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DELTAMETHRIN-INDUCED CHANGES IN GROWTH, HEMATOLOGICAL RESPONSES, AND OXIDATIVE STRESS LEVELS IN *Gallus gallus domesticus*

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ABSTRACT
Deltamethrin, a type-II pyrethroid and synthetic-cyno pyrethroid, is extensively employed as an insecticide in agricultural and pest management practices. This study aims to investigate the toxicological impact of deltamethrin exposure on *Gallus gallus domesticus*, focusing on hematological, biochemical, histopathological, and growth parameters. *Gallus gallus domesticus* (chickens) were divided into four groups, including a control group and three experimental groups exposed to increasing doses of deltamethrin. Hematological parameters, biochemical markers, oxidative stress biomarkers, and histopathological changes in the liver were assessed. The study also examined the influence of deltamethrin on growth parameters, including weight and length. The study revealed significant adverse effects of deltamethrin exposure on various parameters in *Gallus gallus domesticus*, including hematological, biochemical, histopathological, and growth parameters. Dose-dependent reductions in hemoglobin, red blood cells, and hematocrit, coupled with increased oxidative stress biomarkers and liver enzyme levels, indicated potential toxicity. Histopathological examinations underscored substantial liver damage. Moreover, a dose-dependent impact on growth parameters highlighted significant weight and length reduction in exposed chickens. These findings emphasize the need for use of deltamethrin in poultry farming and the development of sustainable pest management practices to safeguard both poultry health and human consumers.

Keywords: Deltamethrin, *Gallus gallus domesticus*, pyrethroid, toxicological impact, hematological responses, oxidative stress biomarkers, sustainable pest management

I- INTRODUCTION
Poultry consumption and production have noticeably increased globally (Del Bosque *et al.*, 2021). People prefer poultry meat because it is rich in proteins and minerals, plus it comes at an affordable price (Valceschini, 2006; Adamski *et al.*, 2017). In Pakistan, the poultry industry is vital, contributing significantly (1.3%) to the national GDP. The commercial poultry production in the

country began in the 1960s and has consistently supplied a substantial portion of daily proteins to the Pakistani population (Hussain *et al.*, 2015).

The poultry industry in Pakistan faces various issues, including the high cost of feed affecting the rearing of specific pathogen-free (SPF) and pest-free hens (Tauqir and Nawaz, 2001). Pests like flies, beetles, and mites pose significant challenges, leading to economic losses and diseases in poultry (Axtell, 1999; Harrington *et al.*, 2011). To combat these issues, the industry relies on pest management strategies, including the use of pesticides. Pesticides like organophosphates, carbamates, and pyrethroids are commonly employed to control pests, especially house flies (Reddy *et al.*, 2024). However, the use of pesticides raises concerns about potential health and environmental impacts. One commonly used pyrethroid is deltamethrin, which is utilized to control a broad spectrum of ectoparasites in poultry, livestock, and aquaculture (Akre, 2016).

Deltamethrin belongs to the type II pyrethroid group and acts on the neurological system of insects by modifying sodium channels (Dong *et al.*, 2022). The use of deltamethrin can affect various aspects, including hematological parameters, oxidative stress, liver histology, and growth performance in poultry. The hematological parameters, such as hemoglobin levels, red blood cell count, and white blood cell count, can be disturbed by deltamethrin exposure (Tewari and Gill, 2014). Additionally, the pesticide may induce oxidative stress, leading to alterations in antioxidant enzyme activities like catalase (Shakir *et al.*, 2018). Liver histology can be affected, potentially causing liver fibrosis (Raina *et al.*, 2009). Finally, the growth performance of poultry, particularly Golden chickens, may be influenced by deltamethrin exposure, impacting the commercial production of meat and eggs (Brundage and Barnett, 2010).

The widespread use of pesticides, including deltamethrin, raises concerns about ecological and health risks, emphasizing the need for careful evaluation and sustainable pest management practices. This study aims to assess the toxicological effects of deltamethrin on various parameters in *Gallus gallus domesticus*, providing valuable insights for the poultry industry and regulatory authorities.

II- MATERIALS AND METHODS

Experimental Birds: Chicks weighing 20-25 g were used in this study. They were housed in cages and raised for three months, with dose exposure starting in the fourth month when their weight reached one kg. The experiment was conducted at the Zoology laboratory, department of Zoology, University of Lahore, Sargodha Campus.

Experimental Design: A complete randomized design (CRD) was employed, with four treatment groups: G (control group, no dose exposure), G1 (60mg/ml/kg in chicken feed), G2 (120mg/ml/kg in chicken feed), and G3 (180mg/ml/kg in chicken feed), each consisting of 20 chickens.

Chicken Feed and Feeding Protocol: Commercial feed No 32 from Punjab Feed Pvt. Ltd. was used, comprising wheat, corn, barley, oats, millets, rye, fine iodized salt, and fish oil. Feeding was done at 9 a.m. and 5 p.m. daily.

Blood Sampling: Blood samples were collected from the brachial wing vein using a 23-gauge plastic syringe, transferred to EDTA vials, and sent to Lahore Medical Laboratories and Research Center for analysis (Olanrewaju and Magee, 2023).

Evaluation of Hematological Parameters: Hematological parameters (Hb, RBCs, hematocrit, MCV, MCHC, platelets, and WBCs) were analyzed in Lahore Medical Laboratories and Research Center. Counts were obtained using Neubauer hemocytometer after diluting blood samples with a sodium citrate solution. Giemsa staining technique was employed, and a light microscope was used to observe blood smears. PCV was determined using capillary tube microhematocrit technique, and

hemoglobin was quantified using cyanmethemoglobin technique. Red blood cell indices were calculated using appropriate formulas (Garg *et al.*, 2004; Azmi *et al.*, 2009)

Organ Recovery for Histology: Liver tissues were fixed in 10% formalin for 24 hours after washing in saline during necropsy. Dehydration followed using alcohol and xylene, and then tissues were embedded in paraffin wax. A rotary microtome cut 4-micron sections, dipped in PBS-0.05% Tween 20, and mounted on clean slides coated with glycerin and egg albumin. Hematoxylin and eosin staining was done, and sections were stored for 1-2 weeks before observation at x400 magnification with a trinocular research microscope (B-350 Optika, Italy).

Biochemical Serum Analysis: Blood centrifuged at 2700g for 15 mins at 4°C; serum samples collected and stored in Eppendorf tubes at 30°C. Serum levels of urea, creatinine, and uric acid determined using Bio Maghreb kits. Commercial kits' instructions followed. ALT, AST, urea, and creatinine tested at Lahore Medical Laboratories and Research Center (Shailajan *et al.*, 2014).

Lipoid Peroxidation: Lipid peroxidation in kidney and liver was assessed using the thiobarbituric acid (TBA) reaction (Ohkawa *et al.*, 1979). A mixture of 0.67% TBA, 15% TCA, and 0.25N HCl was prepared. 6µl of serum or plasma was combined with 120 µl of (TCA/TBA/HCL) in a 1:1:1 ratio (40 µl each). The mixture was boiled at 100°C, cooled, and then centrifuged at 3000 rpm for 10 minutes. After removing flocculent material, absorbance was read at 532 nm. MDA concentration was calculated using the formula (Ohkawa *et al.*, 1979).

Catalase Activity: Catalase activity was assessed using Goth's (1991) method. Ammonium molybdate (0.4 g) was dissolved in 10 ml of distilled water. Sodium Phosphate Buffer (60 mM, pH 7.4) was prepared with 0.55g Na_2HPO_4 and 0.135g KH_2PO_4 in 50 ml distilled water. Hydrogen Peroxide (H_2O_2, 0.065 M) was made by diluting 67µl of H_2O_2 (30%) to a final volume of 10ml in Na-K-phosphate buffer.

Growth Performance: Sampling was conducted 15 days post-exposure to Deltamethrin. Chicken growth parameters, including Net weight and Food Conversion Efficiency (FCE), were calculated using the formulas:

- Net weight = Initial weight of chick - Final weight of chick
- FCE = Weight gain of chicken / Weight of the given feed

Length measurements were taken using a centimeter tape at the beginning of the experiment, after 15 days, and after 30 days of Deltamethrin exposure.

3.12. Statistical Analysis: Data were statistically analyzed using one-way ANOVA and post hoc Tukey's test to assess the toxic effects of Deltamethrin on the selected parameters.

III- RESULTS

In the present study, *Gallus gallus domesticus* (chickens) were divided into four groups: a control group with no deltamethrin exposure and three experimental groups (G1, G2, and G3) exposed to increasing doses of deltamethrin (60mg/ml/kg, 120mg/ml/kg, and 180mg/ml/kg, respectively). The study investigated the impact of deltamethrin exposure on various hematological parameters, including hemoglobin (Hb), red blood cells (RBCs), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets, and white blood cells (WBCs). Results from Table 1 revealed significant effects on hematological parameters in the experimental groups compared to the control group. For instance, Hb levels decreased significantly in G1, G2, and G3, with the lowest level observed in G3. Similarly, RBCs, HCT, MCHC, and WBCs showed significant decreases in the experimental groups, while MCV and platelets exhibited significant increases, with the most pronounced effects observed in G3. The graphical

representations in Figures 1 to 8 visually depict the changes in hematological parameters after deltamethrin exposure. These figures highlight the notable reduction in Hb and RBCs, as well as the decrease in HCT levels. Conversely, MCV, platelets, and WBCs demonstrated an increase, indicating the hematological impact of deltamethrin exposure.

Oxidative Stress Biomarkers

Biochemical parameters such as Urea, Creatinine, ALT, and AST showed elevated values, indicating oxidative stress induced by reactive oxygen species (ROS) generation. The study also assessed the effects on antioxidant enzymes, liver enzymes, and serum biochemical parameters in the liver and kidney. The results revealed a dose-dependent increase in ALT, AST, Urea, and Creatinine levels in the exposed groups compared to the control group. Catalase enzyme activity in the liver and kidney showed a similar trend, with the highest activity observed in the group exposed to the highest dose of deltamethrin. Lipid peroxidation levels in the liver and kidney also increased with deltamethrin exposure.

Histopathological Effect on Liver

Various concentrations of Deltamethrin were observed to have histological effects on the liver of *Gallus gallus domesticus*. The primary effects included cellular degeneration, necrosis, and inflammations. Higher concentrations of Deltamethrin led to additional changes such as acetaminophen, hepatic steatosis fatty change, glycogen accumulation, and centrilobular necrosis. The control group exhibited normal cellular arrangement in the liver, while groups G1, G2, and G3 demonstrated the mentioned histological alterations.

Effect on Growth

The initial weight of the control group was highest (885.7g±61.20), followed by Group1 (875.2g±27.05), Group2 (840g±43.84), and Group3 (860.3g±22.16). After 15 days of exposure, the weight reduction was highest in Group3 (739.1g±28.70), followed by Group2 (775.2g±23.09), Group1 (729.7g±20.75), and the least in the control group (985.7g±61.20). After 30 days, the weight reduction continued to be highest in Group3 (715g±22.65), followed by Group2 (785g±30.16), Group1 (890g±78.28), and the least in the control group (1090g±78.28). At slaughtering, the control group exhibited the highest weight (1180g±43.84), followed by Group1 (935g±53.14), Group2 (812.8g±27.14), and the least in Group3 (630g±42.23). In both cases, the highest effect was observed in Group3, with a significant difference among all groups. This indicates a dose-dependent impact of Deltamethrin on the growth parameter of *Gallus gallus domesticus*, with higher doses leading to more substantial weight reduction.

Effect on Length

In the control group, the initial length was 29.16±0.69, and after 15 days, G1 had a similar length. However, G2 showed a decrease to 26.4±0.34, and the least length was observed in G3 (25.8±0.26), indicating a significant difference among all groups. After 30 days, the control group maintained the highest length (30.5±0.19), but G1 experienced a decrease to 26.7±0.16, G2 decreased to 25.8±0.26, and G3 had the lowest length at 24.3±0.28. Again, a significant difference was found among all groups. These results suggest that deltamethrin exposure had a notable impact on the length parameter of *Gallus gallus domesticus* over the observed periods.

IV- DISCUSSION

The study investigated the impact of deltamethrin exposure on *Gallus gallus domesticus*, a significant source of protein in human diets. Poultry, including chickens, faces challenges from ectoparasites, leading to reduced productivity and economic losses (Axtell and Arends, 1990; Arkle *et al.*, 2006; Akanni, 2007; Harrington *et al.*, 2011). Deltamethrin, a commonly used pesticide in the

poultry industry, is known for its efficacy against ectoparasites (Bradbury and Coats, 1989; McDevitt *et al.*, 2009).

The study focused on hematological parameters, revealing significant changes in Hb, RBCs, HCT, MCV, MCHC, platelets, and WBCs in response to deltamethrin exposure. These alterations, especially the decrease in Hb levels, align with previous findings indicating the potential impact of deltamethrin on immunological alterations and peripheral neurotoxicity (Pimpao *et al.,* 2007). Studies have shown that dimethasone and cypermethrin herbicide dosing reduces haemoglobin and hematocrit levels in sheep (Wedin and Benson, 2003). Pimpao *et al.* (2007) indicated an increase in Urea, Creatinine, ALT, and AST levels, suggesting oxidative stress induced by reactive oxygen species (ROS) generation (Kumar *et al.,* 2016). The dose-dependent elevation of liver enzymes, biochemical parameters, and oxidative stress markers emphasized the potential toxicity of deltamethrin in Gallus gallus domesticus. Pyrethroids, including deltamethrin, have been shown to increase ROS formation and cause lipid peroxidation in various hosts (Ding *et al.,* 2017).

Histopathological examination of the liver revealed significant changes such as cellular degeneration, necrosis, and inflammation, particularly with higher concentrations of deltamethrin. This aligns with previous research highlighting the cytotoxic effects of deltamethrin on various organs and tissues (Yousef *et al.,* 2006; Hussain *et al.,* 2018; Hussain *et al.,* 2021).

The study also demonstrated a dose-dependent impact on growth parameters, with significant weight and length reduction in chickens exposed to deltamethrin. Organ weight increases at certain times could suggest the body's compensatory response to meet the demands of metabolizing and excreting deltamethrin. The decrease in the relative weight of organs like the cockscomb, crop, muscular stomach, liver, lungs, and kidneys aligns with findings from earlier studies, indicating the toxic effects of deltamethrin on these organs (Oda and El-Maddaw, 2012; Chandra *et al.,* 2013; Sharma *et al.,* 2014; Arora *et al.,* 2016). The increase in uric acid and creatinine levels further indicated potential kidney dysfunction. The biochemical serum activity of ALT, AST, urea, and creatinine corroborated the histological findings, suggesting liver and kidney damage due to deltamethrin exposure (Tuzmen *et al.,* 2008; Hamidipoor *et al.,* 2015). Sublethal butachlor exposure in quails has been linked to histological evidence of renal injury and glomerular dysfunction (Hussain *et al.,* 2014). The findings indicate that using deltamethrin can have a harmful impact on the overall health in chickens.

V- CONCLUSION

The study provides comprehensive evidence of the detrimental effects of prolonged deltamethrin exposure on hematological, biochemical, histopathological, and growth parameters in Gallus gallus domesticus. These findings emphasize the need for caution in the use of deltamethrin in poultry farming to mitigate potential risks to both animal health and human consumers.

VI- REFERENCES

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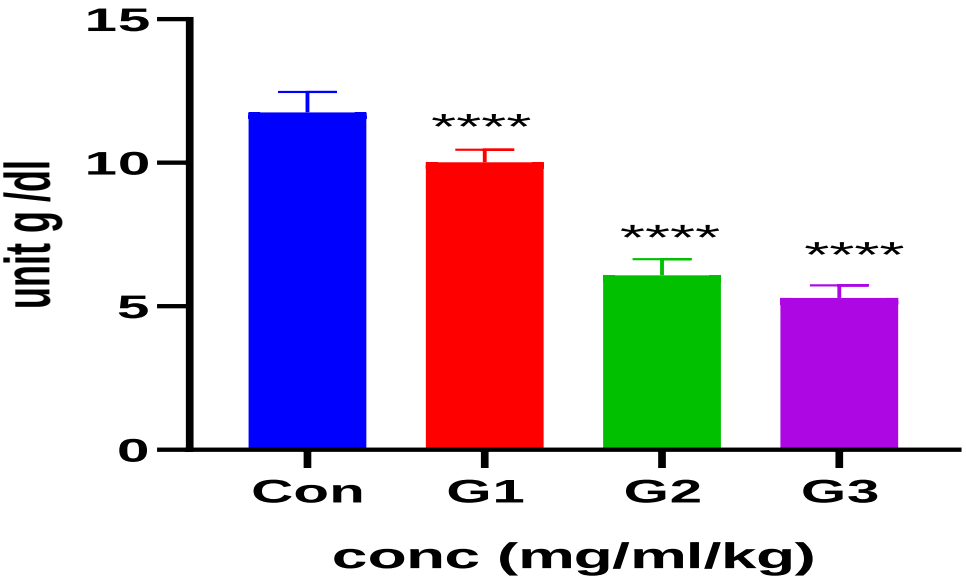


Figure 1: Evaluation of Hb level of *Gallus gallus domestics* after exposure to deltamethrin

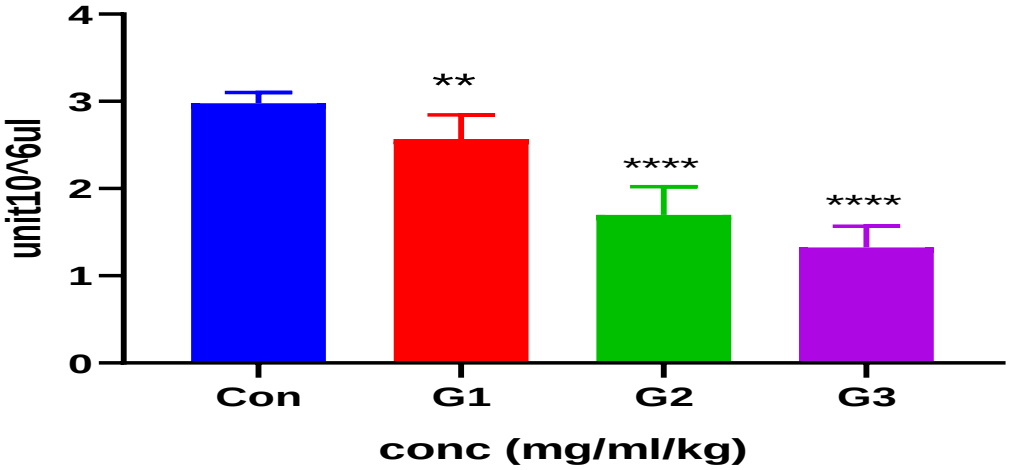


Figure 2: Evaluation of RBCs level of *Gallus gallus domestics* after exposure to deltamethrin

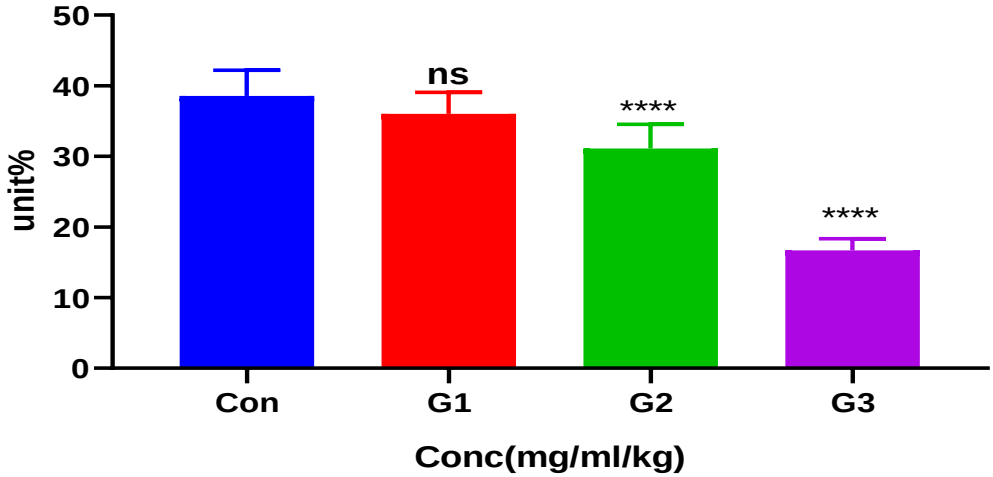


Figure 3: Evaluation of Hematocrit level of *Gallus domestics* after exposure deltamethrin

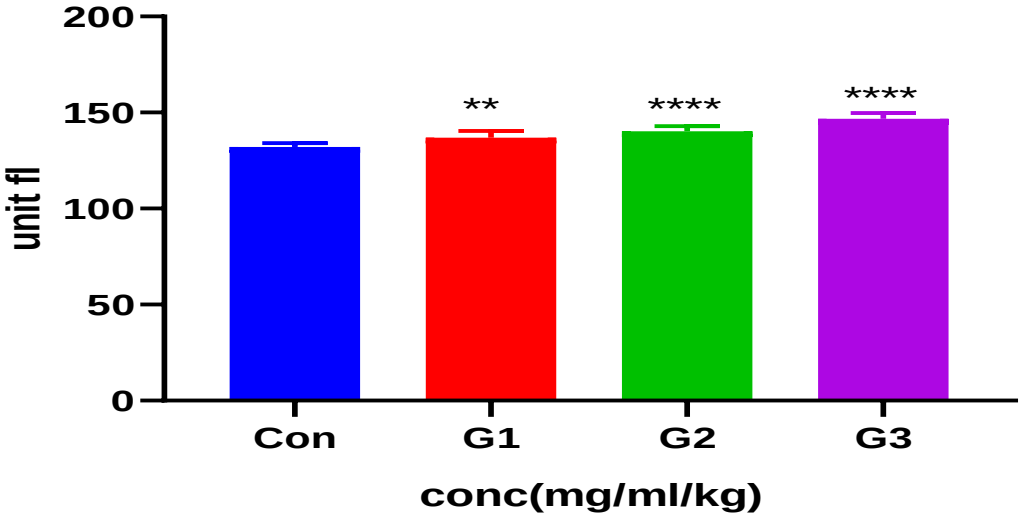


Figure 4: Evaluation of MCV level of *Gallus gallus domestics* after exposure of deltamethrin

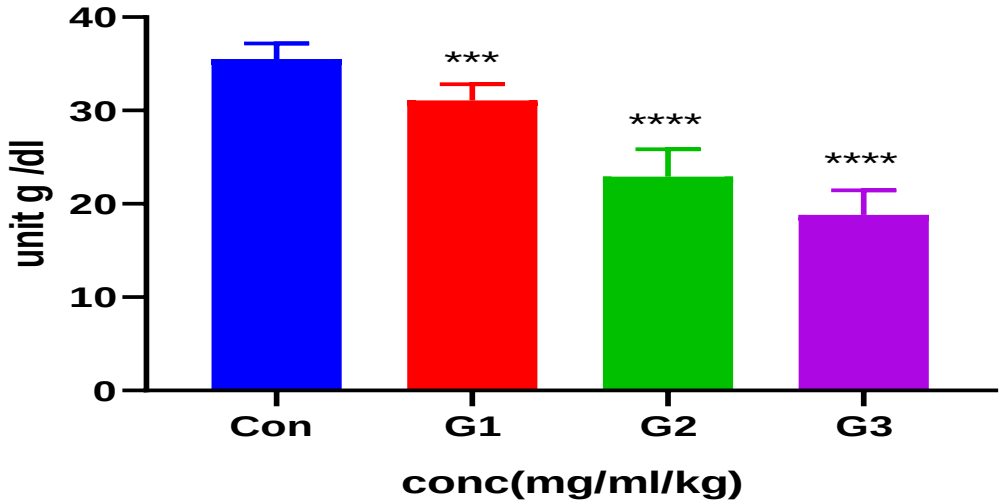


Figure 5: Evaluation of MCHC level of *Gallus gallus domesticus* after exposure of deltamethrin

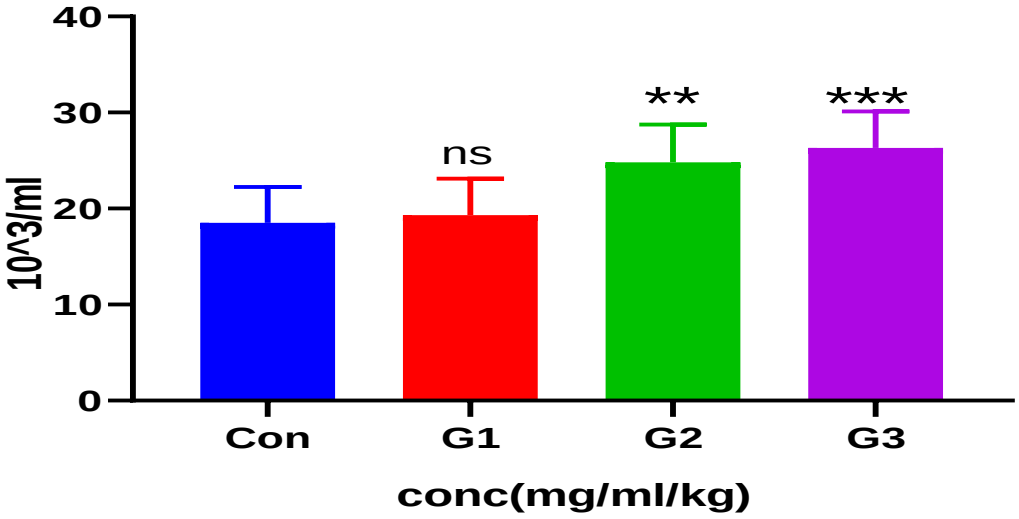


Figure 6: Evaluation of Platelets level of *Gallus gallus domestics* after exposure of deltamethrin

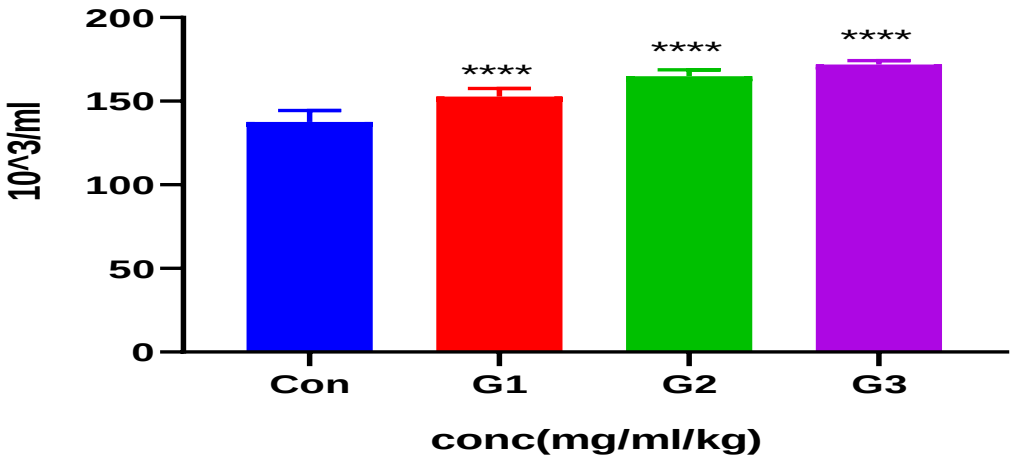


Figure 7: Evaluation of WBCs level of *Gallus gallus domestics* after exposure of deltamethrin

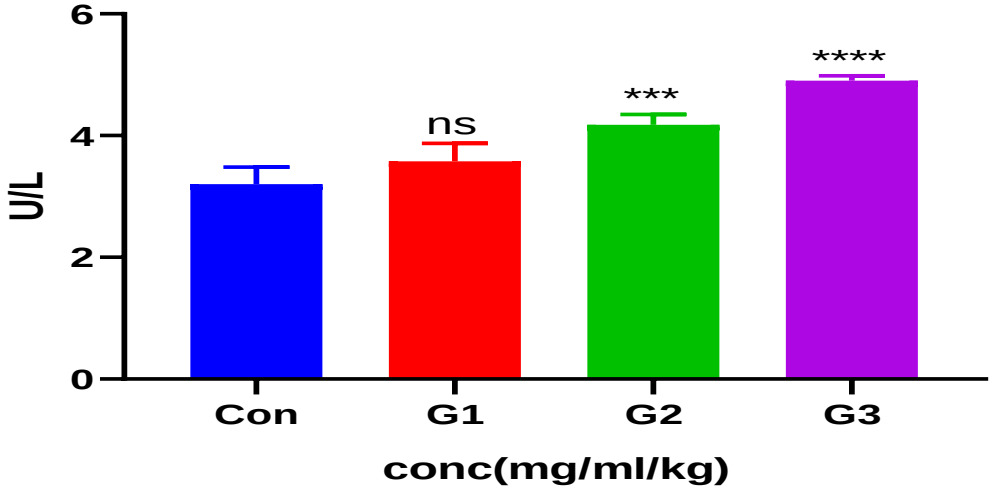


Figure 8: Evaluation of ALT level of *Gallus gallus domestics* after exposure of deltamethrin

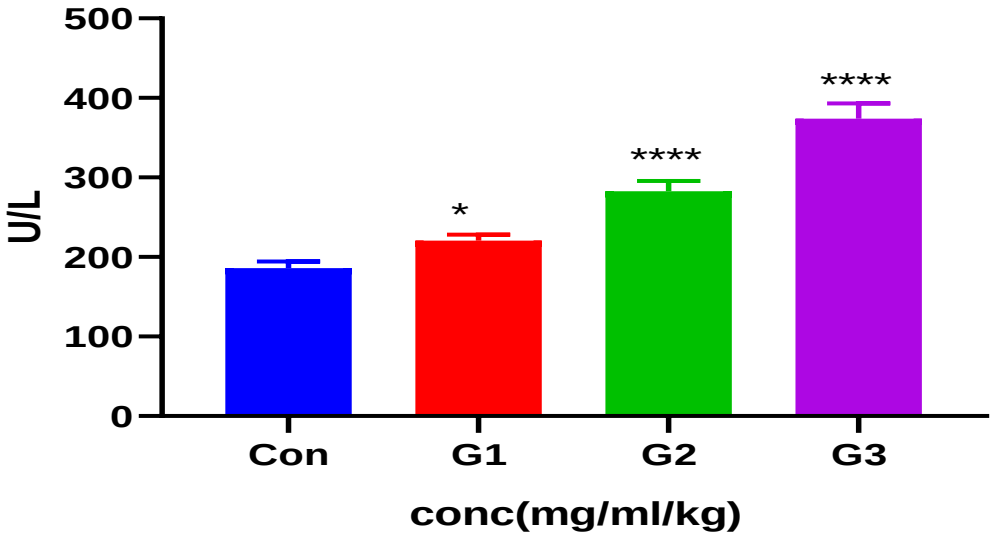


Figure 9: Evaluation of AST level of *Gallus gallus domestics* after exposure of deltamethrin

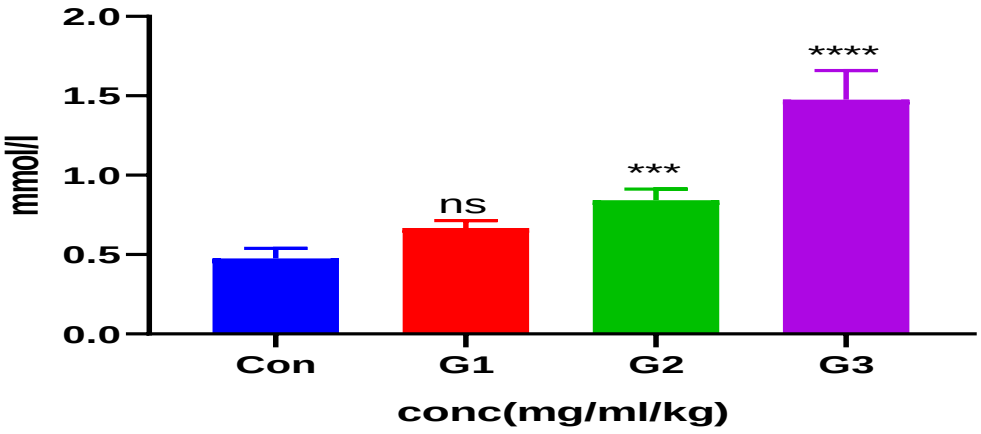


Figure 10: Evaluation of Urea level of *Gallus gallus domestics* after exposure of deltamethrin

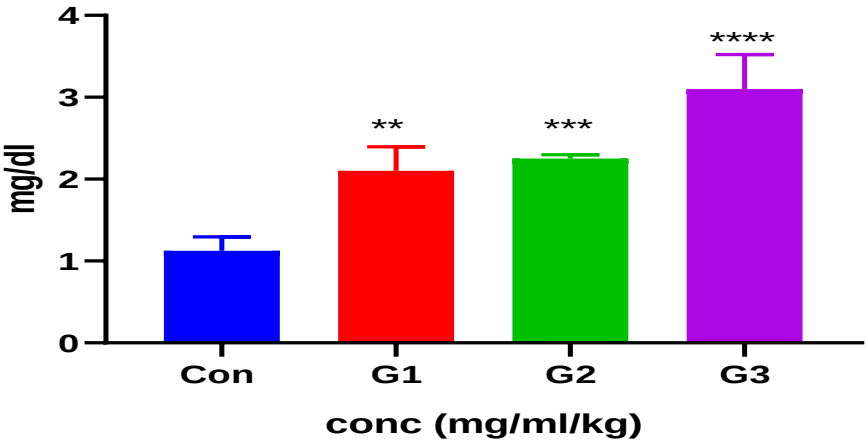


Figure 11: Evaluation of Creatinine level of *Gallus domestics* after exposure of deltamethrin

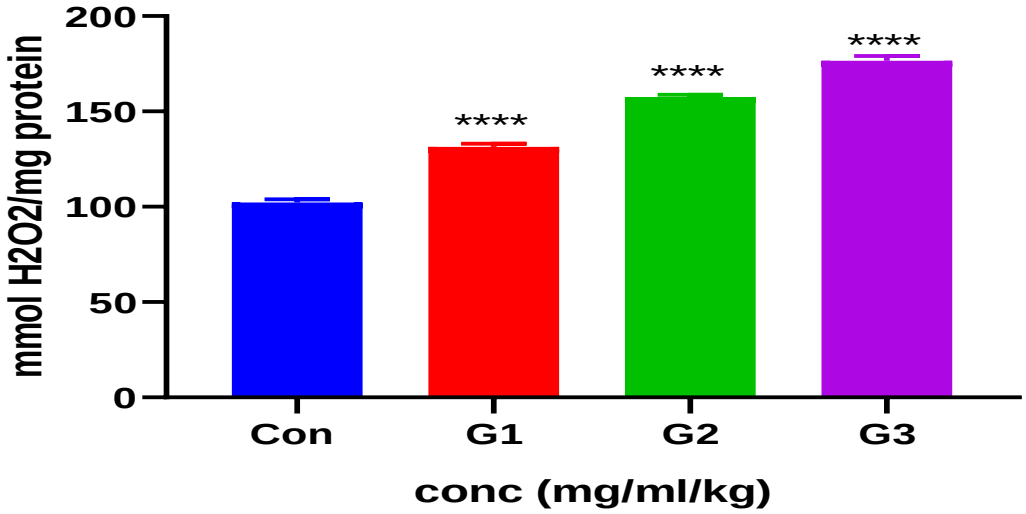


Figure 12: Evaluation of Catalase enzyme activity in liver of *Gallus gallus domesticus* after exposure of deltamethrin

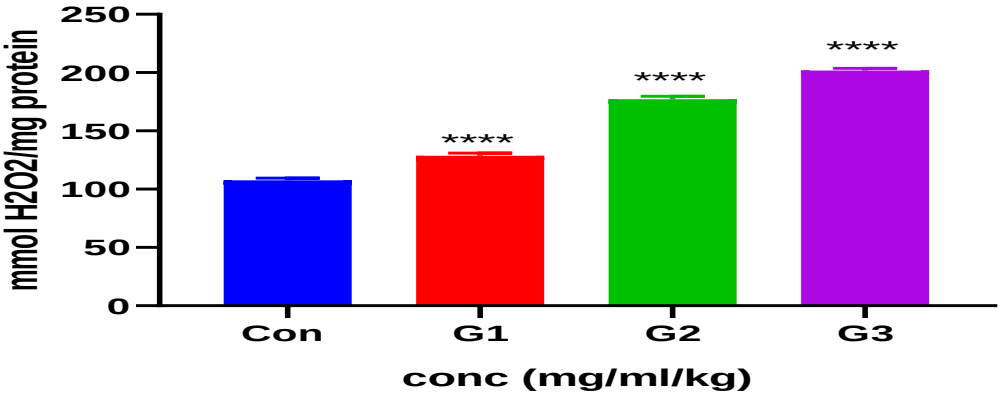


Figure 13: Evaluation of Catalase enzyme activity in kidney of *Gallus domesticus* after exposure of deltamethrin

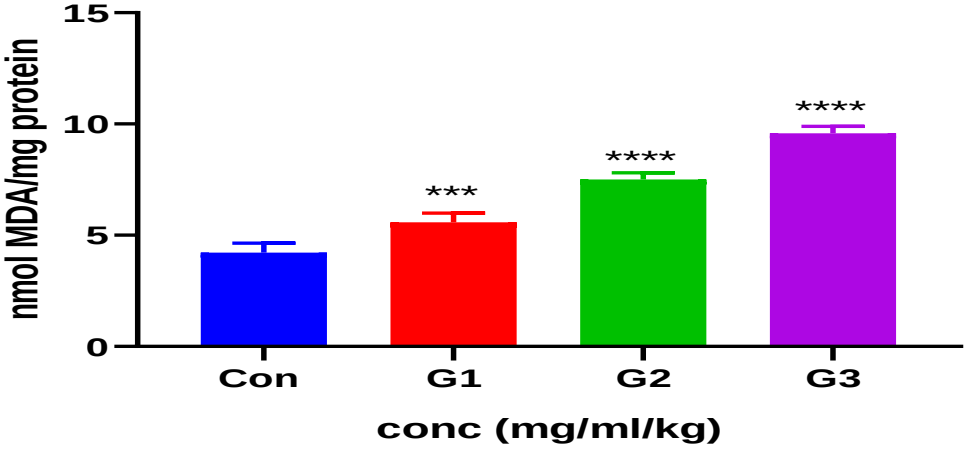


Figure 14: Evaluation of lipid peroxidation level in liver of *Gallus gallus domesticus* after exposure of deltamethrin

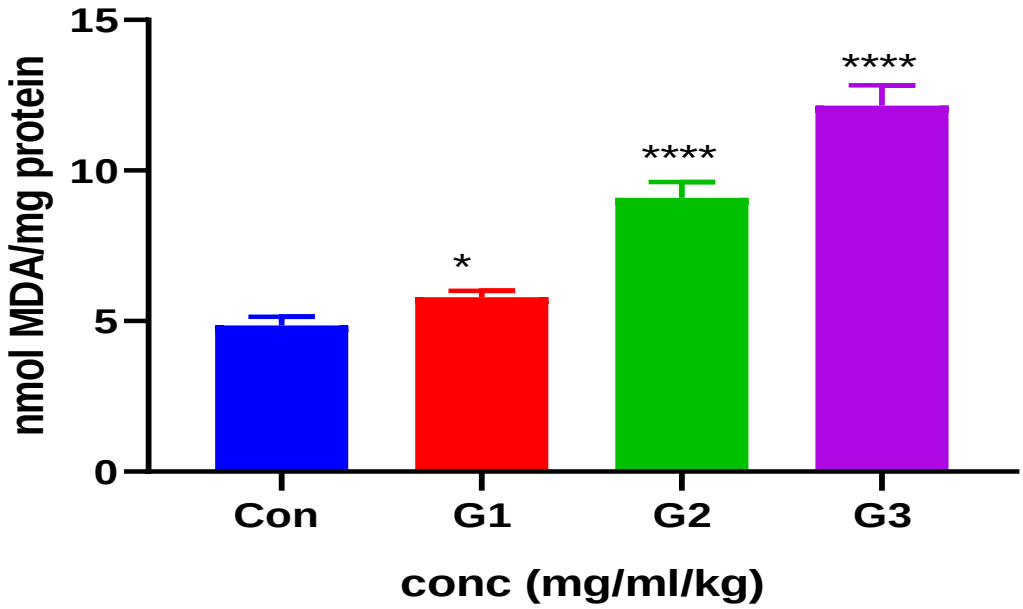
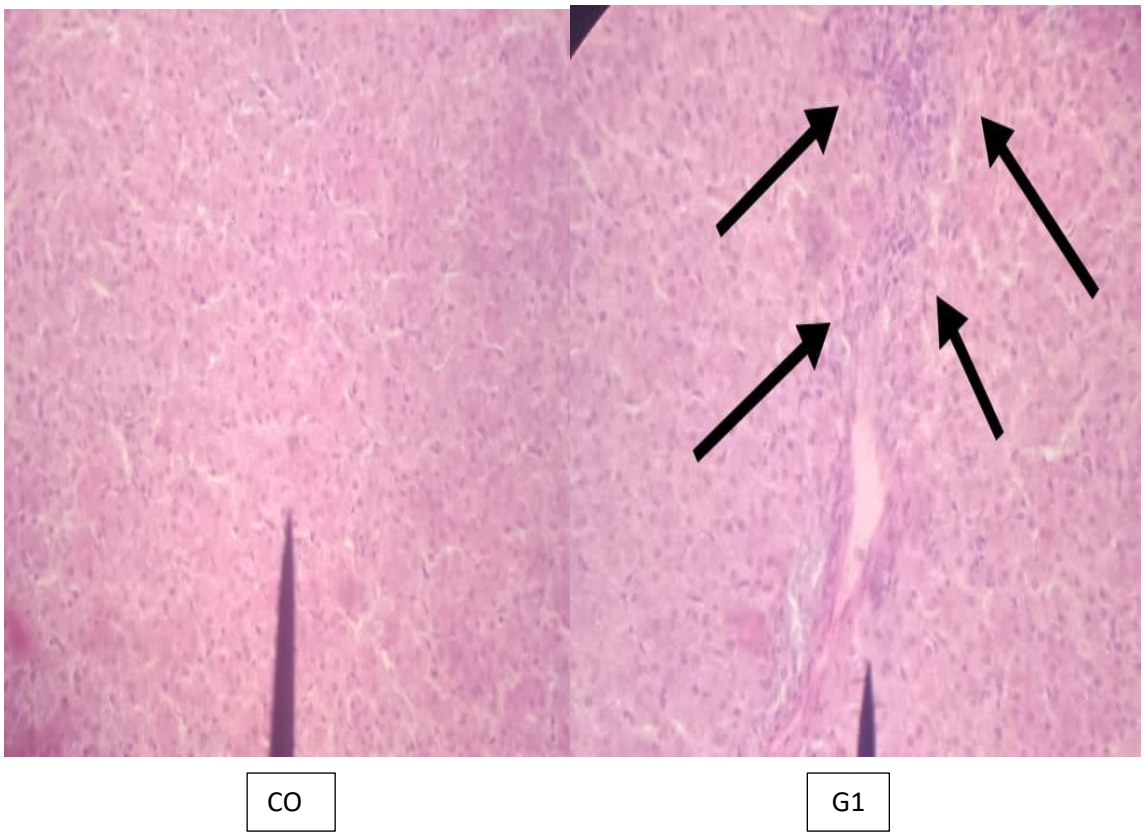


Figure 15: Evaluation of lipid peroxidation level in Kidney of *Gallus gallus domesticus* after exposure of deltamethrin



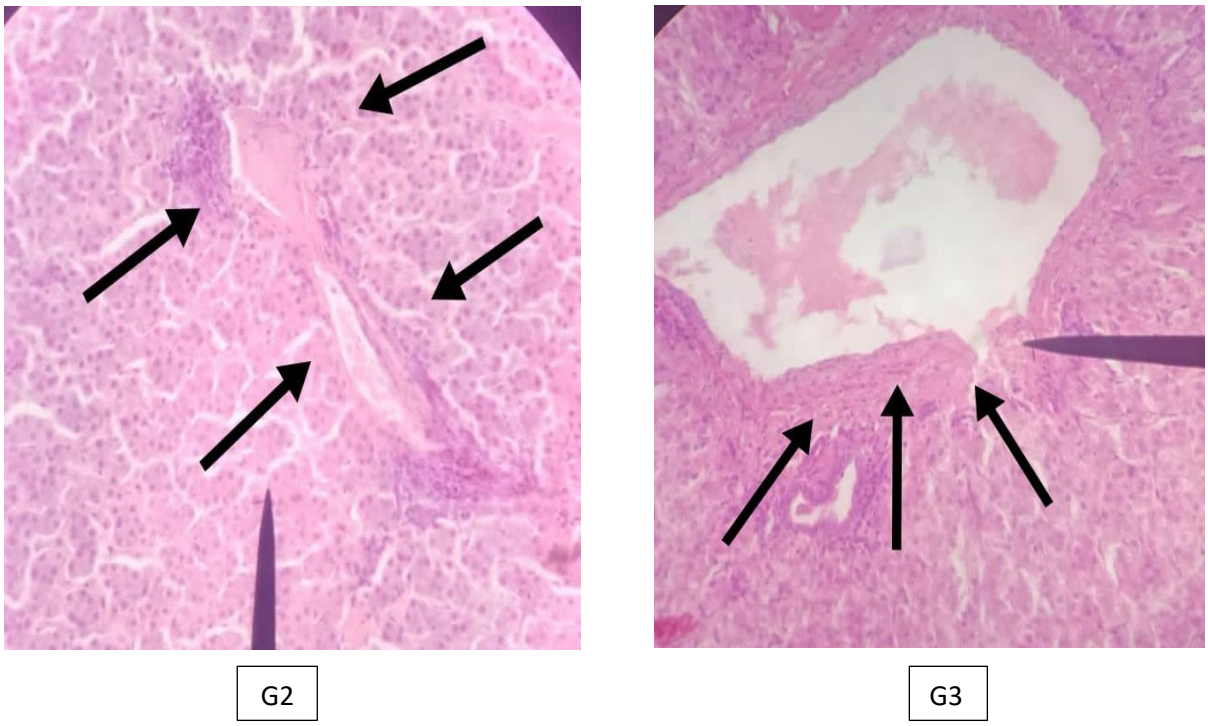


Figure 16: Histological appearance of the Liver: Control group showed the normal arrangement of cells, while comparison between G1, G2 and G3 revealed the signs of cellular degeneration, necrosis, inflammations, hepatic steatosis fatty change and glycogen accumulation

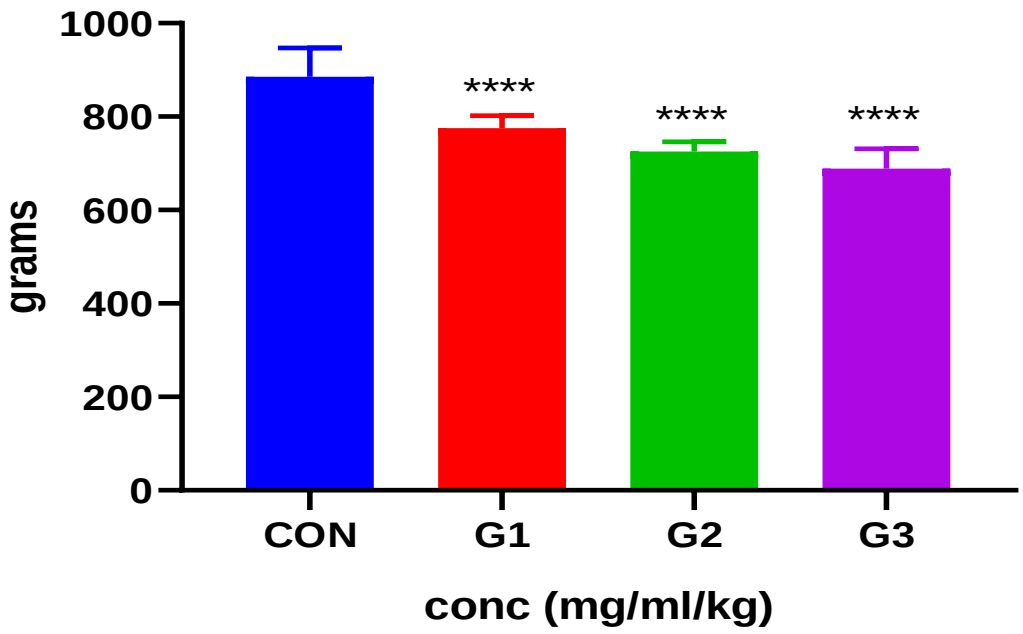


Figure 16: Evaluation of variation in weight of *Gallus gallus domestics* after 15 days' exposure of deltamethrin

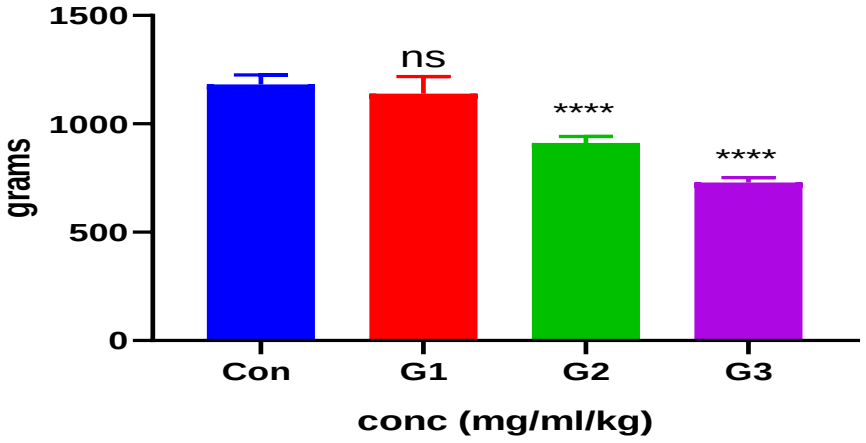


Figure 17: Evaluation of variation in weight of *Gallus gallus domestics* after 30 days' exposure of deltamethrin

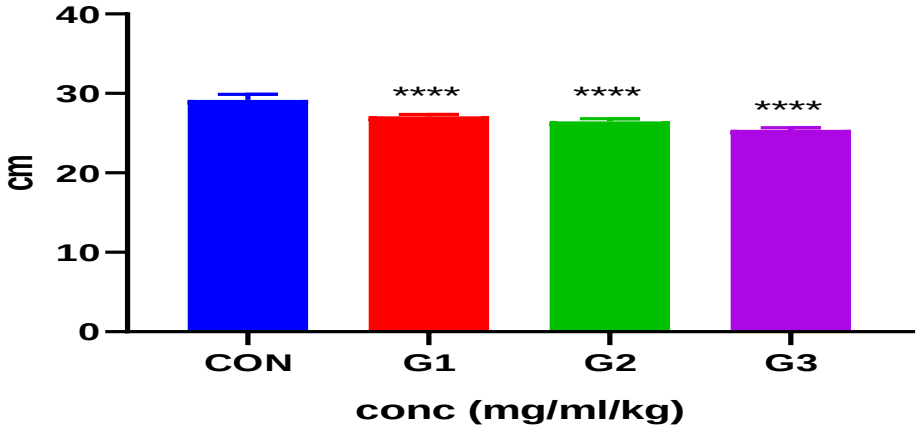


Figure 18: Variation in length of *Gallus gallus domestics* after 15 days exposure of deltamethrin

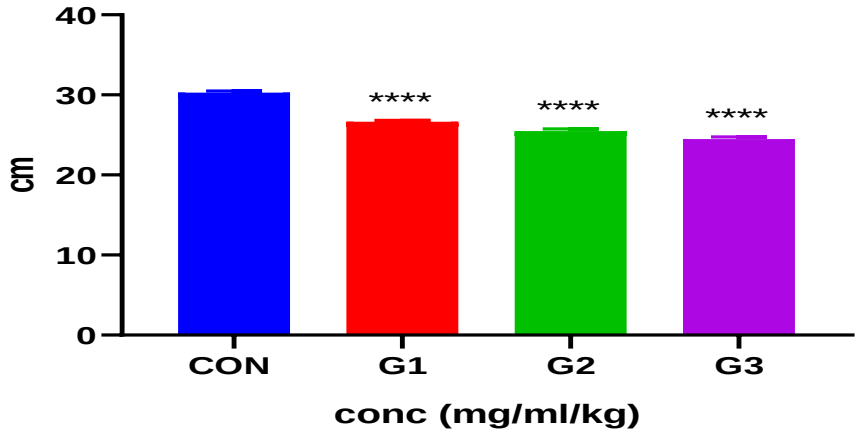


Figure 19: Variation in length of *Gallus gallus domestics* after 30 days exposure of deltamethrin