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Ameliorative Potential of Pumpkin Seeds Against Lead-Induced Toxicity in Poultry Chicken (Gallus gallus domesticus)

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ABSTRACT

Objective: This study aimed to evaluate the protective effects of pumpkin seed supplementation against lead-induced hepatorenal toxicity in Gallus gallus domesticus. **Material and Methods:** Young chickens were divided into four groups: control (G0), lead-exposed (G1), pumpkin seed-treated (G2), and combined lead and pumpkin seed-treated (G3). Lead acetate (0.4 mg/kg body weight) and pumpkin seed powder (300 mg/kg body weight) were administered for four weeks. Liver and kidney function tests (ALT, AST, ALP, bilirubin, urea, creatinine), lipid profiles (LDL, HDL, cholesterol, triglycerides), and antioxidant enzymes (CAT, GPx, GR, SOD) were analyzed. Histopathological evaluations of liver and kidney tissues were conducted. Data were analyzed using SPSS 25, with $P < 0.05$ considered significant. **Results:** Lead exposure increased ALT (14.6 ± 0.01 IU/L), AST (2210.7 ± 10.31 IU/L), ALP (1970.4 ± 1.07 IU/L), bilirubin (0.7 ± 0.00 mg/dL), and LDL (56.9 ± 0.91 mg/dL). Pumpkin seed supplementation reduced AST (110.1 ± 8.84 IU/L), ALP (1360.5 ± 1.00 IU/L), and LDL (22.9 ± 1.04 mg/dL), and improved antioxidant enzyme activities. **Conclusion:** Pumpkin seed supplementation mitigates lead-induced oxidative stress and improves hepatorenal function, presenting a natural strategy to reduce heavy metal toxicity in poultry.

INTRODUCTION

The introduction focuses on understanding lead-induced toxicity, its physiological effects, and the potential therapeutic role of natural interventions like pumpkin seeds. Lead toxicity poses a significant threat to both human and animal health, largely due to its ability to disrupt cellular processes through oxidative stress and the generation of reactive oxygen species (ROS). The binding of lead to thiol-containing compounds in proteins and enzymes impairs their function, leading to widespread damage in vital organs such as the liver, kidneys, brain, and gastrointestinal tract. Accumulation of lead in these organs results in compromised metabolic functions, inflammatory responses, and histopathological changes that are indicative of severe tissue damage. The liver and kidneys, being primary detoxification and filtration organs, are particularly vulnerable to lead-induced toxicity, manifesting as hepatorenal syndrome and structural degeneration (1, 2).

The increasing prevalence of lead contamination through environmental exposure, industrial activities, and contaminated feed presents a global concern. Sources of heavy metals like petrochemical, metallurgical, and plastic industries exacerbate this issue, with lead ions

infiltrating ecosystems and food chains, causing bioaccumulation in living organisms, including poultry (3, 4). These contaminants not only impair animal health but also pose a risk to human health when toxic residues are transferred through the consumption of affected animal products. Research indicates that even low concentrations of lead in biological systems can trigger oxidative damage, impair enzymatic activity, and disrupt lipid metabolism, leading to conditions such as hepatotoxicity and nephrotoxicity (5, 6).

To mitigate the detrimental effects of lead toxicity, dietary antioxidants have emerged as a promising strategy. Antioxidants, known for their ability to neutralize ROS and repair oxidative damage, play a pivotal role in maintaining cellular homeostasis. Among natural interventions, the seeds of Cucurbita maxima (pumpkin) have garnered attention for their rich phytochemical composition, including alkaloids, sesquiterpenes, and glycozoline, which exhibit potent antioxidant, anti-inflammatory, and hepatoprotective properties (7, 8). Phytochemical studies highlight the therapeutic potential of pumpkin seeds, demonstrating their efficacy in enhancing enzymatic antioxidants such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), which are critical in combating oxidative stress (9).

Lead toxicity is associated with disruptions in lipid metabolism, as evidenced by elevated levels of low-density lipoprotein (LDL), cholesterol, and triglycerides, alongside reduced high-density lipoprotein (HDL). These imbalances contribute to cardiovascular risk and are indicative of metabolic distress. Pumpkin seeds, with their hypolipidemic properties, have shown potential in modulating lipid profiles by reducing LDL and cholesterol levels while enhancing HDL concentrations (10). Furthermore, histological studies suggest that dietary supplementation with pumpkin seeds reduces structural damage in the liver and kidneys caused by heavy metal exposure, thereby supporting their protective role at the cellular level (11).

This research aims to explore the ameliorative potential of pumpkin seeds against lead-induced hepatorenal toxicity in Gallus gallus domesticus (chickens), focusing on their biochemical, enzymatic, and histopathological effects. By evaluating liver and kidney function, antioxidant enzyme activities, and lipid profiles, this study seeks to elucidate the protective mechanisms of pumpkin seeds against oxidative and inflammatory damage. These findings may provide a foundation for developing natural dietary interventions to mitigate heavy metal toxicity, ensuring food safety and improving animal health (12).

MATERIAL AND METHODS

This study was conducted to assess the protective potential of pumpkin seeds against lead-induced hepatorenal toxicity in Gallus gallus domesticus. The experimental procedures were designed in compliance with ethical standards, following the guidelines outlined in the Declaration of Helsinki and approved by the relevant institutional ethical committee at the Gomal University. The experimental design was carefully developed to ensure animal welfare, and efforts were made to minimize discomfort during all phases of the study.

Young chickens were procured from the local market and acclimatized to the laboratory environment for one week before the commencement of the experiment. During this period, the chickens were housed under controlled environmental conditions with a 12-hour light/dark cycle, a temperature range of 22–25°C, and adequate ventilation. The birds were provided free access to water and a standard poultry diet. Initial body weights and general health assessments were recorded to ensure homogeneity across the groups before the administration of treatments.

Pumpkin seeds (Cucurbita maxima) were sourced from the local market in DI Khan and processed to obtain the experimental supplement. The seeds were washed, dried, and ground into a fine powder. A daily dose of 60 grams of pumpkin seed powder was administered to the relevant groups, divided into three equal portions given at regular intervals. The lead-induced toxicity was modeled by administering lead acetate solution at a dose of 0.4 mg/kg body weight through oral gavage. This dose was calculated and standardized to ensure consistent exposure across the experimental groups, mimicking chronic lead toxicity conditions as described in previous studies (13).

The experimental setup included four groups: a control group receiving only normal feed and distilled water (G0), a lead-exposed group (G1), a

group receiving pumpkin seed supplementation without lead exposure (G2), and a group treated with both lead acetate and pumpkin seed supplementation (G3). Each group consisted of three chickens, ensuring sufficient replicates for statistical analysis. The experimental duration was four weeks, during which the birds were monitored daily for clinical signs of toxicity or stress.

Acute and chronic toxicity tests were conducted to determine the survival rate and toxic thresholds for the administered lead acetate doses. For acute toxicity testing, lead acetate was administered at doses of 40, 80, and 120 mg/chicken, and survival was assessed over a 48-hour period. Chronic toxicity testing involved administering lead acetate at lower doses (20, 40, and 60 mg/chicken) with survival monitored for 96 hours. These protocols were adapted from previously validated methodologies (14).

Biochemical analyses were performed at three time points: baseline, midway through the experiment, and at the conclusion of the study. Liver function tests (LFTs) included the measurement of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Kidney function tests (RFTs) measured bilirubin, urea, and creatinine levels. Lipid profiles were evaluated by quantifying LDL, triglycerides, cholesterol, and HDL levels. Antioxidant enzyme activities, including catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and superoxide dismutase (SOD), were assessed to determine oxidative stress levels. Histopathological examinations of liver and kidney tissues were conducted to evaluate cellular damage, using microscopy to observe structural changes and cellular integrity (15, 16).

The collection of blood samples for biochemical analysis was performed via venipuncture, using sterilized needles and syringes. Blood was collected in anticoagulant-coated tubes for plasma analysis and plain tubes for serum separation. Tissue samples for histological assessment were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E) for light microscopy.

Data were analyzed using SPSS version 25. Descriptive statistics were used to summarize the data, which were presented as mean ± standard error of the mean (SEM). The normality of data distribution was verified using the Shapiro-Wilk test, and homogeneity of variance was assessed using Bartlett's test. A one-way analysis of variance (ANOVA) was performed to detect significant differences among treatment groups, followed by post hoc comparisons using Tukey's test where applicable. A P-value of less than 0.05 was considered statistically significant, and results were presented with appropriate F-values and degrees of freedom. This study aimed to adhere to rigorous scientific and ethical standards in all aspects of experimental design, data collection, and analysis to ensure the validity and reliability of the findings. All procedures were conducted in accordance with relevant international and institutional guidelines for research involving animals.

RESULTS

The study investigated the ameliorative effects of pumpkin seed supplementation on lead-induced hepatorenal toxicity in Gallus gallus domesticus. The results, including liver enzyme activities, kidney function tests, lipid profiles, and antioxidant enzyme status, are presented in tables and supported by descriptive summaries. All statistical comparisons were performed using SPSS version 25, and a P-value of <0.05 was considered statistically significant.

The liver enzyme activities (ALP, ALT, and AST) across the experimental groups are shown in Table 1. Lead exposure (G1) significantly elevated ALP and AST levels compared to the control (G0), indicating liver stress. Pumpkin seed supplementation (G2 and G3) ameliorated this effect, as evidenced by reduced enzyme activities, with G2 showing the greatest improvement.

Table 1

Liver Function Tests

Treatments	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	F-value	P-value	F crit
G0	1290.7 ± 10.42	12.7 ± 0.01	202.9 ± 12.92	7.173	0.013	4.257
G1	1970.4 ± 1.069	14.6 ± 0.01	2210.7 ± 10.31			
G2	1360.5 ± 1.008	12.7 ± 0.03	110.1 ± 8.84			

G3	1760.0 ± 2.015	12.9 ± 0.01	990.5 ± 8.43

The kidney function parameters, including bilirubin, urea, and creatinine levels, are summarized in Table 2. Lead exposure (G1) caused a significant increase in these markers, indicating renal impairment. Pumpkin seed intervention (G2 and G3) showed a moderate reduction in these values, reflecting its protective effects.

Table 2

Kidney Function Tests

Treatments	Bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	F-value	P-value	F crit
G0	0.5 ± 0.01	16.1 ± 0.02	0.5 ± 0.01	1827.788	0.018	4.257
G1	0.7 ± 0.00	17.6 ± 0.03	0.7 ± 0.00			
G2	0.6 ± 0.00	16.0 ± 0.02	0.6 ± 0.00			
G3	0.7 ± 0.01	16.5 ± 0.03	0.7 ± 0.01			

The lipid profile analysis, presented in Table 3, revealed significant disruptions in G1, with elevated LDL, triglycerides, and cholesterol levels, and decreased HDL. Pumpkin seed supplementation (G2) significantly improved lipid parameters, indicating its hypolipidemic effects.

Table 3

Lipid Profile

Treatments	LDL (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	F-value	P-value	F crit
G0	35.1 ± 0.68	51.4 ± 0.76	84.1 ± 0.82	24.9 ± 0.62	12.160	0.000599	3.490
G1	56.9 ± 0.91	83.6 ± 1.21	106.6 ± 1.67	22.8 ± 0.27			
G2	22.9 ± 1.04	44.5 ± 0.69	61.1 ± 1.24	29.3 ± 0.94			
G3	39.9 ± 0.51	67.8 ± 0.66	81.5 ± 1.04	24.9 ± 0.59			

The antioxidant enzyme activities, including CAT, GPx, GR, and SOD, are presented in Table 4. G1 exhibited significantly elevated oxidative stress markers, while G2 and G3 showed improved antioxidant activities, with G2 demonstrating the most notable effects.

Table 4

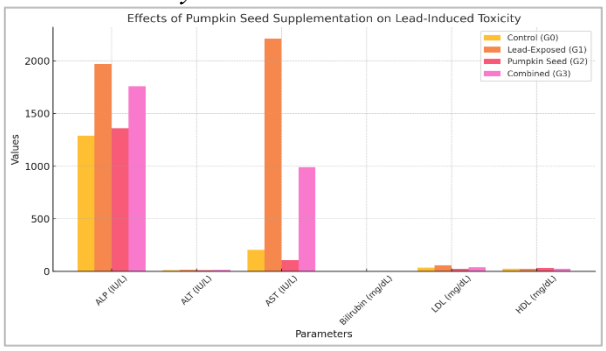
Antioxidant Enzyme Activities

Treatments	CAT (U/mg)	GPx (IU/L)	GR (µmol/mg)	SOD (U/mg)	F-value	P-value	F crit
G0	11.072 ± 0.00	220.86 ± 0.01	0.11 ± 0.04	23.6 ± 23.8	1639.396	0.006	3.490
G1	28.065 ± 0.00	240.65 ± 0.02	0.88 ± 0.05	25.8 ± 18.5			
G2	20.085 ± 0.00	230.99 ± 0.01	0.77 ± 0.09	24.1 ± 38.2			
G3	20.080 ± 0.00	230.78 ± 0.01	0.55 ± 0.12	23.4 ± 32.4			

Histological examination of liver and kidney tissues confirmed the biochemical findings. Severe cellular damage was observed in G1, including hepatocyte degeneration and renal tubular necrosis. In contrast, G2 and G3 exhibited reduced histological abnormalities, indicating protective effects of pumpkin seed supplementation.

Figure 1

Effects of Pumkin Seed Supplementation on Lead Induced Toxicity



The results collectively demonstrate the protective role of pumpkin seeds against lead-induced

hepatorenal toxicity, with significant improvements observed in liver enzyme activities, kidney function, lipid profiles, and antioxidant status, particularly in the group receiving only pumpkin seeds (G2).

DISCUSSION

The findings of this study demonstrated the protective potential of pumpkin seeds against lead-induced hepatorenal toxicity in chickens, aligning with existing literature that highlights the antioxidant and protective properties of natural phytochemicals. Lead toxicity significantly elevated liver enzymes, kidney markers, lipid profiles, and oxidative stress, confirming its deleterious impact on vital organs and metabolic functions. Pumpkin seed supplementation mitigated these effects, supporting its therapeutic role in reducing oxidative damage and improving organ function.

Lead exposure, as observed in this study, caused significant increases in ALP and AST levels, indicative of hepatic damage, consistent with earlier research showing that heavy metals disrupt hepatocyte integrity by generating reactive oxygen species and impairing enzymatic activities (1, 2). Elevated bilirubin, urea, and creatinine levels further corroborated renal impairment due to lead accumulation, which aligns with findings that lead primarily targets the kidneys, leading to structural and functional damage (3). The lipid profile alterations, including elevated LDL and cholesterol levels and reduced HDL, mirrored disruptions in lipid metabolism frequently reported in heavy metal toxicity studies, suggesting heightened cardiovascular risk (4, 5).

Pumpkin seed supplementation demonstrated a significant ameliorative effect, particularly in reducing liver enzyme and kidney marker levels, enhancing antioxidant enzyme activities, and normalizing lipid profiles. These outcomes can be attributed to the high concentration of bioactive compounds in pumpkin seeds, such as carbazole alkaloids, sesquiterpenes, and glycozoline, which have been reported to possess antioxidant and anti-inflammatory properties (6). The observed reduction in oxidative stress markers, particularly catalase and glutathione peroxidase activities, highlighted the role of pumpkin seeds in enhancing cellular defense mechanisms. Similar protective effects of natural antioxidants have been documented, emphasizing their ability to scavenge free radicals and repair oxidative damage in heavy metal toxicity (7, 8).

The histopathological findings provided further evidence of the protective role of pumpkin seeds, with reduced hepatocyte degeneration and renal tubular necrosis in treated groups. These results are consistent with studies reporting that dietary antioxidants preserve cellular architecture and function under toxic conditions (9). While the group receiving pumpkin seed supplementation alone (G2) exhibited the most pronounced improvements, the combination of lead exposure and pumpkin seeds (G3) showed moderate amelioration, suggesting a dose-dependent effect and potential saturation of protective mechanisms.

The study's strengths lie in its comprehensive evaluation of multiple biochemical, enzymatic, and histopathological parameters, providing a robust understanding of the protective mechanisms of pumpkin seeds. The inclusion of antioxidant enzyme assays and lipid profile analysis offered valuable insights into systemic effects beyond hepatic and renal functions. Furthermore, the use of a controlled experimental design and statistical validation enhanced the reliability of the findings.

However, there were limitations. The relatively short duration of the study may not have captured long-term effects or chronic toxicity outcomes. The sample size, while sufficient for initial observations, could limit the generalizability of the findings. Additionally, the study was limited to a single species, and extrapolation to other animals or humans requires caution. Future studies should explore varying doses and durations of pumpkin seed supplementation, as well as its effects in combination with other dietary antioxidants, to determine optimal therapeutic strategies (17).

Despite these limitations, the study provided valuable evidence supporting the use of pumpkin seeds as a dietary intervention against lead toxicity. Recommendations for future research include exploring the molecular mechanisms underlying the observed protective effects, conducting long-term studies to assess chronic outcomes, and evaluating the efficacy of pumpkin seeds in other species and environmental contexts. Additionally, integrating pumpkin seeds into dietary regimens for populations at risk of heavy metal exposure

could have significant public health implications. These findings contribute to the growing body of literature advocating for natural, cost-effective strategies to mitigate heavy metal toxicity and promote organ health (18-20).

CONCLUSION

This study demonstrated that pumpkin seed supplementation effectively mitigates lead-induced hepatorenal toxicity in chickens by reducing oxidative stress, normalizing liver and kidney functions, and improving lipid profiles. The bioactive compounds in pumpkin seeds exhibited strong antioxidant and protective properties, highlighting their potential as a natural intervention against heavy metal toxicity. These findings suggest that incorporating pumpkin seeds into the diet may serve as a cost-effective and accessible strategy to reduce heavy metal-induced health risks, with significant implications for human healthcare. Further research on the long-term effects and applicability in humans is recommended to strengthen the evidence for their therapeutic use.

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