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RESEARCH ARTICLE

Oral administration of *Fumaria officinalis* alleviates Nephrotoxicity and improves behavior in Japanese quails

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Abstract Aromatic hydrocarbons (AHCs) are crucial in petroleum-based transportation and consumer product manufacturing, but their accumulation in the body poses serious threats. Toluene, an important aromatic hydrocarbon, can cause oxidative stress and structural damage in the liver, kidney, and brain due to its lipophilic nature. Fumaria officinalis, an antioxidant with flavonoids and alkaloids, has been shown to protect against renal diseases. The study aims to demonstrate the beneficial effects of F. officinalis on general performance and in reducing nephrotoxicity caused by toluene in Japanese quail. After a period of acclimation, sixteen Japanese quails, weighing between 100 and 140g, were used in the experiment. Four equal groups of birds were created: one group was given tap water and standard food; another group was exposed to toluene (0.2 mL/kg) in distilled water; a combination treatment of toluene (0.2 mL/kg) and F. officinalis (150 mg/kg B.W.) was given to the third group; and F. officinalis (150 mg/kg B.W.) was given to the fourth group. After a month, the birds were slaughtered, and blood samples were collected for analysis. The toluene-exposed group showed a significant increase in uric acid, creatinine, and biliverdin levels, a decline in albumin levels, and a decrease in body weight gain. The enzyme activity of superoxide dismutase and catalase was significantly lower in the toluene group. Furthermore, F. officinalis improved toluene-induced reductions in alertness, feeding activity, crowing, mating activity, and overall behavioral performance. This suggests that F. officinalis protects quails' kidneys against the toxicity of toluene.

Keywords: Toluene; Nephrotoxicity; Oxidative Stress; Antioxidant Enzymes; Renal Protection

Introduction

Environmental toxins are a major concern in modern toxicology because many industrial, agricultural, and transportation-related chemicals persist in air, soil, water, and biological systems [9]. Among these pollutants, aromatic hydrocarbons are considered important environmental contaminants due to their widespread use and potential to affect multiple organs after exposure [9; 5]. Monocyclic aromatic

hydrocarbons (MAHs), including toluene, are commonly released from paints, thinners, adhesives, fuels, solvents, and industrial products [9]. Toluene, also known as methylbenzene, is highly lipophilic, which allows it to pass through biological membranes and accumulate in critical organs such as the kidneys, liver, and brain [9]. Long-term exposure to toluene, even at low concentrations, has been associated with nephrotoxicity, hepatotoxicity, neurological disturbances, reproductive abnormalities, and systemic toxicity [5; 13].

The kidney is one of the main target organs affected by toluene because it plays a central role in filtration, fluid balance, waste removal, and detoxification [Price, 2002; 8]. The nephrotoxic effect of toluene is mainly linked with its biotransformation through the cytochrome P-450 enzyme system, which can increase the production of reactive oxygen species (ROS) [3; 23]. Excessive ROS production weakens cellular antioxidant defenses and causes oxidative stress in renal tissues [3; 23]. Important antioxidant systems, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), become overloaded when ROS such as superoxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) are produced in excess [32; 1]. This imbalance can lead to lipid peroxidation, protein oxidation, DNA damage, and structural injury in kidney cells [32; 1]. Renal tissues are especially vulnerable to oxidative damage because they have high mitochondrial activity and high metabolic demand [Price, 2002; 8]. Histological studies have also reported tubular lesions, glomerular abnormalities, and altered renal biomarkers after toluene exposure in animal and human models [24; 25].

Toluene exposure is not limited to kidney damage because it can also affect the nervous system and behavior due to its ability to reach brain tissues through biological membranes [9]. Neurological problems linked with toluene exposure may appear as altered activity, reduced coordination, stress-related responses, feeding changes, and abnormal behavioral performance in exposed animals [5; 13]. These behavioral changes are important because they may indicate early systemic toxicity before severe tissue damage becomes visible [5; 13]. Therefore, both renal biomarkers and behavioral observations are useful for understanding the broader toxicological effects of toluene exposure [24; 25; 5].

Japanese quail are widely used in avian research because they are small, easy to handle, fast-growing, sensitive to environmental stressors, and suitable for controlled laboratory experiments. Quail also has economic and biological importance as poultry birds, making them useful for studying the effects of environmental contaminants on avian health and productivity. Their sensitivity to toxic substances makes them a suitable model for evaluating biochemical, histopathological, physiological, and behavioral changes caused by environmental pollutants. For this reason, Japanese quail were selected in the present study to evaluate the renal and behavioral effects of toluene exposure under controlled experimental conditions.

Due to the harmful effects of chemically induced oxidative stress, interest in plant-based protective compounds with antioxidant and nephroprotective properties has increased in recent years [26; 15]. Fumaria officinalis, commonly known as fumitory, is an herbaceous plant from the family Fumariaceae and has been used in Asian and European traditional medicine for kidney, liver, and skin-related disorders [26; 15]. This plant contains several bioactive compounds, including alkaloids and flavonoids, which are known for their free radical scavenging ability and protective effects against oxidative organ damage [27; 21]. Flavonoids are polyphenolic compounds with antioxidant, anti-inflammatory, and cytoprotective properties that may help protect tissues from chemically induced oxidative injury [12; 22]. These compounds can stabilize free radicals and support natural antioxidant enzymes such as SOD and CAT, which help reduce oxidative stress in damaged tissues [12; 22]. Previous studies have shown that flavonoids from medicinal plants may protect renal function by reducing creatinine, blood urea nitrogen, and lipid peroxidation levels [17; 18].

The nephroprotective potential of F. officinalis has also been linked with its ability to regulate inflammatory mediators, reduce oxidative damage, and preserve membrane integrity in renal tissues exposed to chemical stressors [4; 29]. Pharmacological evidence suggests that the methanolic extract of F. officinalis has strong antioxidant potential and can reduce oxidative damage caused by ferric and cupric acids [4]. Animal-based findings have also reported that F. officinalis supplementation can improve kidney histology and biochemical profiles under nephrotoxic conditions [29]. These findings suggest that F. officinalis may be a promising natural candidate for reducing oxidative and toxic injury in renal tissues [4; 29]. Although the toxic effects of toluene and the antioxidant potential of F. officinalis have been studied separately, limited information is available on the protective effect of

dietary F. officinalis supplementation against toluene-induced nephrotoxicity and behavioral changes in Japanese quail [3; 23; 4; 29]. The combined evaluation of renal biomarkers, antioxidant enzyme activities, kidney histopathology, behavioral responses, and body weight changes may provide a clearer understanding of how F. officinalis affects toluene-induced toxicity in avian species [24; 25; 29]. This knowledge gap is important because environmental exposure to toluene continues to increase, while safe and effective natural protective strategies remain limited [9; 5; 13].

Based on previous evidence, this study hypothesized that dietary supplementation with F. officinalis extract could reduce toluene-induced nephrotoxicity, improve antioxidant defense, protect renal tissue structure, reduce behavioral disturbances, and support body weight stability in Japanese quail [32; 1; 4; 29]. The main aim of this study was to evaluate the possible nephroprotective effect of F. officinalis extract against toluene-induced nephrotoxicity and behavioral alterations in Japanese quail by assessing biochemical indicators, antioxidant enzymes, renal histopathology, behavioral changes, and body weight fluctuations [24; 25; 29]. The study specifically addressed whether toluene exposure alters creatinine, uric acid, biliverdin, SOD, CAT, kidney histology, behavior, and body weight in Japanese quail [3; 23; 24; 25]. It also examined whether F. officinalis supplementation can reduce these toxic effects and serve as a natural nephroprotective agent against environmental contaminant-induced renal injury [4; 29].

Materials and Methods

Experimental Animals and Ethical Statement

Sixteen healthy quails, aged 4–6 weeks and weighing 110–140 g, were obtained from a nearby hatchery. The birds were acclimatized for seven days under controlled laboratory conditions before the start of the experiment. During acclimatization, the quails were maintained at 25 ± 2°C with 50–60% relative humidity and a 12 h light/12 h dark photoperiod. All birds received commercial feed chow and fresh drinking water ad libitum. The Departmental Animal Ethics Committee, University of Agriculture, Faisalabad, approved the experimental protocol. All procedures were performed according to institutional guidelines for the care and use of experimental animals.

Experimental Design

After acclimatization, the quails were divided into four equal experimental groups, with four birds in each group (n = 4). The experimental period lasted for 30 days. The control group received normal feed and tap water under a clean environment at room temperature [33]. The toluene-treated group received toluene at a dose of 0.2 mL/kg body weight through oral gavage. The co-treated group received both toluene at 0.2 mL/kg body weight and Fumaria officinalis extract at 150 mg/kg body weight through oral gavage. The F. officinalis control group received only F. officinalis extract at 150 mg/kg body weight through oral gavage. All treatments were administered once daily for 30 consecutive days using a sterile feeding needle.

Table 1. Experimental groups and treatments

Group	Treatment	Dose	Route	Duration
Group I	Normal control	Feed and tap water only	Oral intake	30 days
Group II	Toluene-treated group	Toluene, 0.2 mL/kg body weight	Oral gavage	30 days
Group III	Co-treated group	Toluene, 0.2 mL/kg body weight + F. officinalis, 150 mg/kg body weight	Oral gavage	30 days
Group IV	F. officinalis control group	F. officinalis, 150 mg/kg body weight	Oral gavage	30 days

Preparation of Fumaria officinalis Extract

Dried aerial parts of Fumaria officinalis were purchased from a licensed herbal source and authenticated by a plant taxonomist. The plant material was shade-dried and ground into fine powder. The powdered material was subjected to cold maceration in 70% ethanol for 72 h. After maceration, the extract was filtered and concentrated using a rotary evaporator at 40°C. The concentrated extract was stored in amber-colored bottles at 4°C until further use. The required dose of F. officinalis extract was freshly prepared before administration according to the body weight of each bird.

Toluene Administration

Analytical-grade toluene was purchased from Sigma-Aldrich. Toluene was administered orally at a dose of 0.2 mL/kg body weight once daily for 30 days. The dose was calculated according to the body weight of each bird before administration. Oral gavage was

performed carefully using a sterile feeding needle to avoid irritation, regurgitation, or aspiration.

Behavioral Observation

Behavioral changes were observed throughout the experimental period. The birds were monitored for general activity, posture, alertness, feeding behavior, water intake, feather condition, movement pattern, stress response, and abnormal behavioral signs [38]. Observations were recorded regularly during the study period to compare behavioral responses among the control, toluene-treated, co-treated, and F. officinalis control groups.

Body Weight Measurement

Body weight was recorded before the start of treatment and at the end of the experimental period [34]. Weight changes were calculated to assess the effect of toluene exposure and F. officinalis supplementation on growth performance and general physiological condition.

Sample Collection

After 30 days of treatment, all birds were humanely slaughtered according to the approved ethical protocol [36]. Blood samples were collected from each bird and processed for biochemical analysis. Serum was separated and stored at −20°C until further analysis. Kidneys were carefully removed, washed with cold normal saline, and processed for biochemical and histopathological examination. Kidney samples intended for histology were fixed in 10% buffered formalin.

Biochemical Assays

Serum levels of creatinine, uric acid, biliverdin, superoxide dismutase (SOD), and catalase (CAT) were measured using standard enzymatic colorimetric kits according to the manufacturer's instructions. The assay kits were obtained from Merck, Germany. Absorbance was measured using a UV-visible spectrophotometer [37]. These biochemical markers were selected to evaluate renal function, oxidative stress status, and the possible protective effect of F. officinalis against toluene-induced nephrotoxicity.

Histopathological Analysis

Kidney tissues fixed in 10% buffered formalin were processed using the standard paraffin embedding technique. Tissue sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E). The stained sections were examined under an Olympus CX21 light microscope [35]. Histopathological evaluation focused on glomerular structure, tubular morphology, interstitial changes, congestion, cellular degeneration, and other renal tissue abnormalities.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical analysis was conducted using SPSS version 25.0. Differences were considered statistically significant at $p < 0.05$ [37].

Results

Body and Kidney Weight Alterations

There were notable differences ($p < 0.05$) in body weight between the groups. Quails exposed to toluene exhibited a significant decrease in body weight (31.25 ± 9.47 g) in contrast to the control group (46.5 ± 1.85 g). Weight was partially restored (42.25 ± 8.15 g) by co-treatment with Fumaria officinalis, indicating a statistical improvement over the toluene group but not a significant difference from the control. Only F. officinalis-treated quails showed a non-significant increase in weight (33 ± 8.36 g).

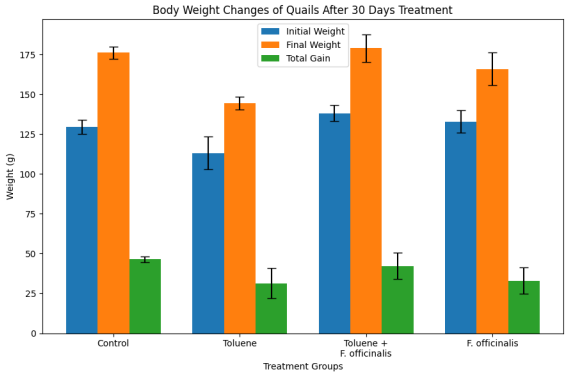

Fig. 1: The graph representing the variations in body weight under different treatments

Likewise, toluene-treated birds had considerably lower kidney weights (0.49 ± 0.07 g) than the control group (0.86 ± 0.03 g), whereas the co-treated group (0.82 ± 0.05 g) had a noticeable recovery. A marginally higher kidney weight (0.92 ± 0.12 g) was obtained by the Positive group, but this difference was not statistically significant from the control.

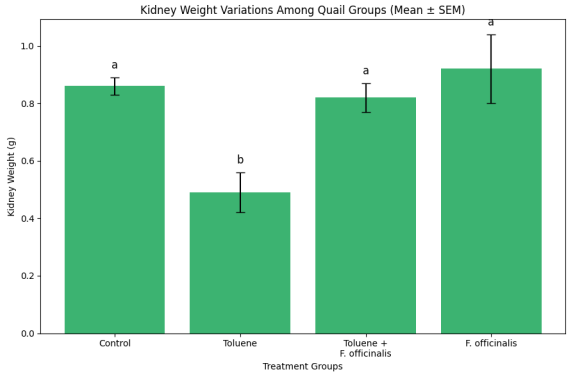

Fig. 2: The graph representing the variations in organ (kidney) weight under different treatments

Renal Function Biomarkers

Serum creatinine (0.75 ± 0.25 mg/dL) and uric acid (7.02 ± 1.53 mg/dL) levels were significantly higher ($p < 0.05$) after toluene exposure than in controls (0.3 ± 0.16 and 3.47 ± 2.00 mg/dL, respectively). Although not to baseline levels, co-treatment with F. officinalis significantly decreased both measures (creatinine: 0.65 ± 0.25; uric acid: 6.27 ± 1.91). The values of the Positive group remained similar to the control group. Likewise, the toluene group had considerably higher biliverdin levels (24.48 ± 1.47 pmol/L) than the co-treatment group (13.69 ± 1.34 pmol/L), which was comparable to the control group (14.23 ± 0.91) and the positive (F. officinalis) group (13.08 ± 1.53).

Table 2: Kidney biochemical parameters and antioxidant enzyme activities in control and treated quail groups after 30 days of treatment.

Parameter	Control	Toluene treated	Toluene + F. officinalis	F. officinalis treated	F value	P value
Albumin (mg/dL)	2.00 ± 0.34^{b}	1.55 ± 0.25^{a}	1.85 ± 0.19^{b}	1.96 ± 0.25^{b}	7.72	P < 0.05
Uric acid (mg/dL)	3.47 ± 2.00^{b}	7.02 ± 1.53^{a}	6.27 ± 1.91^{a}	3.50 ± 2.58^{b}	13.12	P < 0.05
Creatinine (mg/dL)	0.30 ± 0.16^{b}	0.75 ± 0.25^{a}	0.65 ± 0.25^{a}	0.25 ± 0.25^{b}	6.78	P < 0.05
Biliverdin (pmol/L)	14.23 ± 0.91^{b}	24.48 ± 1.47^{a}	13.69 ± 1.34^{b}	13.08 ± 1.53^{b}	16.48	P < 0.05
CAT (U/mg protein)	1.87 ± 0.27^{a}	1.22 ± 0.25^{b}	1.49 ± 0.40^{ab}	1.85 ± 0.21^{a}	41.27	P < 0.05
SOD (U/mg protein)	25.25 ± 3.00^{a}	15.00 ± 7.11^{f}	18.75 ± 4.12^{bf}	24.25 ± 7.54^{ab}	45.91	P < 0.05

Oxidative Stress Markers

Exposure to toluene considerably reduced quails' antioxidant defenses. In contrast to 1.87 ± 0.27 in the control group, CAT activity decreased to 1.22 ± 0.25 U/mg protein in the toluene group. With the F. officinalis group maintaining activity (1.85 ± 0.21) similar to baseline, co-treatment increased catalase levels (1.49 ± 0.40). Under toluene stress, SOD levels similarly decreased (15 ± 7.11 U/mg), which was much lower than the control (25.25 ± 3.0). SOD activity was partially restored by co-treatment (18.75 ± 4.12), but birds that were solely fed F. officinalis showed values that were close to the control (24.25 ± 7.54). Together, these results demonstrate F. officinalis's antioxidative effectiveness.

Table 3: Effect of Toluene and Fumaria officinalis on Kidney Biochemical Parameters and Antioxidant Enzymes in Quail (Mean ± SD, n = 4)

Parameter	Control	Toluene Treated	Toluene + F. officinalis	F. officinalis Treated
Albumin (mg/dL)	2.00 ± 0.34^{b}	1.55 ± 0.25^{a}	1.85 ± 0.19^{b}	1.96 ± 0.25^{b}
Uric Acid (mg/dL)	3.47 ± 2.00^{b}	7.02 ± 1.53^{a}	6.27 ± 1.91^{a}	3.50 ± 2.58^{b}
Creatinine (mg/dL)	0.30 ± 0.16^{b}	0.75 ± 0.25^{a}	0.65 ± 0.25^{a}	0.25 ± 0.25^{b}
Biliverdin (pmol/L)	14.23 ± 0.91^{b}	24.48 ± 1.47^{a}	13.69 ± 1.34^{b}	13.08 ± 1.53^{b}
Catalase (U/mg protein)	1.87 ± 0.27^{a}	1.22 ± 0.25^{b}	1.49 ± 0.40^{ab}	1.85 ± 0.21^{a}
Superoxide Dismutase (U/mg protein)	25.25 ± 3.00^{a}	15.00 ± 7.11^{f}	18.75 ± 4.12^{bf}	24.25 ± 7.54^{ab}

Histopathological Findings

Under a microscope, toluene-treated quails showed signs of severe kidney injury, including capillary congestion, tubular necrosis, epithelial desquamation, Bowman space shrinkage, and bleeding. These lesions were significantly reduced by co-treatment with F. officinalis. Moderate tubular preservation, decreased necrosis, and mild epithelial edema were seen. The histoarchitecture of the F. officinalis-treated group was comparable to that of the control group, indicating that it has nephroprotective properties. These patterns were corroborated by histopathological scoring, which showed mild (*) pathology in the controls and positive group, moderate (**) in the co-treated group, and severe (***) in the toluene group.

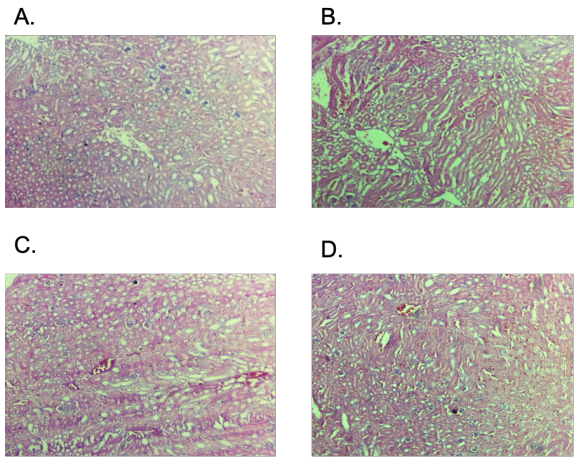

Fig. 2: Showing photomicrographs of quail's H and E-stained kidney sections. A: showing the control group. B: showing treatment with toluene. C: showing cotreatment with toluene + F. officinalis. D: showing treatment with F. officinalis.

Table 4: Histopathological partial quantitative recording of kidney tissues in reference and exposed quails after trial of 30 days

Groups	Control	Toluene	Toluene + F. officinalis	F. officinalis
Tubular degeneration	*	***	**	*
Distance in glomerulus	*	***	**	*
Expansion of tubules	*	***	**	*
Necrosis of tubules	*	***	**	*
Bowman space reduction	*	***	**	*
Hemorrhage	*	***	**	*

Assessment was completed as follows: none (*), moderate (**), Severe (***)

Behavioral Observations

Toluene-induced hypoactivity was discovered through behavioral scoring over 30 days. Birds in the toluene group continuously scored lower on behaviors related to feeding, crowing, vigilance, and mating. Quails that were co-treated and treated with F. officinalis, however, gradually improved. The toluene group (8) had the lowest overall behavioral ratings at week four, whereas the co-treated birds, F. officinalis, and control groups had the greatest scores. This pattern remained consistent over weekly intervals, indicating that F. officinalis lessened toluene-induced neurobehavioral deficits.

Table 5: Weekly behavioral alteration scores in control and treated quail groups during 30 days of treatment.

Week	Maximum Score	Control	Toluene Treated	Toluene + F. officinalis Treated	F. officinalis Treated
1st week	20	5	4	4	5
2nd week	20	9	7	8	9
3rd week	20	11	9	10	13
4th week	20	15	8	11	17
Total score	80	40	28	33	44

Discussion

The current investigation showed that giving toluene to quails causes serious physiological and biochemical problems, especially regarding kidney function and systemic oxidative balance. Birds treated with toluene showed a significant decrease in body weight, which could be explained by systemic toxicity, decreased nutritional absorption, and changed appetite. Our results are in line with those of [14], who found that rats exposed to toluene lost weight in a similar way as a result of its catabolic effects and decreased appetite. Co-treatment with Fumaria officinalis, however, reversed this trend and brought body weight back to more typical levels. This implies that the extract might increase metabolic efficiency, most likely as a result of its bioactive flavonoids and polyphenols, which have been shown by [6] to have

appetite-stimulating and anti-inflammatory properties. Additionally, exposure to toluene resulted in a significant decrease in kidney weight, which may have been brought about by inflammation, cellular degradation, and tissue atrophy. The findings of [10], who reported structural shrinkage and renal mass reduction in rodents treated with toluene, are consistent with these findings. It's interesting to note that quails given F. officinalis in addition to toluene showed a notable recovery in kidney weight. The plant's cytoprotective and antioxidant properties, which may lessen cellular damage and promote tissue regeneration, may be responsible for this improvement.

The toluene-exposed group's biochemical profile revealed markedly higher serum uric acid and creatinine levels, which suggested compromised renal function. These biomarkers are well-known measures of the effectiveness of glomerular filtration. Our findings concur with those of [19], who observed a comparable pattern of renal failure in rats after being administered toluene. Quails co-treated with F. officinalis, on the other hand, showed notable improvements in these parameters, indicating the preservation of nephrons and the restoration of kidney function. According to [20], this protective effect may be due to the phytoconstituents of the plant, which lower oxidative damage and increase mitochondrial activity in renal tissues.

The toluene group also showed a notable increase in biliverdin levels, which could be a result of increased heme catabolism and oxidative hepatic stress. Biliverdin levels returned to normal after co-treatment with F. officinalis, suggesting a decrease in oxidative turnover and hepatic-renal damage. These findings align with those of [16], who demonstrated that plant-based antioxidants can lessen oxidative liver damage and modify bilirubin metabolism. After being exposed to toluene, there was a noticeable drop in antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD), which suggests oxidative stress and weakened endogenous defense. These results are in line with those of [2], who similarly reported that toluene-induced toxicity suppressed these enzymes. In contrast to the toluene-only group, the quails that were given F. officinalis in addition to toluene exhibited noticeably greater SOD and CAT activities, confirming the plant's function in boosting antioxidant capacity. [11] reported a similar antioxidant restorative effect of flavonoid-rich extracts, highlighting their capacity to stimulate enzymatic defenses and scavenge free radicals.

Histopathological analysis revealed significant tubular necrosis, glomerular congestion, and cellular disarray, which further supported the renal impairment in quails treated with toluene. Acute nephrotoxicity is indicated by these microscopic lesions, which are similar to the pathological alterations in nephron-damaging conditions reported by [7]. The co-treatment group remarkably displayed only minor changes with better-preserved renal architecture, demonstrating F. officinalis's capacity to protect tissue. Reduced reactive oxygen species (ROS), enhanced renal perfusion, and potential anti-inflammatory properties of its ingredients could all contribute to this impact.

The toluene group also showed behavioral abnormalities, such as reduced activity, attentiveness, and coordination, which were probably brought on by central nervous system poisoning. These results are consistent with previous research by [30], which showed that toluene interferes with the brain's neurotransmitter systems, specifically the dopaminergic and cholinergic pathways. It's interesting to note that co-administration of F. officinalis lessened these behavioral deficits, suggesting that it has neuroprotective properties. According to [6], proposed phytotherapeutic principles, this effect might be the result of enhanced neurotransmitter balance and decreased oxidative damage to neural tissues.

Conclusion

The present study demonstrated that toluene exposure induces marked nephrotoxicity, oxidative stress, histopathological damage, reduced body and kidney weight, and behavioral disturbances in Japanese quail. Birds exposed to toluene showed elevated serum creatinine, uric acid, and biliverdin levels, along with reduced albumin concentration and decreased antioxidant enzyme activities of catalase and superoxide dismutase. These biochemical changes were supported by kidney tissue damage, including tubular degeneration, necrosis, congestion, hemorrhage, and reduced Bowman's space, confirming the harmful effect of toluene on renal structure and function. Dietary supplementation with Fumaria officinalis showed a protective effect against toluene-induced toxicity. Co-treated quails exhibited improvement in body weight, kidney weight, renal biomarkers, antioxidant enzyme activity, histological architecture, and behavioral performance compared with the toluene-treated group. The findings suggest that the antioxidant and cytoprotective properties of F. officinalis may help reduce oxidative damage, preserve renal tissue integrity, and improve general physiological condition under toxic stress. Overall, this

study supports the nephroprotective potential of Fumaria officinalis against toluene-induced renal injury in Japanese quail. However, further studies with larger sample sizes, different doses, and molecular-level analysis are recommended to confirm its protective mechanisms and evaluate its practical use as a natural dietary supplement against environmental toxin-induced organ damage.

Authors Contribution

Maryam Saleem conducted the experimental research, performed animal handling, treatment administration, sample collection, behavioral observations, data recording, and prepared the initial manuscript draft. Iftikhar Hussain assisted in laboratory work, methodology support, and review of the manuscript. Saba Malik contributed to the study concept, data interpretation, scientific discussion, and manuscript revision. Rohina Tabassam contributed to the interpretation of findings and the critical revision of the manuscript. Hafiza Sadia contributed to scientific writing and manuscript editing. Sheeba Riaz contributed to data interpretation, discussion development, and critical review of the manuscript. Namrah assisted in data organization and manuscript formatting. Muhammad Sakandar Majid supervised the study, designed the research framework, guided the overall research direction, reviewed and finalized the manuscript, and served as the corresponding author. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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