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), leads to depletion of glutathione (GSH) reserves, resulting in impairment of antioxidant defense capacity and increased generation of free radicals due to mitochondrial dysfunction. Consequently, lipid peroxidation of cellular membranes is stimulated, leading to elevated MDA levels, which represent the final product of this process and serve as a biological indicator of oxidative damage severity (Sohail et al., 2024; Murad et al., 2016). Persistent oxidative stress further exhausts the antioxidant defense system, causing reduced catalase (CAT) activity as a result of its continuous utilization in neutralizing accumulated hydrogen peroxide (Elsafty et al., 2024). This effect may also be attributed to the ability of paracetamol to stimulate inflammatory responses. Oxidative stress and cellular injury activate intracellular inflammatory pathways, leading to increased secretion of inflammatory cytokines, which in turn enhance reactive oxygen species (ROS) production. This process promotes membrane lipid peroxidation and elevation of MDA levels, while the continuous consumption of antioxidants results in depletion of glutathione (GSH) reserves and reduction in catalase (CAT) activity (Mittal et al., 2014; Karthivashan et al., 2016). Regarding the T2 group treated with luteolin at a dose of 100 mg/kg body weight for one month, the obtained results revealed no significant differences in MDA, CAT, and GSH levels compared with the control group. These findings are in agreement with those reported by (Albarakati et al., 2024), who observed no significant differences in MDA, GSH, and CAT levels in animals treated with luteolin at a dose of 50 mg/kg body weight compared with the control group. This finding confirms the relative safety of luteolin and indicates that it does not induce oxidative stress when administered at appropriate doses. This may be explained by the fact that the antioxidant activity of luteolin is mainly activated under conditions of disrupted cellular homeostasis, whereas in the absence of harmful stimuli, these parameters remain within normal physiological ranges. This indicates that

luteolin does not disturb the cellular balance of healthy cells (Nabavi et al., 2015). Finally, concerning the T3 group treated with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight for one month, the results of the present study demonstrated a significant decrease ($P<0.05$) in MDA levels accompanied by a significant increase ($P<0.05$) in both GSH and CAT levels compared with the T1 group treated with paracetamol alone, with these markers approaching the values of the control group. These findings are consistent with several previous studies (Tan et al., 2018; Wang et al., 2024), which reported reduced MDA levels and elevated GSH and CAT levels following luteolin administration. This protective effect may be attributed to the antioxidant properties of luteolin (Fernando et al., 2024), as it scavenges free radicals and reduces lipid peroxidation, thereby decreasing MDA levels (Ma, 2013; Khayyat et al., 2025). In addition, luteolin enhances the antioxidant defense system through activation of the Nrf2/ARE signaling pathway, which is one of the major molecular pathways responsible for protecting cells against oxidative stress. Activation of this pathway stimulates glutathione (GSH) synthesis and regulates detoxification enzymes (Alekhya Sita et al., 2019). Furthermore, the beneficial effects of luteolin may also be related to its anti-inflammatory activity. Reduction in inflammatory cytokine secretion suppresses immune cells responsible for free radical generation, leading to decreased lipid peroxidation and consequently lower MDA levels. As a result, antioxidant consumption decreases, contributing to the preservation of glutathione levels and catalase enzyme activity. Therefore, luteolin plays an important role in restoring the balance between oxidants and antioxidants and reducing cellular injury (Liu et al., 2017).

Inflammatory indicators: The results of the present study demonstrated a significant increase ($P<0.05$) in the levels of the inflammatory markers TNF-α and IL-6 in the T1 group treated with paracetamol at a dose of 470 mg/kg body weight for one month compared with the control group. These findings are consistent with those reported by Taslipinar et al. (2013) and Alqahtani et al. (2023), who indicated that paracetamol administration caused a significant elevation in inflammatory cytokine levels. This could be explained by the fact that toxic doses of paracetamol induce oxidative stress through the formation of the reactive toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). This metabolite depletes glutathione reserves and increases free radical generation. Accumulation of free radicals causes lipid peroxidation and cellular damage, thereby stimulating inflammatory responses and increasing cytokine secretion (Haidara et al., 2019). The toxic effects of paracetamol are not limited to the induction of oxidative stress but also extend to the activation of inflammatory pathways. Accumulation of free radicals generated during paracetamol metabolism activates the NF-κB signaling pathway, which is considered one of the principal regulators of cytokine gene expression (Karimi-Dehkordi et al., 2026). Activation of this pathway consequently enhances the release of inflammatory cytokines, thereby amplifying the inflammatory response. The significant elevation in these inflammatory markers may also be explained by the marked tissue injury observed in the renal tubules, as demonstrated in the present study. Necrosis and degeneration of tubular epithelial cells result in the release of damage-associated molecular patterns (DAMPs), which activate inflammatory pathways within renal tissue, particularly the NF-κB pathway, thereby stimulating the gene

expression of inflammatory cytokines (Rock & Kono, 2008). Regarding the T2 group treated with luteolin at a dose of 100 mg/kg body weight for one month, the results demonstrated no significant differences ($P<0.05$) in the levels of the inflammatory markers TNF-α and IL-6 compared with the control group. These findings are consistent with those reported by (Liang et al., 2025), who observed no significant differences in TNF-α and IL-6 levels in the luteolin-treated group compared with the control group. This confirms the relative safety of luteolin and supports its safety when administered at carefully selected therapeutic doses. This may be explained by the fact that luteolin does not stimulate inflammatory responses under normal physiological conditions, and its activity is mainly restricted to the presence of inflammatory stimuli. Luteolin does not activate inflammatory signaling pathways or enhance cytokine gene expression under normal physiological conditions (Jang et al., 2008; Tian et al., 2022), which explains the maintenance of these markers within normal ranges. This finding may also be attributed to the ability of luteolin to maintain immune homeostasis without disturbing the cellular microenvironment, as luteolin selectively suppresses inflammation without adversely affecting normal physiological cellular functions. Concerning the T3 group treated with paracetamol at a dose of 470 mg/kg body weight concurrently with luteolin at a dose of 100 mg/kg body weight for one month. TNF-α and IL-6 levels were significantly elevated ($P < 0.05$) in the T1 group compared with the control group treated with paracetamol alone. These findings are in agreement with those reported by (Boeing et al., 2020; Taweesap et al., 2025), which confirmed that luteolin exerts anti-inflammatory effects and reduces inflammatory cytokine levels. This protective effect may be attributed to the anti-inflammatory activity of luteolin, as it suppresses primary inflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α), which is considered one of the major mediators initiating the inflammatory cascade and stimulating secondary cytokines such as IL-6. Therefore, inhibition of TNF-α contributes to suppression of inflammatory reactions (Okusa, 2002; Hong et al., 2017). In addition, luteolin reduces infiltration of inflammatory cells into renal tissue, thereby limiting renal damage (Seelinger et al., 2008). This effect may also be explained by the ability of luteolin to inhibit the NF-κB signaling pathway, a well-known inflammatory mediator (Dos Santos Pereira et al., 2020). Free radicals play a major role in activating this pathway through stimulation of its phosphorylation, leading to increased production of inflammatory cytokines (Subramanian et al., 2015; Hassanein et al., 2021). However, luteolin, due to its antioxidant properties, reduces oxidative stress levels, thereby inhibiting NF-κB activation and consequently decreasing inflammatory cytokine production.

Study Limitations: The present study was limited by the absence of molecular analyses and detailed histopathological scoring. Further studies are recommended to investigate the molecular mechanisms underlying the nephroprotective effects of luteolin.

Conclusion: The results of the present study showed that administration of paracetamol at a dose of 470 mg/kg body weight for 30 days caused significant nephrotoxicity in male rats. This renal injury was accompanied by a significant increase in renal function indices including urea, creatinine and uric acid, an increase in the levels of malondialdehyde (MDA) and inflammatory cytokines TNF-α and IL-6, as well as a significant decrease in the antioxidant indices glutathione (GSH) and catalase (CAT). In addition, the decrease in body weight in animals receiving

paracetamol indicated the adverse effects of this drug on the general and metabolic status of the animals. In contrast, concomitant administration of luteolin significantly improved these changes, such that renal function indices and inflammatory and oxidative stress markers decreased and the level of endogenous antioxidants increased. Luteolin was also able to partially compensate for the weight loss induced by paracetamol. These findings suggest that the protective effects of luteolin are likely to be exerted through the inhibition of oxidative stress, enhancement of the antioxidant defense system, and reduction of inflammatory responses. Overall, the results of this study indicate that luteolin has a significant capacity to reduce paracetamol-induced renal injury and can be considered as a natural compound with antioxidant and anti-inflammatory properties in the prevention or reduction of drug-induced nephrotoxicity. However, further studies at the molecular and histological levels are necessary to clarify the precise mechanisms of action and evaluate its potential therapeutic applications.

Author Contributions

S. K. S. conceived and designed the study, performed the experimental work, collected the data, analyzed the results, and prepared the original draft of the manuscript. E. M. M. contributed to the study design, supervised the research work, reviewed and edited the manuscript, and approved the final version for publication. All authors read and approved the final manuscript.

Data Availability Statement

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

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Ethical Considerations

All experimental procedures involving animals were conducted in accordance with the ethical guidelines for the care and use of laboratory animals and were approved by the relevant institutional committee.

Conflict of Interest

The authors declare no conflict of interest.

References

Abdel-Hafez, S. M. N., Rifaai, R. A., & Abd Elzaher, W. Y. (2017). Mechanism of grape seeds extract protection against paracetamol renal cortical damage in male Albino rats. *Bratislavske Lekarske Listy*, *118*(4), 233-242. https://doi.org/10.4149/BLL_2017_046

Abdel-Zaher, A. O., Abdel-Hady, R. H., Mahmoud, M. M., & Farrag, M. M. (2008). The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. *Toxicology*, *243*(3), 261-270. https://doi.org/10.1016/j.tox.2007.10.010

Abubakr, S., Ibrahim, M. M., Taha, M., Hussin, E., Hendawy, M., Abdel-Hay, R. I., Elmetwally, M. A., Abdelkareem, M. A., Farage, A. E., Baokbah, T. A. S., Rabei, M. R., Faheem, A. M., & Badawy, A. M. (2025). Luteolin abates ifosfamide-induced nephrotoxicity by downregulating renal oxidative DNA damage, inflammation, and apoptosis in experimental rats. *International Journal of Morphology*, *43*(2), 422-430. https://doi.org/10.4067/S0717-95022025000200422

Ahmad, S. T., Arjumand, W., Nafees, S., Seth, A., Ali, N., Rashid, S., & Sultana, S. (2012). Hesperidin alleviates acetaminophen induced toxicity in Wistar rats by abrogation of oxidative stress, apoptosis and inflammation. *Toxicology Letters*, *208*(2), 149-161. https://doi.org/10.1016/j.toxlet.2011.10.023

Albarakati, A. J. A., Baty, R. S., Aljoudi, A. M., Habotta, O. A., Elmahallawy, E. K., Kassab, R. B., & Abdel Moneim, A. E. (2020). Luteolin protects against lead acetate-induced nephrotoxicity through antioxidant, anti-inflammatory, anti-apoptotic, and Nrf2/HO-1 signaling pathways. *Molecular Biology Reports*, *47*(4), 2591-2603. https://doi.org/10.1007/s11033-020-05346-1

Albrakati, A. (2024). The potential neuroprotective of luteolin against acetamiprid-induced neurotoxicity in the rat cerebral cortex. *Frontiers in Veterinary Science*, *11*, Article 1361792. https://doi.org/10.3389/fvets.2024.1361792

Alekhya Sita, G. J., Gowthami, M., Srikanth, G., Krishna, M. M., Rama Sireesha, K., Sajjarao, M., Nagarjuna, K., Nagarjuna, M., Chinnaboina, G. K., Mishra, A., & SreeHarsha, N. (2019). Protective role of luteolin against bisphenol A-induced renal toxicity through suppressing oxidative stress, inflammation, and upregulating Nrf2/ARE/HO-1 pathway. *IUBMB Life*, *71*(7), 1041-1047. https://doi.org/10.1002/iub.2066

Alqahtani, Q. H., Fadda, L. M., Alhusaini, A. M., Hasan, I. H., & Ali, H. M. (2023). Involvement of Nrf2, JAK and COX pathways in acetaminophen-induced nephropathy: Role of some antioxidants. *Saudi Pharmaceutical Journal*, *31*(10), Article 101752. https://doi.org/10.1016/j.jsps.2023.101752

Aslipinar, M. Y., Aydin, I., Kaldirim, U., Aydin, F. N., Agilli, M., Eyi, Y. E., Tuncer, S. K., Altayli, E., Ucar, F., Macit, E., Toygar, M., Yigit, N., & Cayci, T. (2013). Hyperbaric oxygen treatment and N-acetylcysteine ameliorate acetaminophen-induced liver injury in a rat model. *Human & Experimental Toxicology*, *32*(10), 1107-1116. https://doi.org/10.1177/0960327113499167

Assi, A. D. M., Beli, A. R., Djetouan, K. J. M., Kanga, J. A., Abe, A. S., & Amonkan, K. A. (2024). Effect of ethanolic extract of *Petroselinum crispum* (Mill.) Fuss (Apiaceae) strand on acetaminophen-induced hepatotoxicity in Wistar rat. *Journal of Pharmacognosy and Phytochemistry*, *13*(1), 384-390. https://doi.org/10.22271/phyto.2024.v13.i1e.14856

Baponwa, O., Amang, A. P., Mezui, C., Koubala, B. B., Siwe, G. T., Vandi, V. L., & Tan, P. V. (2022). Antioxidant mechanism of renal and hepatic failure prevention related to paracetamol overdose by the aqueous extract of *Amblygonocarpus andongensis* stem bark. *BioMed Research International*, *2022*, Article 1846558. https://doi.org/10.1155/2022/1846558

Basile, D. P., Anderson, M. D., & Sutton, T. A. (2012). Pathophysiology of acute kidney injury. *Comprehensive Physiology*, *2*(2), 1303-1353. https://doi.org/10.1002/cphy.c110041

Boeing, T., de Souza, P., Speca, S., Somensi, L. B., Mariano, L. N. B., Cury, B. J., Ferreira Dos Anjos, M., Quintão, N. L. M., Dubuqoy, L., Desreumax, P., da Silva, L. M., & de Andrade, S. F. (2020). Luteolin prevents irinotecan-induced intestinal mucositis in mice through antioxidant and anti-inflammatory properties. *British Journal of Pharmacology*, *177*(10), 2393-2408. https://doi.org/10.1111/bph.14987

Chen, L.-Y., Cheng, H.-L., Liao, C.-K., et al. (2023). Luteolin improves nephropathy in hyperglycemic rats through anti-oxidant, anti-inflammatory, and anti-apoptotic mechanisms. *Journal of Functional Foods*, *105*, Article 105461. https://doi.org/10.1016/j.jff.2023.105461

Dar, A. A., Zargar, M. A., Bhat, A. A., Masood, A., Sofi, H. A., & Dar, N. A. (2021). The protective role of luteolin against methotrexate-induced hepato-renal toxicity in rats. *Human & Experimental Toxicology*, *40*(7), 1195-1204. https://doi.org/10.1177/0960327121991905

Domitrović, R., Cvijanović, O., Pugel, E. P., Zagorac, G. B., Mahmutefendić, H., & Škoda, M. (2013). Luteolin ameliorates cisplatin-induced nephrotoxicity in mice through inhibition of platinum accumulation, inflammation and apoptosis in the kidney. *Toxicology*, *310*, 115-123. https://doi.org/10.1016/j.tox.2013.05.015

Dos-Santos-Pereira, M., Guimarães, F. S., Del-Bel, E., Raisman-Vozari, R., & Michel, P. P. (2020). Cannabidiol prevents LPS-induced microglial inflammation by inhibiting ROS/NF-κB-dependent signaling and glucose consumption. *Glia*, *68*(3), 561-573. https://doi.org/10.1002/glia.23738

Fan, X., Lin, F., Chen, Y., Dou, Y., Li, T., Jin, X., Song, J., & Wang, F. (2024). Luteolin-7-O-β-d-glucuronide ameliorates cerebral ischemic injury: Involvement of RIP3/MLKL signaling pathway. *Molecules*, *29*(7), Article 1665. https://doi.org/10.3390/molecules29071665

Fernando, P. D. S. M., Ko, D. O., Piao, M. J., Kang, K. A., Herath, H. M. U. L., & Hyun, J. W. (2024). Protective effect of luteolin against oxidative stress-mediated cell injury via enhancing antioxidant systems. *Molecular Medicine Reports*, *30*(1), Article 121. https://doi.org/10.3892/mmr.2024.13244

Gamal el-din, A. M., Mostafa, A. M., Al-Shabanah, O. A., Al-Bekairi, A. M., & Nagi, M. N. (2003). Protective effect of 646rabic gum against acetaminophen-induced hepatotoxicity in mice. *Pharmacological Research*, *48*(6), 631-635. https://doi.org/10.1016/s1043-6618(03)00226-3

Gu, J., Zhang, P., Li, H., Wang, Y., Huang, Y., Fan, L., Ma, X., Qian, X., Xi, J., & Chen, Z. (2024). Cerium-luteolin nanocomplexes in managing inflammation-related diseases by antioxidant and immunoregulation. *ACS Nano*, *18*(8), 6229-6242. https://doi.org/10.1021/acsnano.3c09528

Haidara, M. A., Al-Hashem, F., El Karib, A. O., Zaki, M. S., Kamar, S. S., El-Bidawy, M. H., & Al-Ani, B. (2019). Inhibition of paracetamol-induced acute kidney damage in rats using a combination of resveratrol and quercetin. *International Journal of Morphology*, *37*(4), 1422-1428. https://doi.org/10.4067/S0717-95022019000401422

Hanif, M. O., Rout, P., & Ramphul, K. (2025). Acute renal tubular necrosis. In *StatPearls*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK507815/

Hassanein, E. H. M., Ali, F. E. M., Kozman, M. R., & Abd El-Ghafar, O. A. M. (2021). Umbelliferone attenuates gentamicin-induced renal toxicity by suppression of TLR-4/NF-κB-p65/NLRP-3 and JAK1/STAT-3 signaling pathways. *Environmental Science and Pollution Research*, *28*(9), 11558-11571. https://doi.org/10.1007/s11356-020-11416-5

Hong, X., Zhao, X., Wang, G., Zhang, Z., Pei, H., & Liu, Z. (2017). Luteolin treatment protects against renal ischemia-reperfusion injury in rats. *Mediators of Inflammation*, *2017*, Article 9783893. https://doi.org/10.1155/2017/9783893

Hu, J., Nieminen, A.-L., Zhong, Z., & Lemasters, J. J. (2024). Role of mitochondrial iron uptake in acetaminophen hepatotoxicity. *Livers*, *4*(3), 333-351. https://doi.org/10.3390/livers4030024

Ibrahim, M. M., Osman, A., Helal, A. I., Faheem, A. M., El-Kattan, M. A., Ibrahim, I., Elmetwally, A. A., Abubakr, S., Badawy, A. M., & Hussin, E. (2024). Celastrol mitigates acetaminophen-induced nephrotoxicity in rats via targeting renal oxidative stress, inflammation, apoptosis with enhancement in aquaporin 1 level. *Reports of Biochemistry & Molecular Biology*, *13*(2), 204-217. https://doi.org/10.61186/rbmb.13.2.204

Jaeschke, H., Ramachandran, A., & McGill, M. R. (2024). Mechanisms of acetaminophen-induced liver injury and its relevance to mitochondrial dysfunction. *Journal of Clinical and Translational Hepatology*, *12*(12), 1057-1066. https://doi.org/10.14218/JCTH.2024.00324

Jang, S., Kelley, K. W., & Johnson, R. W. (2008). Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. *Proceedings of the National Academy of Sciences*, *105*(21), 7534-7539. https://doi.org/10.1073/pnas.0802865105

Kandemir, F. M., Kucukler, S., Eldutar, E., Caglayan, C., & Gülçin, İ. (2017). Chrysin protects rat kidney from paracetamol-induced oxidative stress, inflammation, apoptosis, and autophagy: A multi-biomarker approach. *Scientia Pharmaceutica*, *85*(1), Article 4. https://doi.org/10.3390/scipharm85010004

Kanno, S., Tomizawa, A., & Yomogida, S. (2016). Detecting mRNA predictors of acetaminophen-induced hepatotoxicity in mouse blood using quantitative real-time PCR. *Biological & Pharmaceutical Bulletin*, *39*(3), 440-445. https://doi.org/10.1248/bpb.b15-00734

Karimi-Dehkordi, M., Saghaei, F., Saberian, M., Nejad, H. S., Sohrabi, F., & Zabihi, N. (2026). Feselol ameliorates acetaminophen-induced hepatotoxicity through multi-pathway modulation of oxidative stress, inflammation, and apoptosis in mice. *Journal of Biochemical and Molecular Toxicology*, *40*(2), Article e70710. https://doi.org/10.1002/jbt.70710

Karthivashan, G., Kura, A. U., Arulselvan, P., Md Isa, N., & Fakurazi, S. (2016). The modulatory effect of *Moringa oleifera* leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. *PeerJ*, *4*, Article e2127. https://doi.org/10.7717/peerj.2127

Khayyat, A. I. A., Alabdali, A. N., Alonazi, M., Alzahrani, A. A., Al-Shehri, E., & Ben Bacha, A. (2025). Luteolin mitigates oxidative stress and multi-organ impairment in a propionic acid-induced rodent model of autism. *Frontiers in Nutrition*, *12*, Article 1583119. https://doi.org/10.3389/fnut.2025.1583119

Kour, H., Singh, A., Jaiswal, P., Anusha, & Sharma, R. (2023). Screening models of nephrotoxicity and their molecular mechanism. *World Journal of Biology Pharmacy and Health Sciences*, *13*(3), 234-251. https://doi.org/10.30574/wjbphs.2023.13.3.0142

Latif, A. A. E., Assar, D. H., Elkaw, E. M., Hamza, H. A., Alkhalifah, D. H. M., Hozzein, W. N., & Hamouda, R. A. (2021). Protective role of *Chlorella vulgaris* with thiamine against paracetamol-induced toxic effects on hematological, biochemical, oxidative stress parameters and histopathological changes in Wistar rats. *Scientific Reports*, *11*, Article 3911. https://doi.org/10.1038/s41598-021-83316-8

Li, X., Zhang, Y., Wang, L., Chen, J., & Zhao, H. (2025). Mitochondrial dysfunction in acetaminophen-induced toxicity: Mechanisms and therapeutic targets. *Molecular Medicine Reports*, *31*(4), Article 106. https://doi.org/10.3892/mmr.2025.13471

Liang, D. Y., Cong, S. H., Li, L. H., Yi, Q. Q., Tang, L. P., & Cao, L. O. (2025). Luteolin delays the progression of IgA nephropathy by attenuating inflammation, oxidative stress and reducing extracellular matrix accumulation through activating the Nrf-2/HO-1 pathway. *Frontiers in Pharmacology*, *16*, Article 1530655. https://doi.org/10.3389/fphar.2025.1530655

Liao, J., Lu, Q., Li, Z., Li, J., Zhao, Q., & Li, J. (2023). Acetaminophen-induced liver injury: Molecular mechanisms and treatments from natural products. *Frontiers in Pharmacology*, *14*, Article 1122632. https://doi.org/10.3389/fphar.2023.1122632

Lin, P., Tian, X. H., Yi, Y. S., Jiang, W. S., Zhou, Y. J., & Cheng, W. J. (2015). Luteolin-induced protection of H_2O_2-induced apoptosis in PC12 cells and the associated pathway. *Molecular Medicine Reports*, *12*(5), 7699-7704. https://doi.org/10.3892/mmr.2015.4400

Lin, Y., Shi, R., Wang, X., & Shen, H. M. (2008). Luteolin, a flavonoid with potential for cancer prevention and therapy. *Current Cancer Drug Targets*, *8*(7), 634-646. https://doi.org/10.2174/156800908786241050

Liu, Y. S., Yang, Q., Li, S., Luo, L., Liu, H. Y., Li, X. Y., & Gao, Z. N. (2021). Luteolin attenuates angiotensin II-induced renal damage in apolipoprotein E-deficient mice. *Molecular Medicine Reports*, *23*(2), Article 157. https://doi.org/10.3892/mmr.2020.11796

Liu, Y., Shi, B., Li, Y., & Zhang, H. (2017). Protective effect of luteolin against renal ischemia/reperfusion injury via modulation of pro-inflammatory cytokines, oxidative stress and apoptosis for possible benefit in kidney transplant. *Medical Science Monitor*, *23*, 5720-5727. https://doi.org/10.12659/msm.903253

Lorz, C., Justo, P., Sanz, A., Subirá, D., Egido, J., & Ortiz, A. (2004). Paracetamol-induced renal tubular injury: A role for ER stress. *Journal of the American Society of Nephrology*, *15*(2), 380-389. https://doi.org/10.1097/01.asn.0000111289.91206.b0

Ma, Q. (2013). Role of Nrf2 in oxidative stress and toxicity. *Annual Review of Pharmacology and Toxicology*, *53*, 401-426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Mahwish, Imran, M., Naeem, H., Hussain, M., Alsagaby, S. A., Al Abdulmonem, W., Mujtaba, A., Abdelgawad, M. A., Ghoneim, M. M., El-Ghorab, A. H., Selim, S., Al Jaouni, S. K., Mostafa, E. M., & Yehuala, T. F. (2025). Antioxidative and anticancer potential of luteolin:

A comprehensive approach against wide range of human malignancies. *Food Science & Nutrition*, *13*, Article e4682. https://doi.org/10.1002/fsn3.4682

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxidants & Redox Signaling*, *20*(7), 1126-1167. https://doi.org/10.1089/ars.2012.5149

Mody, H., Ramakrishnan, V., Chaar, M., Lezeau, J., Rump, A., Taha, K., Lesko, L., & Ait-Oudhia, S. (2020). A review on drug-induced nephrotoxicity: Pathophysiological mechanisms, drug classes, clinical management, and recent advances in mathematical modeling and simulation approaches. *Clinical Pharmacology in Drug Development*, *9*(8), 896-909. https://doi.org/10.1002/cpdd.879

Murad, H. A., Habib, H., Kamel, Y., Alsayed, S., Shakweer, M., & Elshal, M. (2016). Thearubigins protect against acetaminophen-induced hepatic and renal injury in mice: Biochemical, histopathological, immunohistochemical, and flow cytometry study. *Drug and Chemical Toxicology*, *39*(2), 190-198. https://doi.org/10.3109/01480545.2015.1070170

Nabavi, S. F., Braidy, N., Gortzi, O., Sobarzo-Sanchez, E., Daglia, M., Skalicka-Woźniak, K., & Nabavi, S. M. (2015). Luteolin as an anti-inflammatory and neuroprotective agent: A brief review. *Brain Research Bulletin*, *119*(Pt A), 1-11. https://doi.org/10.1016/j.brainresbull.2015.09.002

Okusa, M. D. (2002). The inflammatory cascade in acute ischemic renal failure. *Nephron*, *90*(2), 133-138. https://doi.org/10.1159/000049032

Owumi, S. E., Ijadele, A. O., Arunsi, U. O., & Odunola, O. A. (2020). Luteolin abates reproductive toxicity mediated by the oxido-inflammatory response in doxorubicin-treated rats. *Toxicology Research and Application*, *4*, 1-15. https://doi.org/10.1177/2397847320972040

Pakravan, N., Bateman, D. N., & Goddard, J. (2007). Effect of acute paracetamol overdose on changes in serum and urine electrolytes. *British Journal of Clinical Pharmacology*, *64*(6), 824-832. https://doi.org/10.1111/j.1365-2125.2007.02952.x

Parameshappa, B., Ali Basha, M. S., Sen, S., Chakraborty, R., Kumar, G. V., Sagar, G. V., Sowmya, L., Raju, K. K., Sesh Kumar, P. K., & Lakshmi, A. V. (2012). Acetaminophen-induced nephrotoxicity in rats: Protective role of *Cardiospermum halicacabum*. *Pharmaceutical Biology*, *50*(2), 247-253. https://doi.org/10.3109/13880209.2011.596843

Perazella, M. A. (2010). Toxic nephropathies: Core curriculum 2010. *American Journal of Kidney Diseases*, *55*(2), 399-409. https://doi.org/10.1053/j.ajkd.2009.10.046

Rock, K. L., & Kono, H. (2008). The inflammatory response to cell death. *Annual Review of Pathology*, *3*, 99-126. https://doi.org/10.1146/annurev.pathmechdis.3.121806.151456

Roy, S., Pradhan, S., Das, K., Mandal, A., Mandal, S., Patra, A., Samanta, A., Sinha, B., & Nandi, D. K. (2015). Acetaminophen induced kidney failure in rats: A dose response study. *Journal of Biological Sciences*, *15*(4), 187-193. https://doi.org/10.3923/jbs.2015.187.193

Sánchez-González, P. D., López-Hernández, F. J., López-Novoa, J. M., & Morales, A. I. (2011). An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. *Critical Reviews in Toxicology*, *41*(10), 803-821. https://doi.org/10.3109/10408444.2011.602662

Seelinger, G., Merfort, I., & Schempp, C. M. (2008). Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin. *Planta Medica*, *74*(14), 1667-1677. https://doi.org/10.1055/s-0028-1088314

Sohail, N., Azam, M., Farhat, H., Hira, K., Urooj, F., Qureshi, S. A., Ara, J., Ali, M. S., & Ehteshamul-Haque, S. (2024). *Ulva fasciata*, a green alga, attenuates the kidney and liver dysfunctions in rats induced by acetaminophen. *Drug and Chemical Toxicology*, *47*(1), 1-14. https://doi.org/10.1080/01480545.2022.2150206

Subramanian, P., Anandan, R., Jayapalan, J. J., & Hashim, O. H. (2015). Hesperidin protects gentamicin-induced nephrotoxicity via Nrf2/HO-1 signaling and inhibits inflammation mediated by NF-κB in rats. *Journal of Functional Foods*, *13*, 89-99. https://doi.org/10.1016/j.jff.2014.12.035

Tai, M., Zhang, J., Song, S., Miao, R., Liu, S., Pang, Q., Wu, Q., & Liu, C. (2015). Protective effects of luteolin against acetaminophen-induced acute liver failure in mouse. *International Immunopharmacology*, *27*(1), 164-170. https://doi.org/10.1016/j.intimp.2015.05.009

Tan, X., Liu, B., Lu, J., Li, S., Baiyun, R., Lv, Y., Lu, Q., & Zhang, Z. (2018). Dietary luteolin protects against $HgCl_2$-induced renal injury via activation of Nrf2-mediated signaling in rat. *Journal of Inorganic Biochemistry*, *179*, 24-31. https://doi.org/10.1016/j.jinorgbio.2017.11.010

Taweesap, P., Potue, P., Khamseekaew, J., Iampanichakul, M., Jan-O, B., Pakdeechote, P., & Maneesai, P. (2025). Luteolin relieves metabolic dysfunction-associated fatty liver disease caused by a high-fat diet in rats through modulating the AdipoR1/AMPK/PPARγ signaling pathway. *International Journal of Molecular Sciences*, *26*(8), Article 3804. https://doi.org/10.3390/ijms26083804

Tian, X., Yuan, J., Mei, L., Li, P., & Pan, R. (2022). Luteolin suppresses TNF-α-induced inflammatory injury and senescence of nucleus pulposus cells via the Sirt6/NF-κB pathway. *Experimental and Therapeutic Medicine*, *24*(1), Article 469. https://doi.org/10.3892/etm.2022.11396

Vongthip, W., Nilkhet, S., Boonruang, K., Sukprasansap, M., Tencomnao, T., & Baek, S. J. (2024). Neuroprotective mechanisms of luteolin in glutamate-induced oxidative stress and autophagy-mediated neuronal cell death. *Scientific Reports*, *14*(1), Article 7707. https://doi.org/10.1038/s41598-024-57824-2

Wan, C., Liang, Q., Ma, Y., Wang, Y., Sun, L., Lai, J., Wu, J., & Chen, Z. (2025). Luteolin: A natural product with multiple mechanisms for atherosclerosis. *Frontiers in Pharmacology*, *16*, Article 1503832. https://doi.org/10.3389/fphar.2025.1503832

Yan, M., Huo, Y., Yin, S., & Hu, H. (2018). Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. *Redox Biology*, *17*, 274-283. https://doi.org/10.1016/j.redox.2018.04.019

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تعیین سطوح برخی شاخص‌های ایمنی‌شناختی و فیزیولوژیک در موش‌های صحرایی مبتلا به نفروتوکسیسیته القاشده با استامینوفن و تیمار شده با لوتئولین

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چکیده

هدف: این مطالعه با هدف بررسی نقش محافظتی لوتئولین در کاهش نفروتوکسیسیته (سمیت کلیوی) ناشی از استامینوفن از طریق ارزیابی برخی شاخص‌های ایمنی‌شناختی و بیوشیمیایی در موش‌های صحرایی آلبینو انجام شد.

مواد و روش‌ها: در این پژوهش از ۳۲ سر موش صحرایی نر بالغ آزمایشگاهی (سه ماهه با وزن ۱۴۵ تا ۱۶۵ گرم) استفاده شد. آزمایش در حیوان‌خانه گروه زیست‌شناسی، دانشکده علوم، دانشگاه القادسیه انجام گرفت. حیوانات به‌طور تصادفی به چهار گروه، هر گروه شامل ۸ موش، تقسیم شدند. گروه شاهد در طول دوره آزمایش (۳۰ روز) فقط آب معمولی دریافت کرد. در گروه تیمار اول (T1)، حیوانات استامینوفن را با دوز ۴۷۰ میلی‌گرم بر کیلوگرم وزن بدن دریافت کردند. در گروه تیمار دوم (T2)، لوتئولین با دوز ۱۰۰ میلی‌گرم بر کیلوگرم وزن بدن تجویز شد. در گروه تیمار سوم (T3)، حیوانات به‌طور همزمان استامینوفن با دوز ۴۷۰ میلی‌گرم بر کیلوگرم و لوتئولین با دوز ۱۰۰ میلی‌گرم بر کیلوگرم وزن بدن دریافت کردند. استامینوفن و لوتئولین روزانه یک بار به مدت ۳۰ روز متوالی تجویز شدند.

نتایج: نتایج مطالعه نشان داد که در گروه T1 افزایش معنی‌داری ($P<0.05$) در شاخص‌های عملکرد کلیه شامل اوره، کراتینین و اسیداوریک، همچنین در سطح مالون‌دی‌آلدئید (MDA) و شاخص‌های التهابی شامل TNF-α و IL-6 در مقایسه با گروه شاهد مشاهده شد. این تغییرات با کاهش معنی‌دار ($P<0.05$) در افزایش وزن بدن و شاخص‌های آنتی‌اکسیدانی شامل گلوتاتیون احیاشده (GSH) و آنزیم کاتالاز (CAT) همراه بود. در مقابل، در گروه T2 تفاوت معنی‌داری نسبت به گروه شاهد مشاهده نشد. در گروه T3 بهبود قابل توجهی مشاهده شد که شامل کاهش معنی‌دار ($P<0.05$) شاخص‌های عملکرد کلیه، سطح MDA و نشانگرهای

التهابی (TNF-α و IL-6) بود. این بهبودها همراه با افزایش معنی‌دار ($P<0.05$) در افزایش وزن بدن و شاخص‌های آنتی‌اکسیدانی (GSH و CAT) نسبت به گروه T1 بود.

نتیجه‌گیری: نتایج این مطالعه نشان داد که تجویز استامینوفن موجب بروز نفروتوکسیسیته قابل توجهی می‌شود که با اختلال در عملکرد کلیه، افزایش استرس اکسیداتیو و افزایش شاخص‌های التهابی همراه است. درمان با لوتئولین توانست این تغییرات را به‌طور معنی‌داری بهبود بخشد. این اثرات احتمالاً ناشی از خواص آنتی‌اکسیدانی و ضدالتهابی لوتئولین بوده که منجر به بهبود شاخص‌های عملکرد کلیه و بازسازی سیستم دفاع آنتی‌اکسیدانی شده است. بنابراین می‌توان نتیجه گرفت که لوتئولین می‌تواند به‌عنوان یک عامل محافظتی امیدوارکننده در برابر آسیب کلیوی ناشی از استامینوفن مطرح باشد.

کلمات کلیدی: آنتی‌اکسیدان‌ها، استامینوفن، استرس اکسیداتیو، ترکیب لوتئولین، نفروتوکسیسیته

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