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Down-regulation of β-defensins, an immuno-linked gene in quinalphos-exposed birds

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Quinalphos, an organophosphate pesticide, is widely used in agriculture to mitigate pest load and poses risks to non-target species, including poultry. The sublethal and immunotoxic effects of quinalphos on indigenous Kamrupa chickens, vital for Northeast India's rural economy, are poorly understood. In this study an attempt has been made to study the acute toxicity and immunosuppressive effects of quinalphos in Kamrupa chickens. Twenty birds were divided into control (n=10) and treatment groups (n=10), with the latter receiving a single oral LD50 dose (22.5 mg/kg body weight) in corn oil via oral gavage. Birds in the treatment group exhibited rapid onset of neurological symptoms, excessive salivation, reduced feed intake, diarrhoea, and 50% mortality within 36 hours. Massive reduction in lymphocyte counts with elevated haemoglobin; total erythrocyte count, total leukocyte count, and heterophil percentage were evident in haematological findings. Serum biochemistry showed increased alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, cholesterol, and uric acid, and reduced cholinesterase activity. Histopathological findings showed hepatic, renal, and neural degeneration. Gene expression studies showed significant downregulation of β-defensin genes (AvBD1, AvBD6, AvBD7). These findings highlight the need for low-dose or safer pest-control alternatives to protect poultry and the environment as a whole.

Keywords: β-defensins, organophosphate toxicity, quinalphos, avian immunity, oxidative stress

India's economy is largely agrarian, with more than half of the population is depending on agriculture, and the agro-industry contributing 18% to the national GDP[1]. In 2022–23, food grain production reached a record 329.7 million tons from 156 million hectares, ranking India second globally[2]. However, crop pests remain a major obstacle affecting agricultural productivity and food security[3]. They significantly reduce yield and degrade quality, rendering produce unsuitable for consumption or market[4]. Globally, insecticides constitute 45–50% of total pesticide use, followed by herbicides (35–40%) and fungicides (10–15%)[5]. Organophosphate pesticides are widely used due to their high effectiveness, yet concerns persist regarding their toxicity and persistence in the environment[6]. Quinalphos, a commonly used organophosphate against aphids, jassids, and thrips[7], inhibits acetylcholinesterase, leading to acetylcholine accumulation, paralysis, and insect death[7], while also posing risks to non-target organisms[8].

The present investigation plans to investigate the acute exposure to quinalphos in Kamrupa birds and its effects on gene expression corresponding to immunity and its effects on haemato-biochemical changes in Kamrupa birds, which are native to Assam. In addition, the consequent histological and molecular alterations in beta-defensin genes, namely Avian Beta Defensin 1, Avian Beta Defensin 6, and Avian Beta Defensin 7, in these subjects were also studied. The outcomes will offer an understanding of the impact of these pesticides on avian species and highlight the need for vigilance in agricultural methods to protect public health.

Materials and Methods

Chemicals

Pharmaceutical-grade quinalphos (≥98% purity) was obtained from Sigma-Aldrich.

Experimental animals

The experiment was conducted with approval from the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, A.A.U., Khanapara (Approval no. 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IACE/22-23/1043). Twenty (day-old chicks) Kamrupa chickens of both sexes were used in the research. Before the trial, the birds were housed in the

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institutional poultry shed for a period of seven days for acclimatization, temperature maintained at 25–30°C, relative humidity at 60–70%, and a 12-hour light/dark cycle (6 AM–6 PM light). During this time, water and a balanced, nutritious diet (Godrej Feed) were provided without restriction. On the seventh day, the birds were vaccinated against Ranikhet Disease.

Experimental design

A preliminary LD_{50} study was conducted to identify an appropriate acute exposure dose of quinalphos. Small groups of birds were administered graded concentrations of the compound, and clinical signs were monitored using a standardized scoring system that included parameters such as activity level, posture, respiratory distress, tremors, and feed intake. Mortality and time-to-death were recorded for each dose group. Based on the observed clinical scores and mortality pattern, the LD_{50} value was estimated using the OECD 425[9] and probit analysis method. On the eighth day, the birds were split into two groups of ten each. Group I acted as the control group, while Group II received a single oral dosage of quinalphos in corn oil at a rate of 22.5 mg/kg body weight (LD_{50}).

Sample collection and assessment of haematological and enzyme profiles

Blood samples were collected at 0, 3, 6, 12, 24, and 36 h after dosing from the jugular veins of both the control and treatment groups. Haematological parameters such as haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), lymphocyte, and heterophil levels were analysed by an automated haematology cell counter (model MS4S).

Serum enzymes, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total serum cholesterol (TC), Uric acid, and Acetylcholinesterase (AchE) were analysed using commercially available biochemical kits (Kee Diagnostics) with the help of a Double Beam UV-VIS spectrophotometer (Systronics).

Postmortem and Histopathology

In the gross examination, birds that died during the experiment and those that survived till the end of the experiment were sacrificed by cervical dislocation. Any noticeable alterations were recorded through postmortem examinations. For histopathological examination, after the post-mortem, representative pieces of the liver, kidney, and brain were collected in clean sample containers with 10% formalin, taking care not to damage the tissues. The tissues were sliced into 2-3 mm-thick sections and kept for further fixation. They were then washed in tap water overnight, dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in liquid paraffin. The paraffin sections were cut into 5–6-micron thickness and stained using haematoxylin and eosin methods as described by Luna[10].

Gene expression studies using qRT-PCR

As a part of an immunosuppression study, birds' livers were collected to examine three genes, namely, Avian Beta Defensin 1, Avian Beta Defensin 6, and Avian Beta Defensin 7, which have been shown to code for immunity.

Total RNA isolation

To preserve cellular RNA, liver samples were stored in RNA later solution and kept frozen until use. Throughout tissue handling, the tissues were snap-frozen in liquid nitrogen, and total RNA was isolated using an RNA isolation kit (Himedia, India) following the manufacturer's protocol. The purity of the RNA was evaluated using a NanoDrop Spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized as per the method described by Darwish *et al.*[11].

Quantitative Real-Time PCR (q-RT PCR) Reaction

Each sample was tested in duplicate. Each reaction included 20 ng cDNA, 10 pmol of forward and reverse primers, and 2X Fast SYBR Green PCR Master Mix (TB Green Premix Ex Taq II, # RR820A). 10 µL reaction volumes were used according to the Table 1.

A Tata MD CHECK Express real-time PCR machine was used with the following program: (95°C → 30 sec), and 40 cycles (95°C → 5 sec, 58°C → 30 sec) followed by melting curve 60°C to 99°C. MyGo Pro PCR software 3.6 was used for experimental setup and data analysis. Target gene qRT-PCR data were determined by Cq value. The formula outlined by Livak and Schmittgen[12] was used to compute alterations in the expression levels of genes, and the results were presented as fold change, as per the formula shown below:

Table 1 — Reaction volumes used in the study for gene expression study.

Fast SYBR master mix(µL)	Nuclease-free water (µL)	RT product (20 ng/µL)	F+R primer (10pM)	Total volume (µL)
5	3	1	1	10

Fold change = 2 − ΔΔCt, wherein ΔΔCt = [(Ct of gene of interest − Ct of housekeeping gene) treatment group − (Ct of gene of interest − Ct of housekeeping gene) control group]. Table 2 shows the primer sequence for specific genes used in this study[13].

Statistical analysis

The data was displayed as mean ± SE. One-way analysis of variance (ANOVA) was used with SPSS V 26.0 software to assess the statistical analysis of all the grouped data. A value of $P \leq 0.05$ was taken as statistically significant.

Results

Clinical Signs

The chickens exposed to quinalphos exhibited toxicity symptoms such as initial excitement followed by lethargy, wing drooping, uncoordinated movements, frothy salivation, and sitting on their hocks with curled toes. They eventually succumbed to convulsions, with 5/10 birds dying within 36 hours of the experiment.

Haematological alterations

Haemoglobin levels showed a significant increase in the group exposed to quinalphos compared to the control starting from 12 hours onwards (Table 3, $P<0.05$). Total erythrocyte count (TEC) exhibited a time-dependent significant increase in the quinalphos group from 24 hours onward (Table 3, $P<0.05$). Likewise, the total leukocyte count (TLC) in the quinalphos group showed a time-dependent increase from 12 hours onwards (Table 3, $P<0.05$). Moreover, there was a time-dependent rise in the percentage of heterophils observed in the quinalphos group starting from 24 hours onwards (Table 3, $P<0.05$). Conversely, lymphocyte count decreased significantly and progressively from 12 hours onwards (Table 3, $P<0.05$).

Biochemical alterations

A progressive increase in ALT levels was observed in the quinalphos group compared to the control starting from 6 hours onwards, becoming significant at 12 hours (Table 4, $P<0.05$). Similarly, AST levels demonstrated a time-dependent significant increase from 12 hours onwards (Table 3, $p<0.05$). Serum alkaline phosphatase levels began to rise significantly from 12 hours onwards in the quinalphos group (Table 4, $P<0.05$). In contrast, cholinesterase levels decreased significantly from 12 hours onwards (Table 4, $P<0.05$). Furthermore, total cholesterol levels

Table 2 — Shows qRT-PCR primer sequence of Genes of Interest (AvBD1, AvBD6, and AvBD7)

Primer	Accession number	Primer Sequence		Product Size
		Forward	Reverse	
Avian Beta Defensin 1 (AvBD1)	NM_204993.1	CCTGTGAAAACCCGGGACA	GCACAGAAGCCACTCTTTCG	145
Avian Beta Defensin 6 (AvBD6)	NM_001001193.1	TTGCAGGTCAGCCCTACTTT	CCGGTAATATGGCCACCGAC	95
Avian Beta Defensin 7 (AvBD7)	NM_001001194.1	ATTTCACATCCCAGCCGTGG	AGGCCTAGGAATGAAGGGCT	103
ACTB (housekeeping)	L08165	CCCATCTATGAAGGCTACGC	TCCTTGATGTCACGCACAAT	152

Source: Reproduced from[13]

Table 3 — Shows alterations in hematological parameters (Mean ± SE) in acute quinalphos exposed toxicity

Time (h)	Group	Haemoglobin (g%)	TEC (10^6/µl)	TLC (10^3/µl)	Heterophil (%)	Lymphocyte (%)
0	Control	9.56 ± 0.11	1.49 ± 0.22	26.53 ± 0.18	4.71 ± 0.12	86.30 ± 0.13
	Treatment	9.22 ± 0.14^{c}	1.62 ± 0.03^{c}	25.99 ± 0.30^{c}	4.49 ± 0.07^{d}	85.57 ± 0.49^{a}
3	Control	9.88 ± 0.11	1.95 ± 0.17	26.03 ± 0.26	4.32 ± 0.07	86.72 ± 0.22
	Treatment	10.31 ± 0.15^{bc}	2.75 ± 0.12^{c}	27.05 ± 0.97^{c}	4.79 ± 0.35^{d}	85.60 ± 0.33^{a}
6	Control	10.12 ± 0.24	2.14 ± 0.31	25.61 ± 0.26	4.59 ± 0.13	86.28 ± 0.40
	Treatment	10.58 ± 0.08^{b}	2.92 ± 0.14^{b}	27.63 ± 0.75^{c}	4.57 ± 0.20^{d}	84.75 ± 0.48^{bc}
12	Control	10.72 ± 0.13	2.79 ± 0.20	26.47 ± 0.25	4.19 ± 0.12	87.01 ± 0.73
	Treatment	$11.86 \pm 0.39^{*bc}$	3.37 ± 0.14^{b}	$33.52 \pm 0.14^{*b}$	6.12 ± 0.40^{c}	$82.61 \pm 0.56^{*ab}$
24	Control	11.24 ± 0.28	2.63 ± 0.13	27.10 ± 0.26	3.53 ± 0.01	86.10 ± 0.39
	Treatment	$12.44 \pm 0.09^{*ab}$	$4.55 \pm 0.25^{*b}$	$32.52 \pm 0.51^{*b}$	$9.58 \pm 0.18^{*b}$	$76.29 \pm 0.20^{*c}$
36	Control	10.96 ± 0.34	2.67 ± 0.18	27.52 ± 0.05	4.40 ± 0.18	86.15 ± 0.28
	Treatment	$13.58 \pm 0.08^{*a}$	$5.62 \pm 0.07^{*a}$	$33.94 \pm 0.37^{*a}$	$14.96 \pm 0.22^{*a}$	$75.83 \pm 0.29^{*d}$

Means marked with an asterisk (*) are significant between groups

Haemoglobin, TEC, TLC, and heterophil percentage increased progressively, with peak values at 36 hours, accompanied by a corresponding decline in lymphocyte percentage. These changes indicate leukocytosis with heterophilia and relative lymphopenia, suggesting an acute stress or inflammatory reaction.

Table 4 — Shows alterations in biochemical parameters (Mean ± SE) in acute quinalphos exposed toxicity

Time (h)	Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	CHE (IU/L)	Total Cholesterol (IU/L)	Uric Acid (mg/dl)
0	Control	24.83 ± 0.15	50.63 ± 0.20	106.08 ± 0.38	441.68 ± 0.56	142.80 ± 0.23	7.91 ± 0.14
	Treatment	25.33 ± 0.15^{e}	50.97 ± 0.22^{d}	107.19 ± 0.52^{e}	440.10 ± 0.20^{ab}	141.79 ± 0.73^{e}	8.71 ± 0.27^{d}
3	Control	24.98 ± 0.25	51.34 ± 0.28	105.50 ± 0.29	440.82 ± 0.35	143.37 ± 0.66	7.70 ± 0.28
	Treatment	27.46 ± 0.84^{e}	52.67 ± 0.26^{d}	108.50 ± 0.65^{de}	436.48 ± 1.08^{a}	145.93 ± 0.53^{e}	8.18 ± 0.39^{d}
6	Control	25.64 ± 0.40	51.50 ± 0.50	106.28 ± 0.27	440.03 ± 0.51	145.56 ± 0.64	8.36 ± 0.10
	Treatment	33.25 ± 1.18^{d}	55.75 ± 0.95^{cd}	117.55 ± 1.78^{*d}	434.07 ± 0.89^{b}	152.86 ± 1.07^{*d}	11.05 ± 0.72^{c}
12	Control	25.37 ± 0.25	52.56 ± 0.37	107.30 ± 0.72	441.10 ± 0.17	146.58 ± 0.79	7.52 ± 0.11
	Treatment	51.40 ± 0.43^{*c}	82.08 ± 0.60^{*c}	157.50 ± 0.52^{*c}	416.77 ± 0.71^{*c}	173.50 ± 0.65^{*c}	13.88 ± 0.74^{*c}
24	Control	27.13 ± 0.30	52.39 ± 0.25	107.10 ± 0.57	439.81 ± 0.78	146.64 ± 0.41	7.33 ± 0.19
	Treatment	65.79 ± 0.37^{*b}	105.89 ± 0.31^{*b}	243.75 ± 0.41^{*b}	372.95 ± 1.11^{*d}	191.71 ± 0.92^{*b}	20.96 ± 0.30^{*b}
36	Control	26.91 ± 0.54	51.97 ± 0.56	107.21 ± 0.28	439.12 ± 1.16	148.96 ± 0.42	7.59 ± 0.15
	Treatment	85.13 ± 0.29^{*a}	131.15 ± 0.25^{*a}	292.75 ± 0.46^{*a}	304.28 ± 0.82^{*e}	202.41 ± 0.92^{*a}	28.54 ± 0.19^{*a}

Means marked with an asterisk (*) are significant between groups
Means marked with different superscript letters are significant within group (α 0.05)

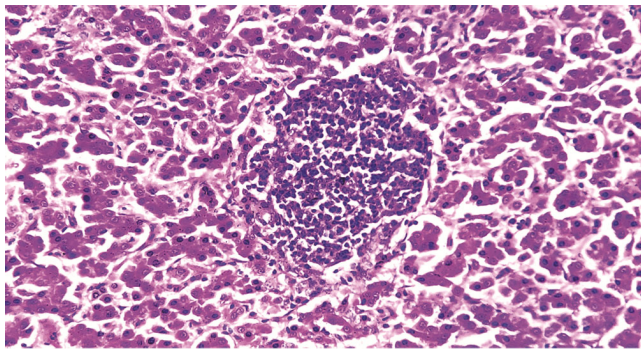

Fig. 1 — Liver section from a quinalphos-exposed bird showing prominent perivascular lymphoid aggregates and foci of necrosis with mononuclear phagocyte infiltration. H&E stain, 40×.

showed a time-dependent increase from 6 hours onwards (Table 4, $P<0.05$), and uric acid levels began to rise significantly from 12 hours onwards in the quinalphos group (Table 4, $P<0.05$).

Histopathological Alterations and scoring

Histopathological changes were assessed using both descriptive microscopy and a semi-quantitative scoring system. Lesions in liver, kidney, and brain tissues were graded as mild (+), moderate (++), or severe (+++) based on the degree of cellular degeneration, necrosis, inflammatory infiltration, and structural disruption. In the liver, quinalphos-exposed birds showed severe perivascular lymphoid aggregation with areas of necrotic tissue replaced by mononuclear phagocytic cells (H&E, 40X) (Fig. 1). Kidney sections revealed moderate degeneration of renal tubules along with areas of coagulative necrosis (H&E, 40X) (Fig. 2). Histopathological examination of the brain showed mild to moderate neuronal degeneration accompanied by dilation of blood vessels (Fig. 3). All lesions were scored independently by two blinded veterinary pathologists,

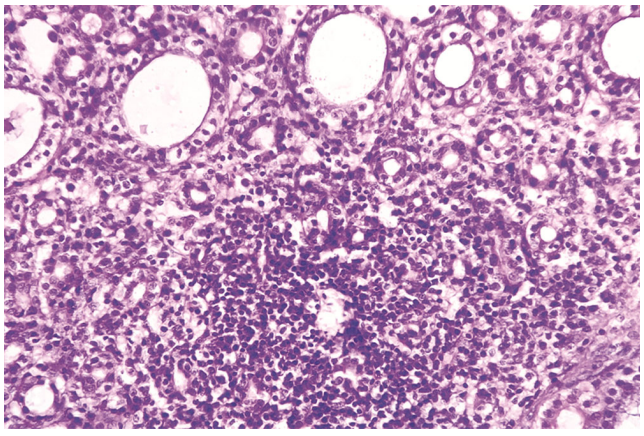

Fig. 2 — Kidney section from a quinalphos-exposed bird showing degeneration and coagulative necrosis of renal tubules. H & E stain, 40×.

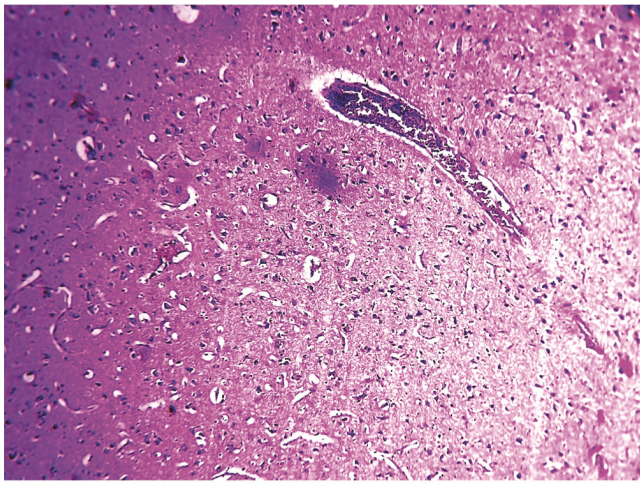

Fig. 3 — Brain section from a quinalphos-exposed bird showing dilatation of cerebral blood vessels. H&E stain, 40×.

and a summary of the semi-quantitative scores has been included to complement the photomicrographs.

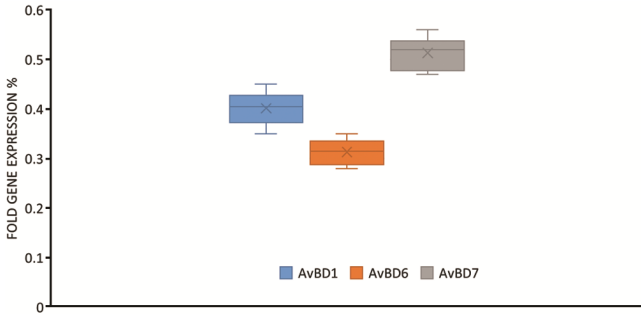

Fig. 4 — Box-and-whisker plot showing relative fold change (%) in mRNA expression levels of AvBD1, AvBD6, and AvBD7 in quinalphos-exposed birds compared to controls.

Gene expression

Quinalphos significantly decreased the expression of AvBD1, showing a fold change in gene expression of 4% with a notable 96.0% down-regulation. Additionally, AvBD6, associated with immune function, was markedly down-regulated by 96.9% with a fold gene expression of only 3.1%. Similarly, AvBD7, responsible for antimicrobial and immunomodulatory functions, showed substantial down-regulation of 94.9%, with a fold change in gene expression of only 5.1% in quinalphos-exposed Kamrupa birds (Fig. 4).

Discussions

Organophosphate (OP) exposure generally results in acute cholinergic symptoms such as salivation, lacrimation, urination, defecation, colic, emesis, and respiratory difficulty due to bronchoconstriction and increased bronchial secretions, typically appearing within minutes to hours[14]. In some cases, delayed effects may occur following either a severe initial phase or a symptom-free interval, beginning with neuromuscular weakness and later progressing to extrapyramidal signs[15] The primary toxic mechanism involves inhibition of carboxyl ester hydrolases, particularly acetylcholinesterase (AChE), responsible for hydrolyzing acetylcholine into choline and acetic acid; this leads to excessive acetylcholine accumulation and subsequent neurological, respiratory, and cardiovascular dysfunction[16].

Haemoconcentration due to diarrhoea and excessive salivation in quinalphos-exposed birds likely contributed to elevated haemoglobin, TEC, and TLC values, consistent with findings in mice administered a single intraperitoneal dose of quinalphos[17]. A reduction in lymphocyte percentage with a corresponding rise in heterophils reflects stress and immunosuppression induced by quinalphos-mediated tissue degeneration, predisposing treated birds to infection[18]. Similar patterns were documented in chlorpyrifos-fed layer birds, where lymphocyte counts decreased from 12 hours onward, while heterophils increased markedly from 24 hours until 36 hours of exposure[19].

Significant increases in ALT, AST, and alkaline phosphatase levels in quinalphos-treated birds indicate hepatocellular damage and leakage of enzymes into circulation. Acute chlorpyrifos toxicity also results in marked elevation of these liver enzymes, while chronic liver disease may show mild or no change. Comparable hepatic enzyme rises were reported in rats subjected to quinalphos exposure. Additionally, oxidative stress–mediated impairment of the free-radical scavenging system may further contribute to enzyme elevation, as previously noted in chlorpyrifos-exposed birds[18]. Dose-dependent increases in ALT and AST on day 28 were similarly observed in experimental studies on rats, attributed to varying degrees of liver degeneration[20].

Marked hyperuricemia in quinalphos-treated birds suggests substantial renal structural damage impairing filtration. Uric acid, the primary avian nitrogenous waste product, accumulates when nephron integrity is compromised. Earlier studies demonstrated podocyte vacuolization, mitochondrial damage in tubular epithelium, and heightened lysosomal activity[21]. Organophosphates are known to cause hydrogen peroxide accumulation and lipid peroxidation in renal tissues, contributing to acute tubular necrosis and renal dysfunction[20]. Disruption of hepatic lipid metabolism may also alter plasma cholesterol levels by affecting hepatocyte membrane permeability, bile duct patency, or lipoprotein lipase activity[22].

Histopathological changes such as mononuclear cell infiltration replacing necrotic hepatic tissue confirm liver injury due to quinalphos. Comparable lesions—including focal fatty alterations, eosinophil accumulation, sinusoidal dilation, and single-cell necrosis—were reported in Japanese quails exposed to malathion[23], and cypermethrin-treated rats showed severe hepatocyte vacuolation, vascular obstruction, and fibrosis[24]. Degenerative renal tubular changes with coagulative necrosis align with reports in albino rats exposed orally to cypermethrin for 30 days[25]. Cerebral congestion and vascular dilation observed in brain sections correspond to earlier findings where profenofos induced cellular infiltration, edema, vacuolation, and vascular congestion in Wistar rats[26].

Defensins are essential components of innate immunity and play a critical role in protecting hosts against bacteria, fungi, protozoa, and certain enveloped

viruses. In birds, only beta-defensins are present, making them particularly important for avian defense mechanisms. Avian Beta Defensin 1 (AvBD1) exhibits strong antibacterial action by disrupting pathogen membranes due to its cationic and conserved structure[27]. Along with its antimicrobial effects, AvBD1 regulates immune cell activity and supports mucosal integrity, with its expression modulated by infection and immune stimuli[28]. Therefore, the significant reduction in AvBD1 gene expression observed in quinalphos-treated birds strongly indicates compromised immune competence.

Similarly, Avian Beta Defensin 6 (AvBD6), a vital innate immune effector with broad-spectrum antibacterial activity, was also markedly downregulated following quinalphos exposure[29]. This reduction highlights that quinalphos may interfere with immune signaling pathways essential for pathogen defense. AvBD7 expression showed a comparable decline, reinforcing the conclusion that multiple immune-protective pathways are suppressed by quinalphos. These observations align with studies in free-range chickens exposed to DDT showing downregulation of immune-related genes, and with experiments using chlorpyrifos where phagocytic activity decreased dose-dependently, whereas chronic malathion exposure produced minimal innate immune suppression[30]. Organophosphates such as quinalphos are known to induce oxidative stress and disrupt key immune-regulatory pathways, particularly NF-κB signaling, which plays a central role in antimicrobial peptide transcription. Suppression of NF-κB activation, coupled with altered cytokine profiles—such as reduced IL-1β and TNF-α or increased anti-inflammatory cytokines—may collectively downregulate β-defensin gene expression. Additionally, pesticide-induced impairment of mononuclear phagocytic activity and epithelial immune function may further contribute to reduced defensin production. These potential pathways provide a plausible mechanistic basis for the observed β-defensin suppression and warrant focused investigation in future studies.

Collectively, the significant downregulation of AvBD1, AvBD6, and AvBD7 clearly demonstrates that quinalphos has a profound immunosuppressive impact on birds, weakening key antimicrobial defenses and heightening susceptibility to infections. These findings emphasize the broader biological risk posed by quinalphos, extending beyond toxicity to vital immune system impairment.

Limitations of the study

This study has several limitations that should be acknowledged. The sample size was relatively small, and only a single breed of chicken was used, which may limit the generalizability of the findings. The experiment focused solely on acute exposure to quinalphos at a single dose level, whereas chronic, low-dose exposures are more representative of field conditions. Additionally, qRT-PCR normalization was performed using only one housekeeping gene (ACTB), which may affect the robustness of gene expression quantification. These limitations should be addressed in future studies by including larger and more diverse populations, multiple exposure regimens, and additional reference genes.

Conclusion

Quinalphos at the dose rate of 22.5 mg/kg body weight produces significant toxicity symptoms; the haematological findings showed significant elevation, except for the lymphocyte count. All the biochemical parameters tested showed marked augmentation, with the exception of cholinesterase level, which is indicative of OP poisoning. Histopathological studies revealed severe toxic alterations in the liver, kidney, and brain. Gene expression study revealed critical down-regulation of the immunity genes in the order AvBD6>AvBD1>AvBD7. These findings underscore the vulnerability of Kamrupa chickens to quinalphos exposure and highlight their role as sentinel species for environmental toxicity. Future studies should explore chronic exposure effects and potential mitigation strategies, such as antioxidant supplementation, to reduce pesticide-induced toxicity. These results necessitate the urgent development of safer pest-control alternatives to protect poultry health, rural economies, and ecosystems from the adverse effects of organophosphate pesticides.

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Conflict of Interest: None

Authors' Contribution

A. Kafle and D. Roy conceptualized the research idea and design the research protocol. D. Deka and J. Sarma conducted the literature review and collected

data on pesticide usage. R. Nath and B.Dutta carried out the experimental methods, including toxicity evaluations of organophosphates and data interpretation. R.Saikia drafted the initial manuscript, while B. Borah critically revised the manuscript for intellectual content and scientific accuracy. K. Debbarma and S. Mohapatra contributed to the preparation of figures and tables while M. Chapagain contributed and interpreted the statistical analysis. All authors reviewed and approved the final version of the manuscript for submission.

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