Haematological and biochemical assay in sub chronic toxicity and ameliorative effects of Ziziphus nummularia leaves extract on wister rat

Ramya Rengaraj
National college (Autonomous) Affiliated to Bharathidasan University
Stalin Arumugam
stalinzoo@nct.ac.in

National college (Autonomous) Affiliated to Bharathidasan University
Praveen Kumar Dharmaraj
KIRND Institute Of Research and Development Pvt
Varadharajan Gokula
National college (Autonomous) Affiliated to Bharathidasan University

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Abstract

The present investigation was conducted to evaluate the recovery effect of *Ziziphus nummularia* leaf extract against hepato-renal toxicity induced by Quinolphos in Wistar rats. The study aimed to assess changes in biochemical and hematological parameters, including liver marker enzymes, renal functions, lipid profile parameters, and hematological indices. Thirty male rats were randomly divided into five groups: control, Quinolphos-induced, *Z. nummularia* high dose (500 mg/kg), *Z. nummularia* low dose (250 mg/kg), and vehicle control (300 mg/kg). Treatments were administered orally for four weeks. Quinolphos and *Z. nummularia* were given in increasing doses relative to body weight. By the end of the second and fourth weeks, rats treated with Quinolphos in combination with *Z. nummularia* extract (250, 300, and 500 mg/kg) showed significant improvements ($p \leq 0.05$) compared to the control group. In the lipid profile, rats receiving a high dose of *Z. nummularia* (500 mg/kg) exhibited significantly higher levels of LDL and HDL compared with controls. Biochemical analysis revealed that, by the end of the fourth week, activities of the liver marker enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) had significantly increased. Hematological studies showed elevated levels of lymphocytes and neutrophils, while basophil levels declined. Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were significantly higher in control animals (35 pg and 35 g/dL, respectively), with differences significant at the 0.05 level. However, no significant rise in triglycerides or total cholesterol was observed compared with controls. In conclusion, the present study demonstrates the hepatotoxic and renotoxic effects of Quinolphos, and suggests that *Z. nummularia* leaf extract may have protective or modulatory potential against such toxicity.

1. INTRODUCTION

Organophosphate pesticides are widely used in agriculture to control a broad range of pests. Among them, Quinalphos is a commonly employed compound noted for its high toxicity and environmental persistence. Its primary mode of action is the inhibition of acetylcholinesterase, which leads to the accumulation of acetylcholine at nerve synapses and consequent disruption of normal nervous system function (Kaur et al., 2017). Beyond neurotoxicity, the harmful effects of Quinalphos are largely mediated through the generation of reactive oxygen species (ROS), resulting in lipid peroxidation, enzyme inactivation, and cellular injury. Chronic exposure has been associated with oxidative stress, hepatotoxicity, nephrotoxicity, and hematological alterations (El-Shenawy, 2010; Rezg et al., 2010). In recent years, medicinal plants with antioxidant and anti-inflammatory properties have gained considerable attention as potential protective agents against pesticide-induced toxicity. *Ziziphus nummularia* (family: Rhamnaceae), traditionally used in Ayurvedic and Unani medicine, is known for its pharmacological activities, including hepatoprotective, nephroprotective, and antioxidant effects (Sharma et al., 2020). Its leaves contain diverse bioactive compounds such as flavonoids, alkaloids, saponins, and tannins, which contribute to free radical scavenging and cellular protection, particularly in liver and kidney tissues that are primary targets of toxic insult (Ahmad et al., 2014).

Experimental studies have further highlighted the therapeutic potential of *Z. nummularia* leaf extracts. Ethyl acetate fractions demonstrated strong antioxidant activity, achieving nearly 97% DPPH radical scavenging in vitro, largely attributed to flavonoid glycosides such as quercetin and kaempferol (Mesmar et al., 2022). Additionally, these extracts exhibited notable anti-inflammatory activity by suppressing TNF-α–induced NF-κB activation and adhesion molecule expression in human vascular cells. In rodent models, topical application reduced edema and promoted wound healing, further supporting its tissue-protective capacity (Fardoun et al., 2017). Given the reported hepatoprotective effects of related *Ziziphus* species, these findings justify exploring its efficacy against Quinalphos-induced liver and kidney toxicity. Therefore, the present study was designed to evaluate the protective effects of *Z. nummularia* leaf extract against Quinalphos-induced hepatic and renal toxicity in Wistar rats, with particular emphasis on biochemical and hematological parameters.

2. Materials and methods

2.1 Chemicals and Instruments

All chemicals and reagents used were of analytical grade. Ethanol, xylene, chloroform, May–Grünwald solution, Giemsa stain solution, Bouin's solution, haematoxylin, eosin, paraffin wax, sodium hydroxide, and distilled water were procured from HiMedia Ltd., Mumbai, India. Plant materials were dried and ground using a ventilation-assisted drying oven (1350FX, USA) and grinding mill (MX-335, Panasonic, Malaysia). Haematological tests were performed using an automated haematology analyser (Coulter T890, Beckman Coulter, Tokyo), and biochemical analyses were carried out on a Dade Behring Dimension RxL analyser (Siemens Healthcare, Camberley, UK).

2.2 Identification, Collection, and Extraction of Plant Material

Fresh leaves of *Ziziphus nummularia* (Burm.f.) Wight & Arn. were collected in January 2025 from Nallur, Thiruvarur district, Tamil Nadu, India. The plant was authenticated by Fr. L. John Peter Arulanandam SJ, St. Joseph's College, Tiruchirappalli. A voucher specimen (Accession No. SJCR.R001) was deposited at the Rapinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India (PIN 620002). Leaves were dried at a moderate temperature (3.0 kg batch), crushed, and powdered. Maceration of the powdered leaves was performed in ethanol (100 mg) for 72 h at room temperature. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator to obtain the crude extract (Zhang et al., 2018).

2.3 Experimental Animals

Male Wistar albino rats (6–8 weeks old, weighing 100–120 g) were procured from the National Institute of Nutrition (Hyderabad, India). Animals were maintained under standard laboratory conditions with a commercial pellet diet and water ad libitum. They were acclimatized for one week before experimentation. All procedures followed the methods described by Nguenang et al. (2020).

A total of 30 rats were divided into five groups (n = 6 per group). Control animals received distilled water via oral gastric intubation, while treatment groups (Groups II–V) received respective interventions for 28 consecutive days.

2.4 Experimental Design

The study design followed OECD guideline 407 (OECD, 2008). Rats were divided as follows: Group I – Control: Received distilled water (normal saline), Group II – Toxicant-induced: Received Quinalphos only, to induce hepato-renal toxicity, Group III – High-dose treatment: Received Quinalphos + *Z. nummularia* extract (400 mg/kg body weight), Group IV – Low-dose treatment: Received Quinalphos + *Z. nummularia* extract (200 mg/kg body weight), Group V – Vehicle control: Received Quinalphos + vehicle used for plant extract delivery, to exclude effects due to the solvent.

2.5 Sacrifice of Animals and Collection of Samples

At the end of the 28-day treatment (day 29), rats were anesthetized by intraperitoneal injection of a ketamine–xylazine cocktail (100 mg/kg ketamine + 12 mg/kg xylazine, ratio 10:1). Blood was collected by retro-orbital puncture into heparinised capillary tubes. Serum was separated by centrifugation at 3000 rpm for 10 min and stored at − 20°C until biochemical analysis. This preservation ensured reliable haematological and biochemical evaluation.

2.6 Biochemical Assays

Biochemical parameters were analyzed following Nguenang et al. (2020). Serum proteins, urea, creatinine, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and alkaline phosphatase (ALP) were measured using commercial kits (SPINREACT, Spain). Liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)] were determined by enzyme immunoassay (EIA) kits (Sigma-Aldrich, St. Louis, MO) following Reitman and Frankel (1957). Serum albumin was quantified, and renal function was assessed by measuring urea and creatinine levels (Bartels et al., 1972).

2.7 Haematological Parameters

Haematological indices were measured using an automated analyser (QBC Auto-Read Plus; Beckman Coulter JT series). Parameters included leukocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes, red blood cells (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), haematocrit (HCT), and red cell distribution width (RDW-CV and RDW-SD).

2.8 Red Blood Cell Indices

Red blood cell indices were calculated using Wintrobe's formulas (1929) with Hb (g/dL), packed cell volume (PCV, %), and RBC count (million/μL):

MCV (fL) = (PCV × 10) / RBC

MCH (pg) = (Hb × 10) / RBC

MCHC (g/dL) = (Hb × 100) / HCT (or PCV)

2.9 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using the General Linear Model in IBM SPSS software. Post-hoc comparisons were carried out with the Waller–Duncan test. Statistical significance was set at $p \leq 0.05$.

3. Results

This study evaluated the recovery from Quinolphos-induced toxicity following treatment with *Ziziphus nummularia* leaf extract. Biochemical assays assessed hepatic, renal, and lipid parameters, while haematological indices were also examined. Rats were divided into five groups (n = 6 each): control, induced, high dose (500 mg/kg), low dose (250 mg/kg), and vehicle (300 mg/kg).

3.1 Effect on Hepatic Function

3.1.1 Liver Marker Enzymes (ALT, AST, ALP)

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are summarized in Table 1 and Figs. 1–2.

Table 1 : ANOVA statistical analysis of liver functions test in rats recovery of chronic toxicity analysis in quinolphos induced ziziphus nummularia leaves extract

PARAMETERS	Mg/Kg				
	CONTROL VEHICLE	INDUCED 300	HIGH DOSE 500	LOW DOSE 250	
AST(U/L)	13.35± 3.0	10.88 ± 1.41	14.06±1.66	12.10±1.91	13.46±1.10
ALT(U/L)	15.1 ±3.4	13.4±0.8	16.4±3.53	16.5±2.05	16.9±2.45
ALP(U/L)	44.7±2.20	53.5 ± 3.75	52.2 ± 2.36	52.4 ±1.35	52.6 ± 8.74
Albumin(g/dl)	1.17 ± 0.14[b]	1.17 ± 0.02[b]	1.17 ± 0.3[b]	1.03 ± 0.1[b]	1.13 ± 0.09[b]
Globulin (g/dl)	1.78 ± 0.2[b]	2.26 ± 0.10[a]	1.33 ± 0.43[c]	1.60 ± 0.42[b]	1.68 ± 0.9[b]
Albu globulin Ratio(g/dl)	0.98±0.19[a]	0.75±0.02[a]	0.36±0.07[b]	0.32±0.18[b]	0.28±0.10b
Total protein(g/dl)	3.51 ± 0.05[b]	3.97 ± 0.13[a]	3.56 ± 0.81[b]	3.65 ± 1.33[b]	3.41 ± 0.83[b]
Direct bilirubin(mg/dl)	0.0567 ± 0.0351[b]	0.0667 ± 0.0058[b]	0.0700 ± 0.0200[b]	0.1333 ± 0.0231[a]	0.0767 ± 0.0116[b]
Indirect bilirubin (mg/dl)	0.8667 ± 0.0153[a]	0.6367 ± 0.1350[b]	0.5933 ± 0.0666[b]	0.5933 ± 0.2290[b]	0.7000 ± 0.1952[b]
Bilirubin total (mg/dl)	0.9200 ± 0.0500[a]	0.7000 ± 0.1400[b]	0.6633 ± 0.0862[c]	0.7267 ± 0.2136[b]	0.8767 ± 0.1950[a]

Table:1. Data represented as mean + SD (ANOVA)(n=6)[a] significant at p< 0.05, compared to control,[b] significant at p< 0.05 of the 500 mg/kg (high dose)treated groups compared to control and [c] significant at p< 0.05 of the 250 mg/kg (low dose)treated groups compared to control.,where p-value significant at p< 0.05

ALT

Quinolphos induction significantly reduced ALT activity (10.88) compared to the control (13.35, $p < 0.05$). High-dose treatment restored ALT to 14.06 ($p < 0.01$ vs. induced), comparable to control values ($p >$

0.05). The low-dose group (12.10) showed partial recovery, while the vehicle group (13.46) was similar to control.

AST

The induced group recorded a significant decline (13.40 ± 0.46) compared with control (15.10 ± 1.96, $p < 0.05$). Both high-dose (16.40 ± 2.04) and low-dose (16.56 ± 1.18) treatments significantly improved AST activity ($p < 0.01$ vs. induced), approaching vehicle values (16.90 ± 1.41). No significant differences were observed between treatment and vehicle groups ($p > 0.05$).

ALP

A slight elevation was observed in the induced group (53.55) compared to control (52.70), but this was not significant ($p > 0.05$). High-dose (52.20) and low-dose (52.40) treatments normalized ALP activity, with no differences from control or vehicle ($p > 0.05$).

Quinolphos altered liver enzyme activities, while *Z. nummularia* extract, particularly at the high dose, restored ALT and AST levels toward normal, with ALP remaining largely unaffected.

3.1.2 Serum Protein Profile (Albumin, Globulin, Total Protein, A/G Ratio)

Table 1 presents the effects of Quinolphos and *Z. nummularia* on serum protein levels.

Albumin

Quinolphos induction significantly increased albumin (1.72 ± 0.05 g/dL) vs. control. High-dose treatment restored levels to 1.23 ± 0.05 g/dL ($p < 0.05$ vs. induced), comparable to control.Low dose (1.03 ± 0.05 g/dL) showed partial recovery.

Globulin

The induced group showed elevated globulin (2.26 ± 0.06 g/dL) vs. control. High dose significantly reduced globulin (1.33 ± 0.06 g/dL; $p < 0.001$ vs. induced). Low dose showed moderate improvement (1.60 ± 0.06 g/dL).

Total Protein

Quinolphos induction elevated total protein (3.97 ± 0.07 g/dL). High dose (3.56 ± 0.07) and low dose (3.65 ± 0.07) normalized values close to control.

A/G Ratio

Both high-dose (0.36 ± 0.07) and low-dose (0.32 ± 0.18) groups showed marked decreases in the A/G ratio compared to control and induced groups ($p < 0.01$).

Treatment with the high dose markedly restored these parameters toward normal values, with albumin (1.23 ± 0.05 g/dl) and total protein (3.56 ± 0.07 g/dl) being comparable to the control group, while globulin levels were significantly reduced (1.33 ± 0.06 g/dl; $p < 0.001$ compared to induced). The low dose group showed partial improvement, recording albumin (1.03 ± 0.05 g/dl), globulin (1.60 ± 0.06 g/dl), and total protein (3.65 ± 0.07 g/dl), which were significantly lower than the induced group but remained above high dose values. The vehicle group did not differ significantly from control.In albu globulin ratio g/dl where both the High Dose (0.36 ± 0.07) and Low Dose (0.32 ± 0.18) groups showed a marked decrease in the A/G ratio, indicating significant changes compared to both Control and Induced groups ($p < 0.01$).

3.1.3 Bilirubin Levels (Direct, Indirect, Total)

Direct Bilirubin

Only the low-dose group showed a significant elevation (0.1333 ± 0.0231 mg/dL; $p < 0.01$ vs. control). High dose (0.0700 ± 0.0200) and vehicle (0.0767 ± 0.0116) remained comparable to control.

Indirect Bilirubin

Induced, high-dose, low-dose, and vehicle groups all showed significant declines compared to control ($p < 0.01$–0.05), suggesting altered bilirubin metabolism.

Total Bilirubin

Both induced and treatment groups recorded reductions compared with control, except the vehicle group, which remained statistically similar.

3.2 Renal Function Parameters (Urea, Creatinine, Uric Acid, Glucose)

Urea

Serum urea levels ranged from 15.40 ± 1.60 to 19.03 ± 2.92 mg/dL across groups. No significant differences were observed ($p > 0.05$).

Creatinine

Levels remained stable (0.70–0.80 ± 0.10 mg/dL) with no significant differences across groups ($p > 0.05$).

Glucose

Serum glucose levels varied widely (33.05 ± 5.55 to 61.33 ± 24.17 mg/dL). The vehicle group showed a significant increase compared to control.

In Table 2 Urea levels are not significantly different among groups. The mean serum urea levels ranged from 15.40 ± 1.60 mg/dL **to** 19.03 ± 2.92 mg/dL across the experimental groups. It is indicated that the differences were not statistically significant ($p > 0.05$). This suggests that quinolphos exposure and treatment alter urea levels. Creatinine is stable, no significant effect across groups. Serum creatinine values were maintained between 0.70 ± 0.10 mg/dL and 0.80 ± 0.10 mg/dL across groups. No significant difference was detected among groups compared to control **(**$p > 0.05$**).** Glucose is significantly higher in vehicle compared to control. Serum glucose levels varied more widely, ranging from 33.05 ± 5.55 mg/dL to 61.33 ± 24.17 mg/dL.

Table 2
ANOVA statistical analysis of renal functions test in rats chronic recovery of toxicity analysis in quinolphos induced ziziphus nummularia leaf extract

Parameter	Control	Quinolphos Induced	High Dose (500 mg/kg)	Low Dose (250 mg/kg)	Vehicle
Urea (mg/dL)	18.1 ± 2.7	15.4 ± 1.6	15.6 ± 2.3	18.0 ± 4.5	19.0 ± 2.9
Creatinine (mg/dL)	0.70 ± 0.10	0.80 ± 0.10	0.70 ± 0.10	0.73 ± 0.15	0.70 ± 0.10
Uric Acid (mg/dL)	1.65 ± 0.15	1.25 ± 0.05	1.60 ± 0.30	1.57 ± 0.25	1.53 ± 0.38
Glucose (mg/dL)	33.8 ± 17.9	33.1 ± 5.6	35.3 ± 16.9	61.3 ± 24.2	41.2 ± 14.8
Table:2 Data represented as mean + SD (ANOVA)					
[a] significant at $p < 0.05$, compared to control					
[b] significant at $p < 0.05$ of the 500 mg/kg (high dose)treated groups compared to control					
[c] significant at $p < 0.05$ of the 250 mg/kg (low dose)treated groups compared to control.,where p-value significant at $p < 0.05$					

3.3 Lipid Profile Parameters

Treatment with *Z. nummularia* extract significantly improved HDL levels ($p < 0.05$), particularly in the high-dose group (500 mg/kg). The low-dose group (250 mg/kg) also showed significant improvement. LDL/HDL and TC/HDL ratios approached normal in extract-treated groups compared to Quinolphos-induced rats (Table 3, Fig. 3).

Table 3
ANOVA statistical analysis of lipid parameters in rats chronic recovery of toxicity analysis in quinolphos induced ziziphus nummularia leaf extract

Parameter	Control	Quinolphos Induced	High Dose (500 mg/kg)	Low Dose (250 mg/kg)	Vehicle
Total Cholesterol (mg/dL)	136.0 ± 12.0	124.0 ± 5.0	153.0 ± 17.1	157.7 ± 10.5	149.7 ± 17.5
Triglycerides (mg/dL)	111.0 ± 22.0	117.5 ± 5.5	89.7 ± 12.0	138.3 ± 16.9	106.0 ± 8.2
HDL (mg/dL)	13.4 ± 2.2	21.2 ± 2.3	19.6 ± 7.6	25.6 ± 6.2	22.7 ± 3.8
LDL (mg/dL)	100.4 ± 18.6	79.4 ± 3.9	115.5 ± 24.1	104.4 ± 9.0	105.7 ± 13.0
VLDL (mg/dL)	22.2 ± 4.4	23.5 ± 1.1	17.9 ± 2.4	27.7 ± 3.4	21.2 ± 1.6
TC/HDL Ratio	10.6 ± 2.6	5.9 ± 0.4	8.7 ± 3.5	6.4 ± 1.4	6.5 ± 0.2
Non-HDL (mg/dL)	122.6 ± 14.2	102.9 ± 2.8	133.4 ± 24.6	132.1 ± 8.7	126.9 ± 13.7
LDL/HDL Ratio	7.9 ± 2.7	3.8 ± 0.2	6.7 ± 3.2	4.3 ± 1.3	4.7 ± 0.3

Table 3 : Data represented as mean + SD (ANOVA)(n=6).

[a] significant at p< 0.05, compared to control

[b] significant at p< 0.05 of the 500 mg/kg (high dose)treated groups compared to control

[c] significant at p< 0.05 of the 250 mg/kg (low dose)treated groups compared to control.,where p-value significant at p< 0.05

3.4 Haematological Indices

As shown in Table 4, high-dose treatment significantly increased Hb, RBC, PCV, and platelet counts compared with control ($p < 0.05$). Quinolphos exposure altered leukocyte distribution: neutrophils increased ($p < 0.05$), lymphocytes rose ($p > 0.05$), and eosinophils, monocytes, and basophils declined significantly ($p < 0.05$ vs. control; Fig. 6).

Table 4
Effect of Haematological indices in Quinolphos induced Ziziphus nummularia leaves extract ;

Group	Hb (g/dL)	PCV% RBC (×10⁶/µL)	WBC (cells/µL)	Platelets (×10³/µL)
Control	6.97 ± 0.46	19.90 ± 2.09[a] 1.99 ± 0.66	12,533	332 ± 45.54
Induced	8.13 ± 0.46*	27 ± 0.46[b*] 2.71 ± 0.15*	9,600 *	333 ± 60.61
High Dose	7.27 ± 1.0	24.33 ± 1.0[bc] 2.42 ± 0.33*	8,900 ± 721*	384 ± 34.67*
Low Dose	7.60 ± 0.96	25.33 ± 0.96[bc] 2.53 ± 0.32	12,467	400 ± 27.07**
Vehicle	6.83 ± 0.11	22.77 ± 0.12[ac] 2.28 ± 0.04	11,000	356 ± 62.32

Values are expressed as Mean ± SD (n = 6).p < 0.05 considered statistically significant.Superscripts (a, b, c) indicate statistical comparisons where a: not significantly different from Control,b: significant compared to Control (p < 0.05) c: significant compared to Induced (p < 0.05): p < 0.05, ** : p < 0.01

Z. nummularia extract moderated these effects. The low-dose group reduced neutrophil elevation (p < 0.05 vs. induced) and restored lymphocyte levels toward normal. The high-dose group prevented further deterioration but did not fully normalize values. The vehicle group remained comparable to control (not significant).

3.4.1 Red Blood Cell Indices (MCV, MCH, MCHC)

MCV

All groups remained at ~ 100 fL; no significant differences were observed (p > 0.05).

MCH & MCHC: Control animals recorded significantly higher values (MCH: 35 pg; MCHC: 35 g/dL). Quinolphos induction caused a marked reduction (30 pg; 30 g/dL, p < 0.05 vs. control).

4. Discussion

Ziziphus species, including *Z. nummularia*, are generally regarded as non-toxic at therapeutic doses, although further rigorous toxicity studies are required to confirm their safety profile. Previous investigations have not reported target-organ toxicity or mortality associated with *Z. nummularia* at minimal dosing (El Maaiden et al., 2020). The phytochemical composition of *Z. nummularia* leaf extract includes terpenoids, flavonoids, and alkaloids, which are known to possess pharmacological and phytopharmaceutical significance. A recent study (Imran et al., 2024) demonstrated that fractionated

ethanolic leaf extracts exhibit strong antioxidant and anticancer properties, particularly against triple-negative breast cancer cells.

In the present study, quinolphos exposure altered liver enzyme activity, while *Z. nummularia* leaf extract—particularly at the high dose—significantly restored ALT and AST levels, with ALP remaining largely unaffected. There was significant decrease observed in serum albumin and total protein level of rat in lead acetate only compared to control.In contract other than certain rats treated with DSALME-MD there was a significant increase level compared control is investigated in (E.F Isoje et.,al 2025). This hepatoprotective effect is consistent with the observed normalization of lipid parameters. Since reductions in HDL-C and elevations in TG/VLDL-C are markers of atherogenic risk, reversal of these alterations suggests that *Z. nummularia* may mitigate cardiometabolic risk secondary to toxicant exposure. While rodent lipid values cannot be directly extrapolated to human clinical risk, the trend toward normalization strongly indicates hepatoprotection.

Quinolphos exposure also significantly impaired renal function, as evidenced by elevated serum urea and creatinine. Treatment with *Z. nummularia* leaf extract mitigated these changes in a dose-dependent manner, with the 500 mg/kg dose demonstrating superior renoprotective effects. These findings are consistent with earlier reports where extracts from other *Ziziphus* species (*Z. jujuba*, *Z. mauritiana*) reduced urea and creatinine in nephrotoxicity models (Alsayari et al., 2022; Labban, 2013). Antioxidant-rich plants are known to counteract renal injury (Kumar et al., 2021), and our results extend this protective role to *Z. nummularia* leaves. Importantly, creatinine is considered a more precise biomarker of renal function compared to urea, which may vary with diet and exercise (Ebohon et al., 2020). Quinolphos exposure significantly impaired renal function as evidenced by increased serum urea and creatinine(Table 3). Treatment with *Z. nummularia* leaf extract mitigated these alterations in a dose-dependent, with 500 mg/kg showing superior protective effects. Artesunate treatment for 45 days significantly increased glucose where non- significant effect on urea and creatinine (P.Bigoniya et al., 2015). The findings indicate that *Z. nummularia* leaf extract exerts a renoprotective effect against quinolphos-induced toxicity), indicating preserved renal clearance capacity Their normalization by *Z. nummularia* suggests that the extract may safeguard the kidneys against toxicant-induced injury, potentially lowering risk of chronic renal impairment.

Our results extend these observations to *Z. nummularia* leaves, highlighting a dose-dependent renoprotective effect. Renal dysfunction is evaluated using signs such as urea, creatinine, and electrolyte measurements. However, compared to blood urea nitrogen, the creatinine test is a more precise indicator of renal impairment. This is due to the fact that a high-protein diet and exercise might affect blood urea levels (Ebohon,O et al.,2020).Overall, high dose treatment demonstrated the most effective normalization of serum protein markers, indicating a protective effect against quinolphos-induced alterations.

Lipid profile analysis further supports the protective potential of *Z. nummularia*. Quinolphos exposure produced dyslipidemia, characterized by increased TC, LDL-C, and non-HDL cholesterol, alongside

decreased HDL-C ($p < 0.05$ vs. control). Treatment with *Z. nummularia* leaf extract improved these indices in a dose-dependent manner, with the high-dose group showing the strongest recovery. These effects are attributed to bioactive flavonoids and saponins, which enhance HDL-C and reduce LDL-C levels (Kumar et al., 2021; Alsayari et al., 2022). Similar protective effects of antioxidant-rich plant extracts against organophosphate-induced dyslipidemia have been reported previously (Patel et al., 2021). Total Cholesterol (TC): Quinolphos induction increased TC compared with control (124 increases in 153 mg/dL). Administration of *Z. nummularia* extract, particularly at high dose, tended to reduce cholesterol toward near-normal values (157.7 in induced when compared to 144.0mg/dL in treated).Triglycerides (TG): Induced rats showed fluctuations, with a notable reduction compared to control (111 directly increases to 89.6 mg/dL). Treatment with *Z. nummularia* extract normalized TG levels (138.3 in high doses compared to 106.0 in vehicle), showing recovery toward physiological levels. The sub chronic oral toxicity investigation of plant showed dose dependent variation LDL cholesterol in rats treated with crude extract of H.roeperianum (ngaffo et al., 2022) statistically signicant level were observed.

Quinolphos exposure also produced hematological alterations. Significant leukocyte redistribution was observed, with increased neutrophils and lymphocytes, reflecting systemic inflammation and immune stress. High- and low-dose *Z. nummularia* treatment groups demonstrated partial to near-complete normalization of leukocyte counts, with the low dose showing slightly better lymphocyte recovery. These results align with previous findings of organophosphate-induced immunotoxicity (Acharya et al., 2024). RBC indices (MCV, MCH, and MCHC) revealed hypochromia in the induced group, with treatment unable to fully restore values to control levels. Nonetheless, Z. nummularia treatment improved erythropoietic balance and platelet counts, suggesting hematopoietic modulation. Similar effects have been observed in other plant-based interventions (Lodish et al., 2010; Yakubu et al., 2024). Mean Corpuscular Volume (MCV) All groups remained at ~ 100fL non-significant difference indicating a normocytic profile across treatments and induced conditions. MCH & MCHC which is Control animals had significantly higher MCH (35 pg) and MCHC (35 g/dL). Induced group showed a marked reduction (30 pg / 30 g/dL, $p < 0.05$), consistent with hypochromia due to quinolphos toxicity(Table 5). Both high and low dose treatment groups, as well as the vehicle group, maintained values closer to the Induced group rather than Control. This suggests that while treatment modulated RBC and Hb counts, it did not completely restore RBC indices.

Table 5
Mean Corpuscular Volume (MCV),Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) in quinolphos induced Ziziphus nummularia leaves extract

Group	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control	100.0 ± 0.2[a]	35.01 ± 0.43[a]	35.01 ± 1.20[a]
Induced	100.0 ± 0.1[a]	30.01 ± 0.23[b]*	30.01 ± 1.30[b]*
High Dose	100.0 ± 0.2[a]	29.99 ± 0.33[b]*	29.99 ± 0.2[b]*
Low Dose	100.0 ± 0.3[a]	30.00 ± 0.34[b]*	30.00 ± 1.7[b]*
Vehicle	100.0 ± 0.7[a]	30.01 ± 0.34[b]*	30.01 ± 0.5[b]*

Values expressed as Mean ± SD(ANOVA).[a]: no significant change compared to Control,[b]: significant compared to Control ($p < 0.05$) *: where p-value is significant at < 0.05, MCV = Mean Corpuscular Volume (MCV),Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)

In (Table 4) Induced group showed a significant rise in RBC, possibly due to compensatory erythropoiesis. Treatment (high dose more than low dose) reduced RBC values closer to normal, suggesting protective effect. Total RBC, haemoglobin, platelets, lymphocytes, and mean corpuscular haemoglobin concentration have not changed. Significant increases in WBC, neutrophil, eosinophil, packed cell volume, and mean cell haemoglobin were observed at the 8mg/kg/day dose level ($p < 0.01$))(P.Bigoniya et al., 2015).

In (Fig. 6) Treatment with *Z. nummularia* leaf extract demonstrated a dose-dependent corrective effect is the high-dose group (500 mg/kg) exhibited near-control values in both neutrophils and lymphocytes, while the low-dose group showed moderate improvement. Minor variations in eosinophils and basophils were observed but remained within physiological ranges. Quinolphos-induced group which shows neutrophilia and lymphocytopenia of stress-induced leukocyte shift, consistent with systemic inflammation and immunotoxicity. High- and low-dose *Z. nummularia* extract Improved lymphocyte levels. Reduced neutrophil rise compared with induced group

Taken together, the hepatoprotective, renoprotective, hypolipidemic, and immunomodulatory effects observed in this study indicate that *Z. nummularia* extract can effectively counteract quinolphos-induced toxicity.

Conclusion

This study highlights the importance of integrating biochemical and hematological indices to assess systemic toxicity and therapeutic interventions. Parameters such as RBC, Hb, WBC, and platelet counts provided insight into oxygen transport, immune regulation, and hemostasis, while liver and kidney function markers (ALT, AST, ALP, urea, creatinine, electrolytes) revealed the metabolic integrity of vital organs. The ethanolic extract of *Ziziphus nummularia* leaves demonstrated a significant protective effect

against quinolphos-induced hepato-renal toxicity and dyslipidemia in rats. The high dose (500 mg/kg) consistently showed stronger recovery trends compared to the low dose (250 mg/kg), indicating a clear dose-dependent response. Importantly, no lethality or severe adverse effects were observed at the tested concentrations, suggesting a favorable safety profile. Unlike previous works that focused on isolated endpoints, this study provides a multiparametric evaluation of hepato-renal protection, lipid regulation, and hematological balance. The results highlight the immunomodulatory potential of *Z. nummularia* in mitigating quinolphos-induced systemic toxicity. Overall, this research adds novel mechanistic evidence supporting *Z. nummularia* as a promising natural candidate for preventing or alleviating organ damage caused by environmental toxicants. Further studies—particularly chronic toxicity and mechanistic pathway analyses—are warranted to translate these findings into clinical applications.

Abbreviations

Parameter	Abbreviation
Alanine Aminotransferase	ALT / SGPT
Aspartate Aminotransferase	AST / SGOT
Alkaline Phosphatase	ALP
Globulin	GLOB
Albu globulin Ratio	A/G Ration
Cholesterol	TC
High-Density Lipoprotein	HDL-C
Low-Density Lipoprotein	LDL-C
Very Low-Density Lipoprotein	VLDL-C
White Blood Cell Count	WBC
Red Blood Cell Count	RBC
Hemoglobin	Hb
Hematocrit	HCT / PCV
Mean Corpuscular Volume	MCV
Mean Corpuscular Hemoglobin	MCH
Mean Corpuscular Hemoglobin Concentration	MCHC
Platelet Count	PLT
Neutrophils	NEUT / ANC
Lymphocytes	LYM
Monocytes	MONO
Eosinophils	EOS
Basophils	BASO

Declarations

Ethical Approval:

The research/study was approved by the IAEC of J'J College of Arts and Science (Autonomous), Pudukkottai, number **JJC/BC/AH/010/2025** dated May 2025.

Consent for Publication -Not applicable.

Consent for Participation -Not applicable

Availability of data:

The datasets generated and/or analyzed during the current study are available from the corresponding author .

Author contribution statement

RR led the research design, analysis, and manuscript preparation. AS and DP supervised the methodology GV contributed to revisions, supported literature review and result interpretation.

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Conflict of interest

The authors declare no conflict of interest

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Figures

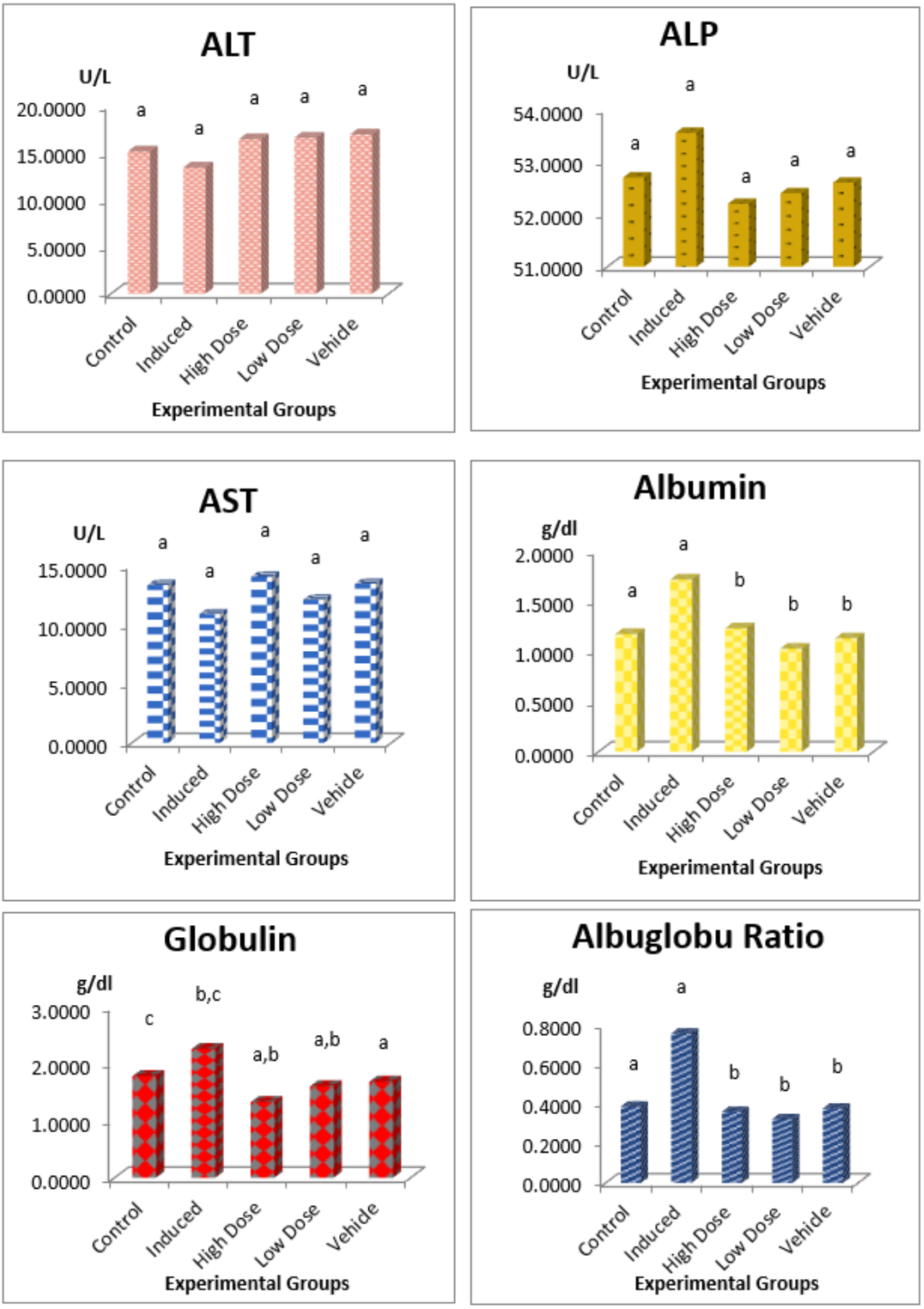

Figure 1

effect of quinolphos induced ziziphus nummularia leaves extract in ALT, ALP,AST, Albumin, globulin and albu globulin ratio among five experimental groups containing each six in numbers (n=6) following 28 days of oral administration as mean + SD (ANOVA). [a] significant at p< 0.05, compared to control .[b] significant at p< 0.05 of the 500 mg/kg (high dose)treated groups compared to control. [c] significant at p<

0.05 of the 250 mg/kg (low dose)treated groups compared to control.,where p-value significant at p< 0.05

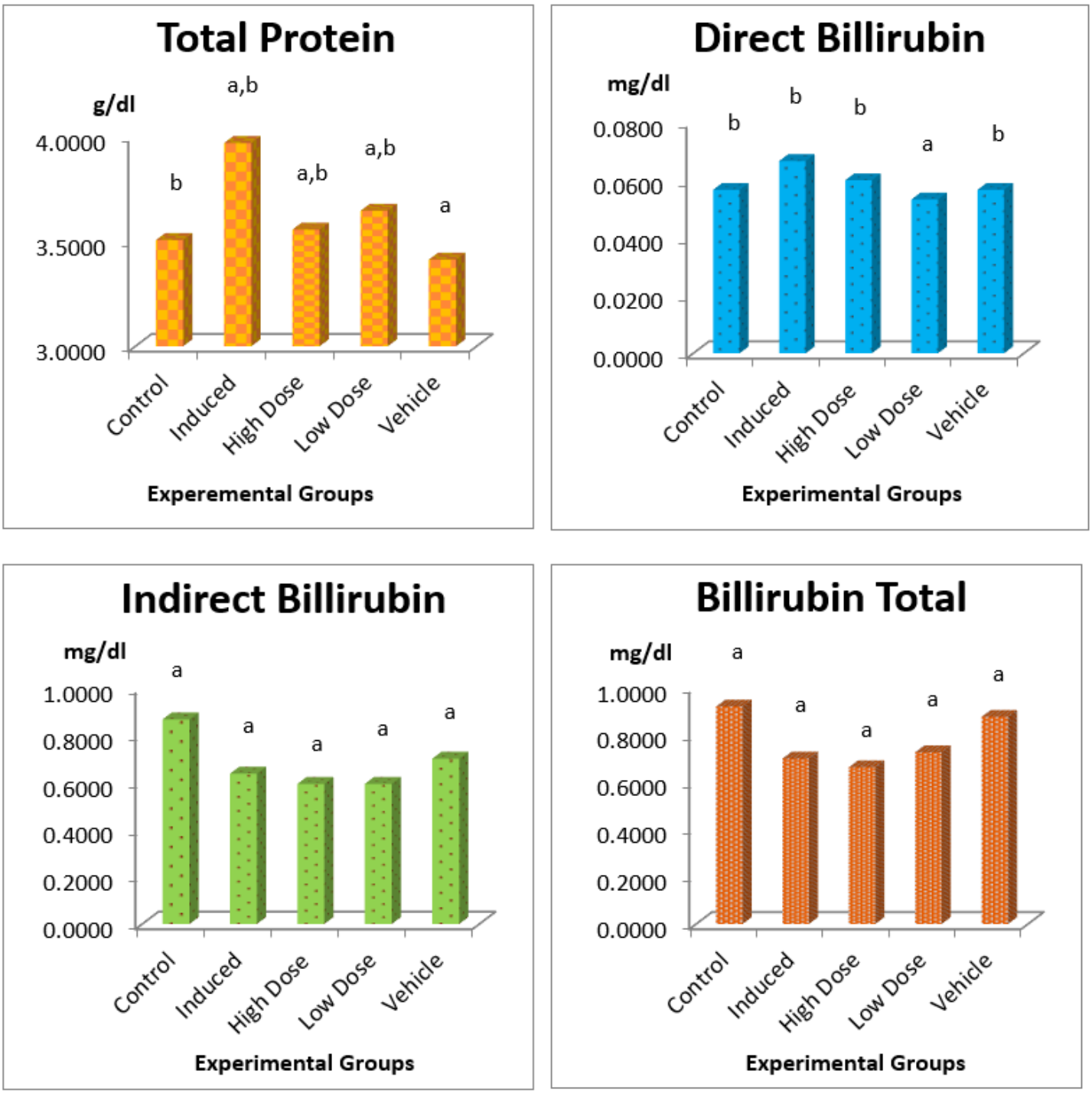

Figure 2

Effect of chronic toxicity analysis in quinolphos induced ziziphus nummularia leaves extract in total protein,Direct bilirubin,indirect bilirubin and bilirubin total among five experimental groups containing each six in numbers (n=6) following 28 days of oral administration as mean + SD (ANOVA). [a] significant at p< 0.05, compared to control .[b] significant at p< 0.05 of the 500 mg/kg (high dose)treated groups

compared to control. [c] significant at p < 0.05 of the 250 mg/kg (low dose)treated groups compared to control, where p-value significant at p < 0.05

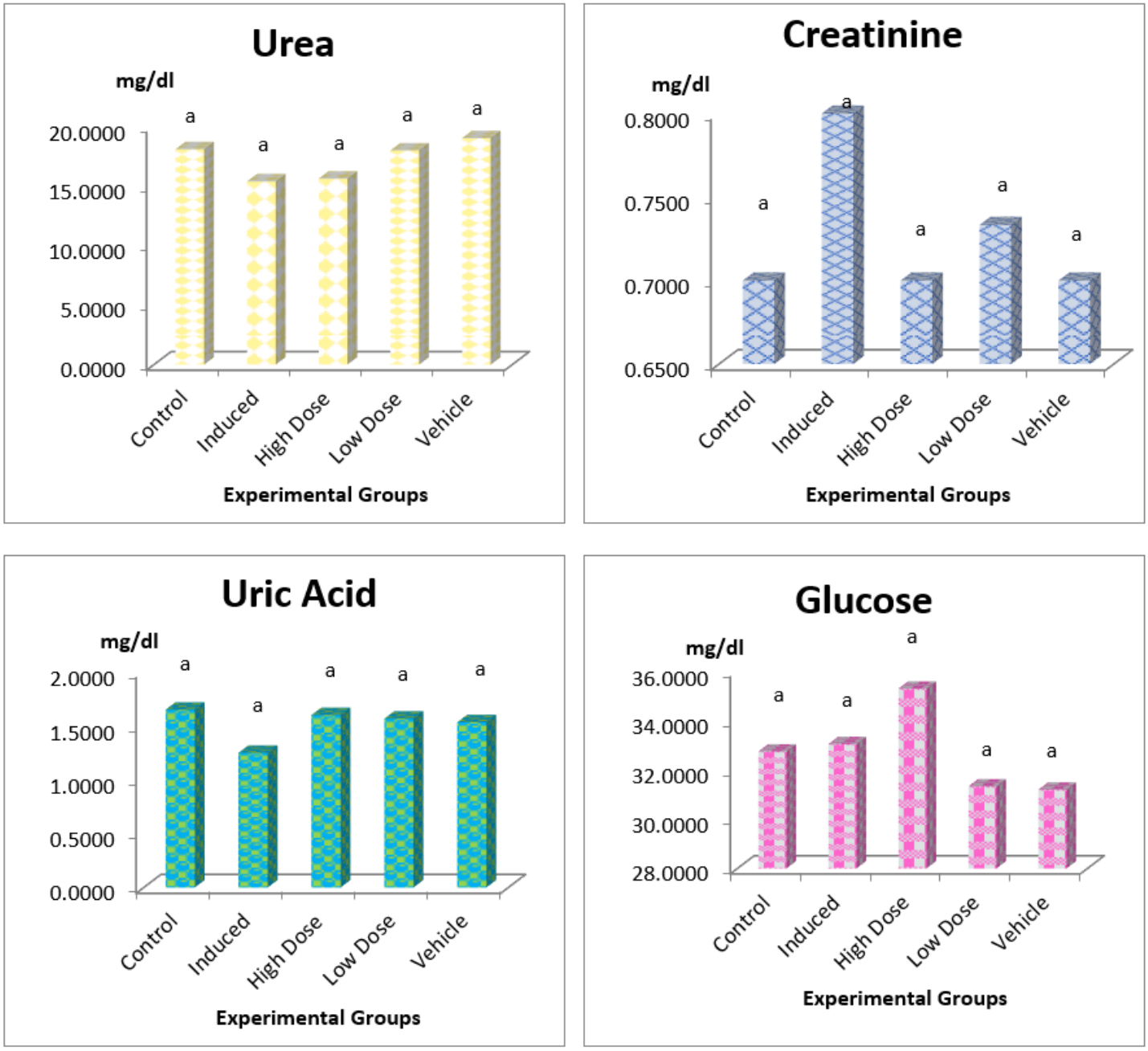

Figure 3

effect of quinolphos induced ziziphus nummularia leaves extract in Urea, creatinine, uric acid and glucose among five experimental groups containing each six in numbers (n=6) following 28 days of oral administration as mean + SD (ANOVA). [a] significant at p< 0.05, compared to control .[b] significant at p< 0.05 of the 500 mg/kg (high dose)treated groups compared to control. [c] significant at p< 0.05 of the 250 mg/kg (low dose)treated groups compared to control, where p-value significant at p< 0.05

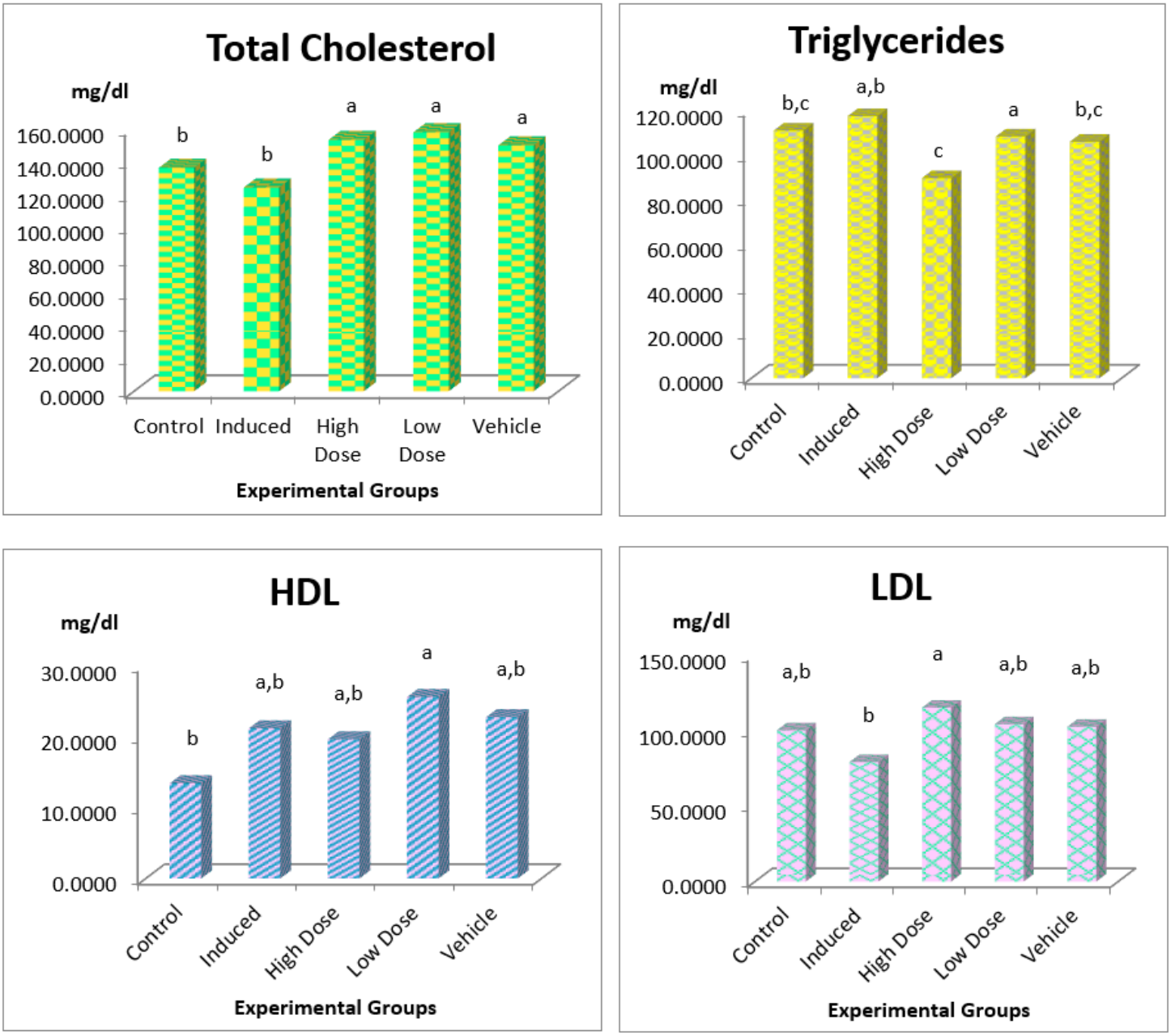

Figure 4

effect of quinolphos induced ziziphus nummularia leaves extract in lipid serum parameters Total cholesterol, triglycerides, HDL and LDL among five experimental groups containing each six in numbers (n=6) following 28 days of oral administration as mean + SD (ANOVA). [a] significant at p< 0.05, compared to control .[b] significant at p< 0.05 of the 500 mg/kg (high dose)treated groups compared to control. [c] significant at p< 0.05 of the 250 mg/kg (low dose)treated groups compared to control, where p-value significant at p< 0.05

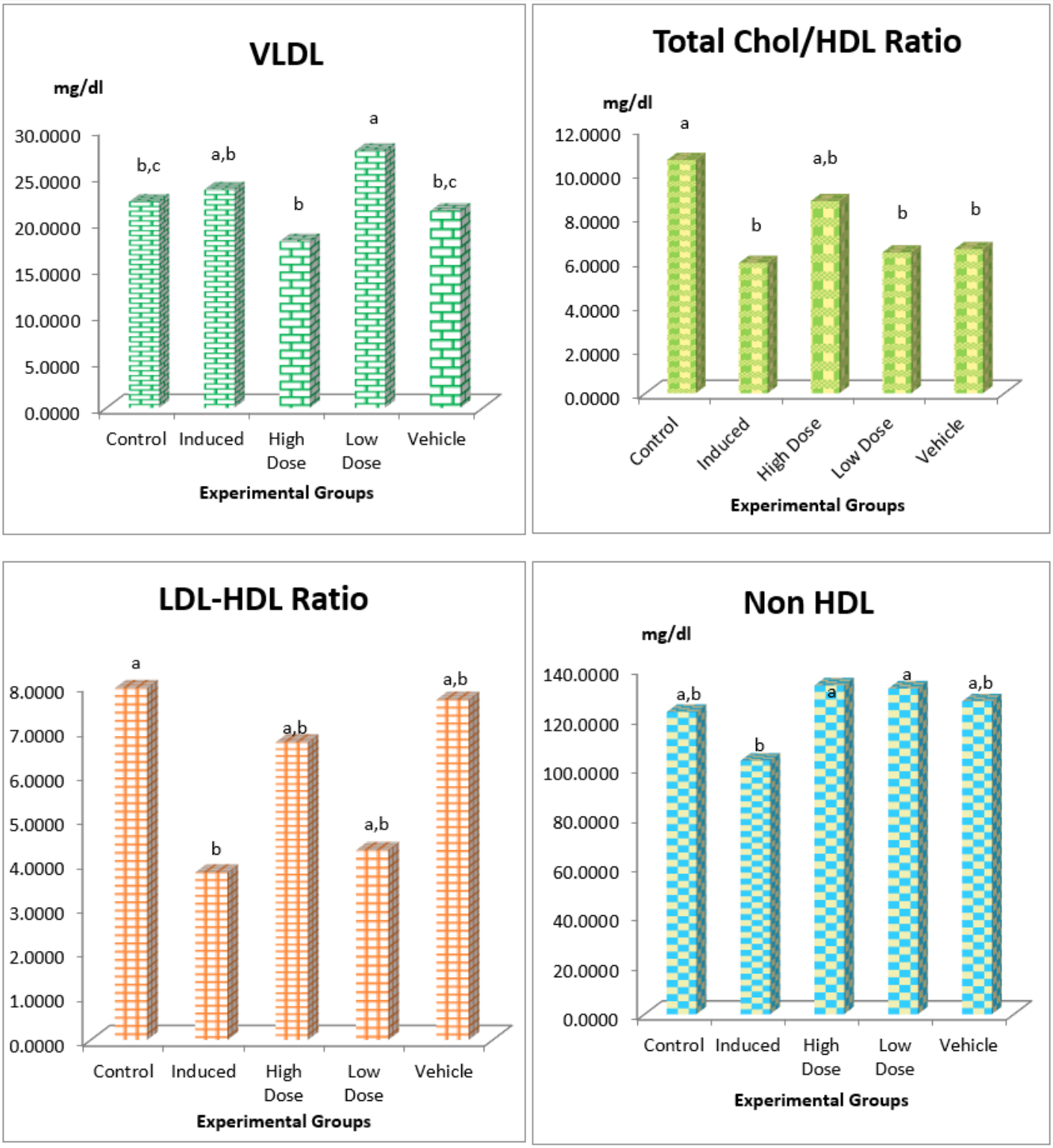

Figure 5

effect of Quinolphos induced Ziziphus nummularia leaves extract in lipid serum parameters VLDL, total chol/HDL ratio, LDL-HDL ratio and Non HDL among five experimental groups containing each six in numbers (n=6) following 28 days of oral administration as mean + SD (ANOVA). [a] significant at p< 0.05, compared to control .[b] significant at p< 0.05 of the 500 mg/kg (high dose)treated groups compared to

control. [c] significant at p < 0.05 of the 250 mg/kg (low dose)treated groups compared to control, where p-value significant at p< 0.05

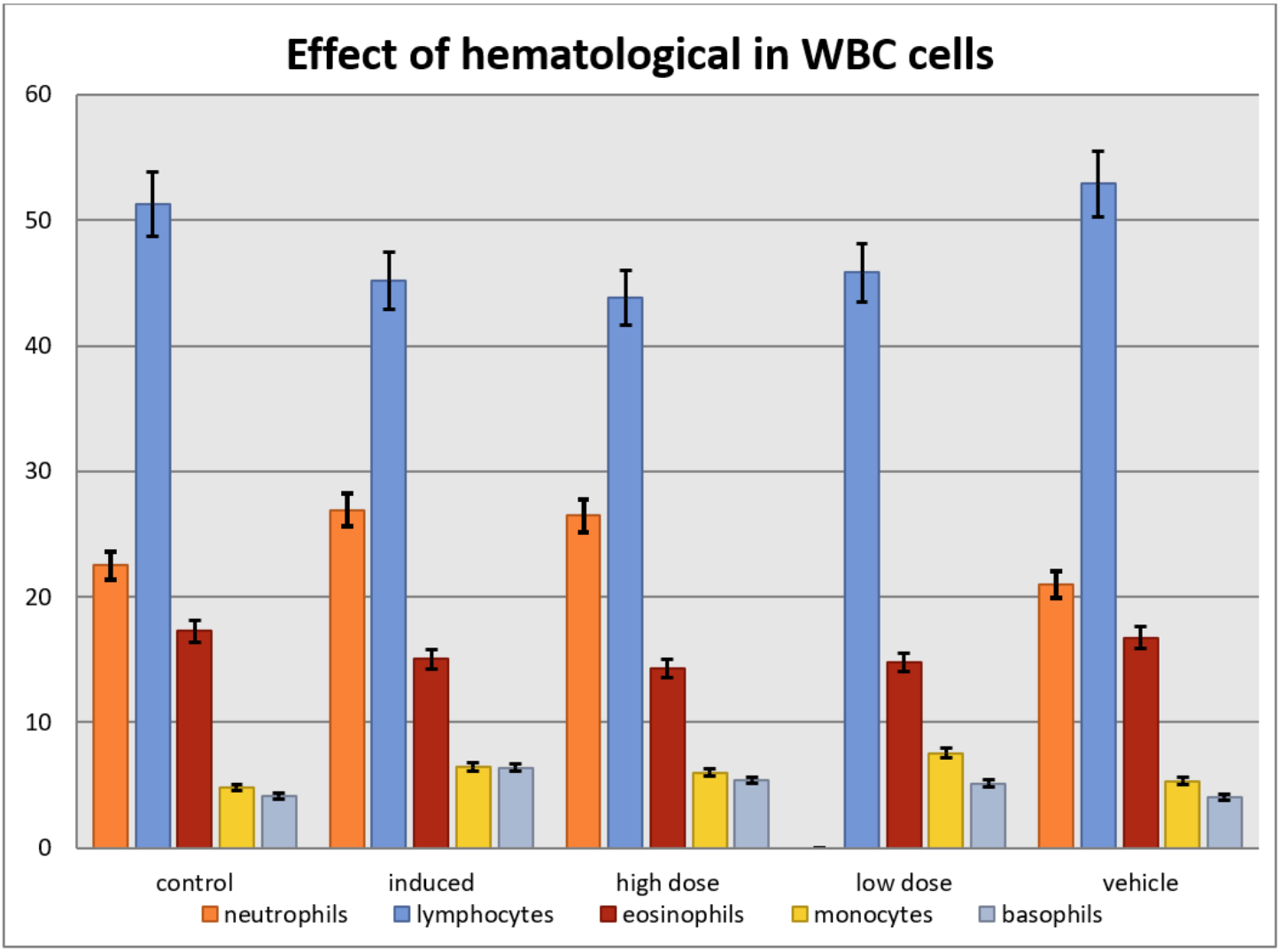

Figure 6

Effect of Haematological indices in quinolphos induced ziziphus nummularia leaves extract

WBC – (Monocytes,Lymphocytes,Basophils,Eosinophils and Neutrophils) in %

Supplementary Files

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