Effects of Ascorbic Acid and Silver Nanoparticles Against Pyrazole and Fipronil Induced Liver Toxicity in *Gallus gallus domesticus*: A Comparative Study

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ABSTRACT

Background: The liver is a major metabolic and detoxification organ and is particularly susceptible to chemical-induced oxidative damage. Pyrazole compounds and the phenylpyrazole insecticide fipronil may induce hepatic injury, while antioxidant agents such as ascorbic acid and silver nanoparticles (AgNPs) may provide protective effects.
Methods: A randomized experimental study was conducted using 90 healthy *Gallus gallus domesticus* (Asil breed) chicks aged 3–4 days and weighing 25–30 g. The chicks were randomly assigned to nine groups (n = 10 per group) and treated orally for 28 days. Group I served as the control and received 0.9% normal saline. Groups II–IV received pyrazole alone or in combination with ascorbic acid or AgNPs, respectively. Groups V and VI received ascorbic acid and AgNPs alone, respectively. Groups VII–IX received fipronil alone or in combination with ascorbic acid or AgNPs, respectively. Serum ALT, AST, and ALP were analyzed, while liver tissues were examined histopathologically.
Results: Pyrazole and fipronil increased serum AST, ALT, and ALP levels compared with the control group, with fipronil producing the greatest elevation in liver enzymes. Histopathological examination revealed hepatocellular degeneration, vascular congestion, and necrosis, particularly in the fipronil-treated group. Co-treatment with ascorbic acid reduced biochemical and histopathological alterations associated with both toxicants. AgNPs also produced protective effects, although their effects varied according to the toxicant and biochemical parameter. Significant positive correlations were observed among AST, ALT, and ALP.
Conclusions: Pyrazole and fipronil produced hepatotoxic effects in growing chicks, with fipronil showing greater toxicity. Ascorbic acid provided substantial protection against toxicant-induced hepatic injury, whereas AgNPs showed variable protective effects.

Keywords: Chicken; Pyrazole; Fipronil; ALT; AST; ALP; Liver; Hepatotoxicity; Silver nanoparticles; Ascorbic acid

INTRODUCTION

Liver is a central metabolic and detoxifying organ in chicks and plays important roles in digestion, nutrient metabolism, waste elimination, and maintenance of homeostasis. In avian species, the liver is the primary site of de novo lipogenesis and contributes substantially to fatty acid synthesis, which is particularly important during rapid post-hatch growth. During late embryonic development and the first week after hatching, chicks rely heavily on hepatic metabolism and derive a substantial proportion of their energy from yolk lipid oxidation[19]. Following hatching, a major metabolic transition occurs as hepatic metabolism shifts from predominantly lipid-based to carbohydrate-based energy metabolism. This transition is regulated by complex transcriptional and post-transcriptional mechanisms, including microRNAs and oxidative stress-responsive pathways [11].
In addition to its metabolic functions, the liver is a major organ of detoxification and is therefore highly susceptible to environmental and chemical insults [19,15]. Hepatic injury in poultry can adversely affect growth, feed efficiency, and immune function and may consequently have important economic implications [1]. Among the chemical stressors of concern are pyrazole compounds and phenylpyrazole insecticides such as fipronil. These compounds have been associated with oxidative stress and hepatocellular damage through disruption of redox balance and inflammatory pathways [21,18,25]
Pyrazoles are heterocyclic aromatic compounds characterized by a five-membered ring containing two adjacent nitrogen atoms. Although pyrazoles are relatively uncommon in nature, their derivatives have numerous pharmaceutical and agricultural applications. Certain pyrazole compounds have been associated with toxic effects, including hepatotoxicity and hematological abnormalities[5]. Experimental evidence indicates that pyrazole exposure can increase susceptibility to liver injury by altering enzymes involved in oxidative stress pathways. Pyrazole has also been reported to potentiate tumor necrosis factor-alpha (TNF-α)-mediated hepatotoxicity, resulting in oxidative stress, mitochondrial dysfunction, and apoptosis [25].
Fipronil is a widely used phenylpyrazole insecticide that has

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attracted considerable attention because of its environmental persistence and potential entry into the food chain. It has been extensively used in agriculture and veterinary medicine for pest control. Its insecticidal activity is primarily associated with inhibition of γ-aminobutyric acid (GABA)-gated chloride channels, resulting in neuronal hyperexcitation in target organisms [21]. Fipronil and its metabolites may also affect non-target organisms and have been associated with neurotoxicity and oxidative stress [18]

Fipronil exposure in poultry has been associated with oxidative stress, lipid peroxidation, altered antioxidant enzyme activity, and hepatic injury. Oxidative stress is an important mechanism underlying hepatotoxicity caused by chemical compounds because excessive production of reactive oxygen species (ROS) can result in lipid peroxidation, protein damage, and DNA alterations. Biomarkers such as malondialdehyde (MDA), together with altered activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are commonly associated with oxidative injury[26]

Ascorbic acid (vitamin C) is a water-soluble antioxidant that contributes to the maintenance of cellular redox balance. It acts as a reducing agent and cofactor in several enzymatic reactions and contributes to collagen synthesis, immune function, and metabolic stability. Experimental studies have demonstrated that ascorbic acid can reduce oxidative damage and tissue injury in different toxicological models [16]. In avian species, vitamin C supplementation has also been associated with improved physiological performance and reduced oxidative stress.

Silver nanoparticles (AgNPs) have received considerable attention because of their distinctive physicochemical properties, including a high surface area and enhanced biological reactivity. They have demonstrated antimicrobial and anti-inflammatory activities and have been investigated for several biomedical applications. AgNPs may influence oxidative stress and cellular antioxidant pathways, including pathways associated with Nrf2 signaling [24]

Despite their potential applications, the biological effects of AgNPs are dose-dependent, and their safety requires careful evaluation [27,9] Experimental studies have reported that appropriately prepared AgNPs may influence liver function, inflammation, and tissue architecture under specific experimental conditions [7,22,24]

Given the susceptibility of the avian liver to chemical-induced oxidative injury and the potential exposure of birds to environmental toxicants such as pyrazole and fipronil, there is a need to investigate protective interventions. Therefore, the present study aimed to investigate the hepatotoxic effects of pyrazole and fipronil in *Gallus gallus domesticus* and to evaluate the potential protective effects of ascorbic acid and silver nanoparticles.

METHODOLOGY

Study design and experimental animals

The present randomized experimental study was conducted from February to June 2026 at the Department of Zoology, Rawalpindi Women University, Pakistan. The study was conducted following approval from the Zoology Research Lab, Zoology Department Rawalpindi Women University.

A total of 90 healthy *Gallus gallus domesticus* (Asil breed) chicks, aged 3–4 days and weighing 25–30 g, were obtained from a local hatchery. The chicks were acclimatized under standard laboratory conditions and randomly divided into nine experimental groups (n = 10 per group). All treatments were administered orally for 28 days.

Group I served as the control and received 0.9% normal saline. Group II received pyrazole at 25 mg/kg body weight. Group III received pyrazole at 25 mg/kg body weight combined with ascorbic acid at 250 mg/kg body weight. Group IV received pyrazole at 25 mg/kg body weight combined with silver nanoparticles at 0.02 ml/animal. Group V received ascorbic acid alone at 250 mg/kg body weight, whereas Group VI received silver nanoparticles alone at 0.02 ml/animal. Group VII received fipronil at 4.5 mg/kg body weight. Group VIII received fipronil at 4.5 mg/kg body weight combined with ascorbic acid at 250 mg/kg body weight. Group IX received fipronil at 4.5 mg/kg body weight combined with silver nanoparticles at 0.02 ml/animal. All doses were administered orally based on the initial body weight measurements.

Blood collection and biochemical analysis

At the end of the experimental period, the birds were anesthetized using chloroform and sacrificed. Blood samples were collected through cardiac puncture into vacuum tubes. Serum was separated by centrifugation at 10,000 rpm for 5 min.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using a Microlab 300 chemistry analyzer based on spectrophotometric methods.

Histopathological examination

Liver tissues were excised, washed with normal saline, and fixed in 10% neutral buffered formalin. The tissues were dehydrated through graded ethanol, cleared in xylene, embedded in paraffin wax, and sectioned at 4–5 µm thickness using a rotary microtome. Sections were stained with hematoxylin and eosin (H&E) and examined using a light microscope at 400× magnification. Cellular morphology was further evaluated using a digital trinocular microscope.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) and analyzed using SPSS version 28.0. One-way analysis of variance (ANOVA), was used to compare experimental groups. Correlation analysis was performed to assess relationships among AST, ALT, and ALP. A p-value <0.05 was considered statistically significant.

RESULT

Body weight

Weekly body-weight measurements differed among the experimental groups. The control group showed progressive increases in body weight throughout the observation period. Differences were also observed among the pyrazole-, fipronil-, ascorbic acid-, and AgNP-treated groups.

Table 1. Mean body weight of chicks in different experimental groups.

Week	Control	Pyrazole	Pyrazole + Ascorbic Acid	Pyrazole + AgNPs	Ascorbic Acid	AgNPs	Fipronil	Fipronil + Ascorbic Acid	Fipronil + AgNPs
1	26.00 ± 1.73	33.00 ± 1.53	26.66 ± 1.20	24.33 ± 2.33	28.66 ± 1.20	37.66 ± 1.86	29.66 ± 2.91	30.66 ± 1.86	34.33 ± 2.19

2	38.33 ± 1.86	43.00 ± 4.73	36.00 ± 1.00	50.66 ± 3.18	40.33 ± 0.88	49.66 ± 7.86	66.00 ± 0.58	53.33 ± 4.98	56.00 ± 4.58
3	91.33 ± 9.35	83.00 ± 5.86	77.66 ± 5.93	83.33 ± 10.84	84.33 ± 2.33	83.00 ± 7.64	69.33 ± 4.18	67.66 ± 4.18	70.66 ± 3.84
4	113.33 ± 13.33	91.33 ± 5.17	83.00 ± 9.02	92.66 ± 11.46	97.33 ± 5.84	98.00 ± 14.00	92.33 ± 9.60	89.00 ± 1.73	83.33 ± 1.76
5	129.67 ± 13.86	103.33 ± 6.01	124.66 ± 22.64	116.66 ± 10.53	115.33 ± 3.93	119.33 ± 18.52	104.33 ± 10.93	107.66 ± 3.93	91.00 ± 3.79

Liver function parameters

Serum AST, ALT, and ALP levels varied among the experimental groups. Pyrazole treatment increased AST and ALP levels compared with the control group. Fipronil treatment produced the greatest elevations in AST, ALT, and ALP. Co-treatment with ascorbic acid reduced the biochemical alterations associated with both pyrazole and fipronil. AgNP treatment also altered the liver-function parameters, although the magnitude and direction of the effects varied among the treatment groups.

Table 2. Liver function parameters in different experimental groups.

Group no.	Groups	Mean Values (mg/dl)		
		AST	**ALT**	**ALP**
1	Control	312.67 ± 35.48	13.67 ± 1.67	1467.97 ± 574.85
2	Pyrazole	518.33 ± 19.62^{a***}	33.33 ± 3.38	2993.90 ± 161.77
3	Pyrazole & Ascorbic Acid	366.67 ± 49.84^{b***} ^{c**}	12.67 ± 1.33	2856.23 ± 58.44
4	AgNPs and Pyrazole	417.33 ± 163.18 ^{b***}	14.00 ± 3.79	2456.67 ± 620.58
5	Ascorbic Acid	290.00 ± 41.94 ^{b**} ^{c*}	18.00 ± 2.00	1492.97 ± 230.39
6	AgNPs	403.00 ± 82.35 ^{b**} ^{c*}	18.33 ± 3.18	1998.83 ± 719.72
7	Fipronil	625.33 ± 18.77 ^{b**} ^{d**e*}	37.67 ± 5.33^{a*e*}	3843.33 ± 141.70^{a**b**}
8	Fipronil & Ascorbic Acid	381.00 ± 67.56 ^{b**}	13.67 ± 2.40	2290.57 ± 322.30
9	Fipronil and AgNPs	223.33 ± 21.36 ^{b***}	20.67 ± 4.06	792.37 ± 155.52

a= significantly different from group 1, b= significantly different from group 2, c= significantly different from group 7, d= significantly different from group 8, e= significantly different from group 9. ***p<0.0001;** = p<0.01; *= p<0.05

Correlation among liver function parameters

Significant positive correlations were observed among AST, ALT, and ALP. AST showed a positive correlation with ALT (r = 0.617, p = 0.001), ALT showed a positive correlation with ALP (r = 0.530, p = 0.004), and ALP showed a strong positive correlation with AST (r = 0.844, p = 0.0001).

Table 3. Correlation among liver function parameters.

Parameter	Correlated parameter	Correlation coefficient (r)	p-value
AST	ALT	0.617	0.001
ALT	ALP	0.530	0.004

ALP	AST	0.844	0.0001

Group-wise correlation analysis

Group-wise correlation analysis was also performed for the control, pyrazole, pyrazole + ascorbic acid, and pyrazole + AgNP treatment

Table 4. Correlation analysis of liver function parameters among the experimental groups

Experimental Group	Parameters	Correlated to	Correlation Coefficient (r)	P value
Control Group	AST	ALT	0.738	0.472
	ALT	ALP	0.936	0.229
	ALP	ALT	0.453	0.700
Pyrazole Treated Group	AST	ALT	0.994	0.071
	ALT	ALP	0.920	0.256
	ALP	ALT	0.958	0.158
Pyrazole + Ascorbic Acid Treated Group	AST	ALT	1.000*	0.011
	ALT	ALP	0.999*	0.032
	ALP	ALT	0.999	0.021*
Pyrazole + Silver Nanoparticle Treated Group	AST	ALT	0.360	0.766
	ALT	ALP	0.213	0.864
	ALP	ALT	0.988	0.098
Ascorbic Acid Treated Group	AST	ALT	0.620	0.547
	ALT	ALP	0.979	0.131
	ALP	ALT	0.767	0.443
Silver Nanoparticle Treated Group	AST	ALT	0.908	0.275
	ALT	ALP	0.852	0.350
	ALP	ALT	0.555	0.625
Fipronil Treated Group	AST	ALT	0.941	0.220
	ALT	ALP	1.000*	0.013
	ALP	ALT	0.934	0.233
Fipronil + Ascorbic Acid Treated Group	AST	ALT	0.957	0.188
	ALT	ALP	0.900	0.288
	ALP	ALT	0.733	0.476
Fipronil + Silver Nanoparticle Treated Group	AST	ALT	−0.852	0.351
	ALT	ALP	−0.703	0.503
	ALP	ALT	0.972	0.152

Histopathological examination

Following were the findings in the liver tissues observed under microscope.

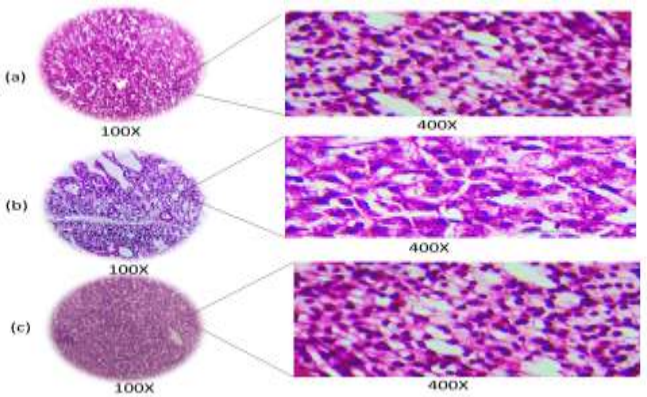


Figure 1.

Histological photomicrographs of liver sections stained with H&E showing: (A) control group with normal hepatic architecture, polygonal hepatocytes with clear cytoplasm and centrally located nuclei; (B) pyrazole-treated group showing hepatocellular degeneration, cytoplasmic vacuolation, and central vein congestion; and (C) pyrazole + ascorbic acid-treated group showing mild hepatocellular changes with largely preserved hepatic cords.

Figure 2.
Histological photomicrographs of liver sections stained with H&E showing: (A) pyrazole + AgNPs-treated group with moderate hepatocellular degeneration, focal necrosis, and sinusoidal dilation; (B) ascorbic acid-treated group showing normal hepatic architecture with intact hepatocytes; and (C) AgNPs-treated group showing mild vacuolar changes with largely preserved hepatocellular arrangement.

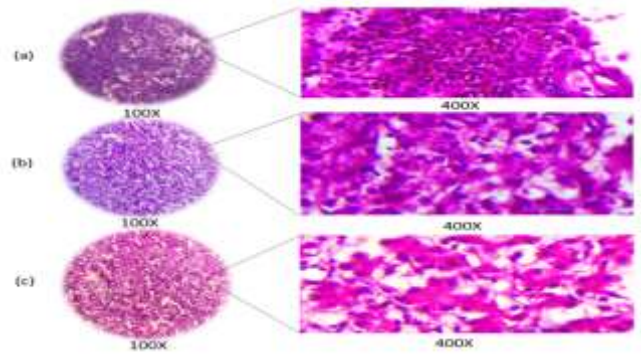
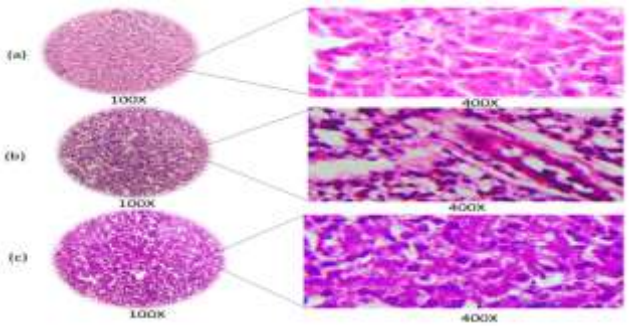


Figure 3.
Histological photomicrographs of liver sections stained with H&E showing: (A) fipronil-treated group with severe hepatocellular degeneration, necrosis, and vascular congestion; (B) fipronil + ascorbic acid-treated group showing and (C) fipronil + AgNPs-treated group showing moderate hepatocellular degeneration and congestion, less severe than in the fipronil-only group.



DISCUSSION

The body-weight data showed differences among the experimental groups during the study period. Exposure to pyrazole and fipronil was associated with altered growth patterns compared with the control group. Previous studies have reported adverse effects of fipronil exposure on growth and physiological parameters in birds. Khalil et al. [13] reported inflammatory and oxidative effects following fipronil exposure in Japanese quail, while Hussain et al. [12] reported reduced feed intake and body-weight gain following exposure to fipronil in cockerels

Antioxidants, including vitamin C, have been investigated for their ability to improve physiological performance and reduce oxidative stress [8]. AgNPs have also been reported to influence growth performance and immune responses in poultry [10, 14]. In the present study, treatment with ascorbic acid in combination with pyrazole was associated with greater body weight than pyrazole alone. AgNP-treated groups also showed variable changes in body weight.

Pyrazole treatment increased AST and ALP levels compared with the control group, indicating altered hepatic function. Fipronil produced the highest AST, ALT, and ALP values among the toxicant-only groups, suggesting greater hepatic injury. These findings are consistent with reports describing oxidative stress and tissue injury following fipronil exposure [26, 6].

Co-treatment with ascorbic acid reduced several of the biochemical alterations observed following pyrazole and fipronil exposure. The antioxidant properties of vitamin C may contribute to its protective effect by scavenging reactive oxygen species and supporting cellular antioxidant defenses. Similar protective effects of antioxidant supplementation have been reported in experimental toxicological models [16].

AgNPs also altered liver enzyme levels. In the fipronil + AgNP group, AST and ALP values were lower than those observed in the fipronil-only group, whereas ALT remained higher than the control value. These findings suggest that the protective response to AgNPs may differ according to the biochemical parameter and toxicant involved. Because AgNP effects are dose- and formulation-dependent, their potential hepatoprotective effects should be interpreted cautiously [27, 9].

The significant positive correlations among AST, ALT, and ALP indicate that changes in these biochemical markers occurred in association with one another. The strong correlation between ALP and AST further supports their usefulness as indicators of altered hepatic function under the experimental conditions.

The control group showed normal hepatic architecture, with organized hepatic cords and apparently normal hepatocytes. This finding is consistent with the normal structural characteristics described for the chicken liver [2].

Pyrazole treatment resulted in hepatocellular degeneration, cytoplasmic vacuolation, and vascular congestion. These findings are compatible with chemically induced hepatic injury associated with oxidative stress. Fipronil treatment produced more pronounced histopathological alterations, including hepatocellular degeneration, necrosis, and vascular congestion. Similar tissue injury has been reported following fipronil exposure [20, 4].

The pyrazole + ascorbic acid group showed comparatively preserved hepatic architecture, suggesting a protective effect of ascorbic acid. Vitamin C may reduce oxidative damage through free-radical scavenging and stabilization of cellular membranes. The fipronil + ascorbic acid group should, however, be interpreted according to the final verified histological observations because the current figure description and discussion are not fully consistent. The AgNP-treated group showed largely preserved hepatic architecture with mild vacuolar alterations. The pyrazole + AgNP and fipronil + AgNP groups showed less severe alterations than the corresponding toxicant-only groups, suggesting partial protection. Nevertheless, the effects of AgNPs were variable across biochemical and histological endpoints and should not be characterized as uniformly hepatoprotective without further dose-response and mechanistic studies.

CONCLUSION

The present study demonstrates that pyrazole and fipronil can adversely affect liver function and hepatic morphology in growing *Gallus gallus domesticus* chicks. Fipronil produced greater alterations in serum liver enzymes and more pronounced histopathological damage than pyrazole. Co-treatment with ascorbic acid reduced several biochemical and histological changes, indicating a potential hepatoprotective effect. Silver nanoparticles also produced protective effects in some experimental groups, although their effects were less consistent. Further studies are required to establish the dose-dependent effects, mechanisms of action, and long-term safety of AgNPs in poultry.

Ethical Approval

Ethical approval for the conduct of this study was obtained from the relevant institutional ethics review committee.

Data Availability
The data generated and analyzed during this study are available from the corresponding author upon reasonable request. The data are not publicly available to protect the privacy and confidentiality of the study participants.

Author Contributions
Laiba Saifullah: Conceptualization, study design, data acquisition, interpretation of results, manuscript drafting, critical revision, final approval, and accountability for the work.
Fareeha Ambreen: Study conception and design, supervision, interpretation of findings, critical revision of the manuscript, final approval, and accountability for the work.
Anas Bilal: Laboratory investigations, data acquisition, biochemical analysis, interpretation of results, critical revision, final approval, and accountability for the work.
Madeeha Chaudhry: Methodology, data acquisition, interpretation of findings, critical revision of the manuscript, final approval, and accountability for the work.
Farzana Aziz: Data analysis, interpretation of results, manuscript review, critical revision, final approval, and accountability for the work.
Rabea Ejaz: Data collection, literature review, manuscript drafting, critical revision, final approval, and accountability for the work.
Muqaddas Raina: Laboratory investigations, data collection, literature review, critical revision, final approval, and accountability for the work.
Qurat ul Ain Siddique: Data analysis, interpretation of findings, manuscript review, critical revision, final approval, and accountability for the work.
All authors contributed substantially to the study, reviewed the manuscript critically, approved the final version, and agreed to be accountable for all aspects of the work.

Informed Consent
We obtained written informed consent from all participants before enrollment in the study. Participants were informed about the study objectives, procedures, potential risks and benefits, and their right to refuse participation or withdraw at any time without affecting their employment or access to services. The study maintained the confidentiality and anonymity of all collected information throughout.

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Conflict of Interest
The authors declare no conflict of interest associated with this study.

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Declaration of AI-Assisted Language Editing
Grammarly AI was used for language and grammar correction.

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