Original Articles
Toxicology International, *Sept-Dec 2015 / Vol-22 / Issue-3, 46-51*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2015/v22/i3/137624

Toxic Evaluation of Subchronic Exposure to Cadmium, Imidacloprid, and their Combination on Hematological Parameters in Buffalo Calves

Tsewang Dorjay*, Harpal Singh Sandhu and Rajdeep Kaur

Department of Pharmacology and Toxicology, College of Veterinary Science, GADVASU, Ludhiana - 141012, Punjab, India; goldiesidhu@rediffmail.com

Abstract

The present investigation was undertaken to evaluate hematological alterations induced by oral subchronic exposure to cadmium, imidacloprid, and their combination in buffalo calves. Cadmium exposure produced a significant decrease in hemoglobin (Hb) concentration, Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and MCH Concentration (MCHC). There was a significant increase in Total Leukocyte Count (TLC) but it did not produce any change in Erythrocyte Sedimentation Rate (ESR). Repeated oral administration of imidacloprid produced a significant decrease in Hb, PCV, TEC, MCV, MCH, and MCHC; however, there was a significant elevation in the levels of ESR and TLC. The combined exposure of cadmium and imidacloprid produced a significant decline in Hb concentration, PCV, TEC, MCV, and MCHC. It produced a significant increase in ESR and TLC, but no changes were observed in the levels of MCH.

Keywords: Buffalo Calves, Cadmium, Imidacloprid, Subchronic, Toxicity

Received: 14-06-2015 **Revised:** 10-10-2015 **Accepted:** 21-10-2015

1. Introduction

Cadmium (Cd) is known to be one of the most toxic environmental and industrial pollutants. It is a ubiquitous toxic heavy metal and, unlike organic compounds, is not biodegradable and has a very long biological half-life (Goyer, 1995). Cadmium toxicity is dependent on dose, duration as well as route of exposure (Järup *et al.,* 1998) and is associated with several clinical complications including renal dysfunctions, bone diseases, and hepatic dysfunctions (Järup *et al.,* 2000). Cadmium-induced peroxidation causes the release of free oxygen radicals (Llobet *et al.,* 1998), which further stimulate and destroy the sensitive macromolecules and tissues (Lafuente *et al.,* 2000). In addition, cadmium inhibits the activity of enzymes by binding to their sulfhydryl groups, alters the carbohydrate metabolism, and competes with other metals such as zinc and selenium for inclusion into metalloenzymes.

Imidacloprid is a systemic, chloro-nicotinyl insecticide with soil, seed, and foliar uses (Quintas *et al.,* 2004). Since its introduction in the insecticide market in 1992, the use of imidacloprid has increased immensely. For the most part, it has replaced the acetylcholinesterase inhibitors, the organophosphorus compounds and methylcarbamates, which display higher mammalian toxicity and decreased effectiveness due to pest resistance. Imidacloprid produces its action by interfering with the transmission of stimuli in nervous system of insect. Specifically, it causes a blockage of nicotinergic pathway that is more abundant in insects as compared to warm-blooded animals thereby making it selectively more toxic to insects than warm-blooded animals (Kidd and James, 1994). In mammals, the acute neurotoxicity of imidacloprid is largely due to action at the α4β2 and the homomeric-α7 receptors in the brain (Shimomura *et al.,* 2002).

The unchecked and indiscriminate usage of pesticides has been correlated with several un-explained health

* *Author for correspondence*

problems in both humans and animals. Although pesticides alone cannot be blamed for all the ills, there are strong indications that pesticides associated with some heavy metals and nonmetals have a pivotal role in these health problems. The potential hazard due to pesticide residues on the health of livestock, in particular, interaction of pesticides with the heavy metals is a growing concern. Although some works on the toxicity of insecticides and minerals have been done in different animals, their interaction studies are limited.

2. Materials and Methods

The experiments were performed on 16 healthy male buffalo calves of 6–12 months age and weighing between 60 and 120 kg. The animals were acclimatized in the animal shed of department under uniform conditions for 2 weeks before the commencement of the study. During this period, all animals were subjected to regular clinical examination and treated with anthelmintics for deworming. The animals were maintained on green fodder and wheat straw and water was provided ad libitum. These animals were randomly divided into four groups of four animals each. Animals of Group I were kept as healthy control, Group II animals were orally administered cadmium at the rate of 0.1 mg/kg/day for 90 consecutive days, Group III animals were administered imidacloprid at the rate of 0.5 mg/kg/day for 90 days, and Group IV animals were given a combination of and imidacloprid for the same period of time. To study the hematological parameters, blood samples were collected in ethylenediaminetetraacetic acid vials via jugular venipuncture of experimental animals on 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 days of treatment and on 100 and 110 days post-treatment periods. The hematological parameters were assayed using standardized methods (Benjamin, 1985). The data collected was analyzed using.

3. Results and Discussion

Oral administration of cadmium at the dose rate of 0.1 mg/kg/day for 90 consecutive days produced significant decrease in the level of hemoglobin, and this decrease was maximum (30.9%) on the 30th day of exposure. Imidacloprid at the dose rate of 0.5 mg/kg/day for the same period also produced a significant decline (34.1%; 40th day) in the level of hemoglobin. The maximum decline produced by combined exposure of cadmium and imidacloprid was 29.1% in buffalo calves (Table 1). Administration of cadmium caused no significant change in the level of Erythrocyte Sedimentation Rate (ESR) in buffalo calves whereas imidacloprid and combined exposure of cadmium and imidacloprid caused a significant elevation in ESR.

Repeated oral administration of cadmium, imidacloprid, and their combination produced a significant decrease in the level of Packed Cell Volume (PCV) (Table 1) and total erythrocyte count (Table 2); however, the pattern and extent of changes varied significantly among the various groups. In contrast, repeated administration of cadmium, imidacloprid, and their combination produced a significant increase in the level of Total Leukocyte Count (TLC).

The effect of repeated oral administration of cadmium, imidacloprid, and their combination on various erythrocytic indices in buffalo calves is depicted in Table 3. Cadmium caused significant decrease in the levels of Mean Corpuscular Volume (MCV) (8.68%), mean corpuscular hemoglobin (MCH) (34.4%), and Mean Corpuscular Hemoglobin Concentration (MCHC) (30.9%). The changes produced by imidacloprid were MCV (58.6%), MCH (23.7%), and MCHC (40.1%) whereas in the combined exposure group, the changes induced were to the tune of MCV (20.2%), and MCHC (19.7%), whereas no significant changes were observed in MCH (23.7%).

It is well known that the presence of cadmium in organism decreases the level of iron in blood (Kostic *et al.,* 1993) and causes the decrease of hemoglobin (Hb) concentration. Cadmium may compete with iron, leading to the occurrence of anemia due to iron deficiency whereas on the other hand renal failure, developing under the influence of cadmium, results in erythropoietin deficiency which may cause normochromic and normocytic anemia without a proportional increase of reticulocytes (Hiratsuka *et al.,* 1996). Cadmium ions significantly affect regulatory genes for erythropoietin, which could be the cause of inhibiting its expression (Chun *et al.,* 200). Moreover, cadmium inhibits the bone marrow to make hemoglobin by interfering with several enzymatic steps in the heme synthesis. In addition, cadmium has been found to have direct effect on blood hemoglobin by decreasing its formation as a result of two basic red cell defects: shortened life span and impaired heme synthesis. The mechanisms by which synthesis of the red cell pigment

Table 1. Effect of repeated oral administration of cadmium (0.1 mg/kg/day; 90 days), imidacloprid (0.5 mg/kg/day; 90 days), and their combination on hemoglobin content, ESR, and PCV in buffalo calves

	Treatment										Posttreatment	
Days	0	10	20	30	40	50	60	70	80	90	10	20
Hemoglobin (g/dL)												
Group I	11.90±0.29^{a}	12.94±0.49^{a}	11.49±0.57^{a}	10.98±0.62^{a}	11.53±0.63^{a}	10.90±0.25^{a}	12.28±0.25^{a,b}	11.85±0.24^{a}	11.73±0.40^{a}	12.29±0.92^{a}	11.86±0.76^{a}	11.71±0.24^{a}
Group II	11.91±0.07^{a}	12.25±0.14^{a}	11.34±0.29^{a}	8.22±0.69^{b}	11.95±0.95^{a}	11.91±0.65^{a}	11.64±0.54^{a}	8.88±0.24^{b}	10.99±0.46^{a,b}	12.64±0.61^{a}	12.14±0.22^{a}	12.50±0.33^{a}
Group III	12.46±0.16^{a}	12.05±0.24^{a}	11.39±0.95^{a}	8.55±0.07^{b}	8.21±0.54^{b}	11.95±0.51^{a}	11.63±0.53^{a}	8.35±0.08^{b}	10.34±0.11^{b}	11.20±0.32^{a}	11.35±0.34^{a}	12.20±0.47^{a}
Group IV	12.32±0.37^{a}	12.76±0.19^{a}	12.80±0.35^{a}	12.38±0.53^{a}	12.68±0.41^{a}	8.74±0.43^{b}	13.13±0.22^{b}	8.99±0.96^{b}	10.24±0.47^{b}	11.58±0.27^{a}	12.02±0.25^{a}	12.32±0.32^{a}
ESR (mm/24 h)												
Group I	112.2±9.63^{a}	109.0±9.92^{a}	115.7±2.84^{a}	127.7±4.97^{a}	133.5±5.31^{a}	126.2±3.75^{a}	130.2±7.86^{a}	131.0±7.38^{a}	132.2±4.49^{a}	135.0±3.19^{a}	127.7±5.12^{a}	131.0±4.30^{a}
Group II	135.5±0.50^{a}	104.2±2.17^{a}	118.0±4.93^{a}	122.5±4.64^{a}	137.2±3.33^{a}	123.7±4.19^{a}	127.5±2.22^{a}	129.7±9.92^{a}	133.5±2.25^{a}	126.2±3.47^{a}	119.0±3.67^{a}	136.7±5.37^{a}
Group III	136.0±3.65^{a}	141.0±7.71^{b}	148.2±5.45^{b}	143.7±4.21^{b}	142.7±2.25^{a}	139.7±1.03^{b}	139.2±0.75^{a}	135.7±3.90^{a}	135.0±4.14^{a}	132.2±4.21^{a}	135.5±1.66^{a}	140.0±5.66^{a}
Group IV	121.7±10.47^{a}	109.25±3.25^{a}	115.5±2.72^{a}	116.5±6.24^{a}	133.0±2.64^{a}	147.0±1.08^{b}	139.7±5.86^{a}	130.0±9.13^{a}	130.0±3.00^{a}	131.0±1.08^{a}	127.5±8.54^{a}	140.0±6.16^{a}
PCV (%)												
Group I	30.08±1.93^{a}	36.39±1.79^{a}	30.70±0.71^{a}	39.62±1.22^{a}	35.55±1.70^{a}	32.47±1.28^{a}	32.54±1.30^{a}	32.19±2.57^{a}	31.91±2.45^{a}	32.99±1.86^{a}	32.59±1.11^{a}	33.01±1.12^{ab}
Group II	35.13±1.28^{a}	37.12±1.46^{a}	33.49±0.87^{a}	34.72±2.06^{b}	33.73±1.39^{a,b}	32.55±2.30^{a}	31.85±1.99^{a}	30.94±2.57^{a}	34.47±1.87^{a}	35.49±1.11^{a}	33.12±1.06^{a}	34.02±0.35^{b}
Group III	31.03±2.74^{a}	32.21±1.29^{a}	29.13±1.55^{a}	30.26±1.04^{c}	28.42±1.91^{b}	23.22±2.05^{b}	29.64±1.78^{a}	29.23±0.99^{a}	34.22±3.48^{a}	33.27±1.43^{a}	33.12±2.06^{a}	34.69±1.52^{b}
Group IV	36.04±0.79^{a}	36.81±2.13^{a}	30.73±1.97^{a}	33.46±0.73^{b,c}	31.91±1.54^{a,b}	27.35±0.84^{a,b}	30.37±2.14^{a}	33.14±2.17^{a}	32.33±1.34^{a}	34.61±0.89^{a}	34.38±1.56^{a}	29.40±1.87^{a}

Values given represent the mean ± SE of 4 animals. Values lacking a common superscript in a given column differs significantly from each other ($P < 0.05$). SE = Standard error, ESR = Erythrocyte sedimentation rate, PCV = Packed cell volume

Table 2. Effect of repeated oral administration of cadmium (0.1 mg/kg/day; 90 days), imidacloprid (0.5 mg/kg/day; 90 days), and their combination on TEC and TLC in buffalo calves

	Treatment										Posttreatment	
Days	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**10**	**20**
TEC ($\times10^6$/mm^3)												
Group I	6.34±0.41^a	7.40±0.22^a	6.98±0.44^a	7.77±0.34^a	6.78±0.36^a	7.23±0.20^a	7.85±0.52^a	6.94±0.30^a	7.09±0.32^a	7.06±0.61^a	7.28±0.58^a	6.96±0.56^a
Group II	7.01±0.12^a	6.66±0.24^a	6.73±0.51^a	6.80±0.22^{a,b}	7.38±0.44^a	7.60±0.43^a	7.71±0.20^a	5.88±0.35^b	7.13±0.22^a	7.27±0.48^a	7.82±0.51^a	6.60±0.31^a
Group III	6.76±0.58^a	7.00±0.36^a	6.18±0.32^a	5.86±0.60^b	7.11±0.23^a	7.02±0.48^a	6.49±0.47^a	7.62±0.48^a	6.45±0.41^{a,b}	7.82±0. 81^a	6.43±0.62^a	6.99±0.48^a
Group IV	6.70±0.19^a	7.55±0.51^a	7.58±0.70^a	7.93±0.22^a	7.61±0.36^a	7.19±0.40^a	7.79±0.45^a	4.96±0.16^b	5.46±0.35^b	7.05±0.27^a	7.74±0.29^a	5.72±0.61^a
TLC ($\times10^3$/mm^3)												
Group I	7.21±0.47^a	8.31±0.78^a	9.67±0.31^a	9.19±0.52^{a,b}	8.47±0.55^a	9.60±0.90^a	9.99±0.64^a	8.52±0.43^a	8.99±0.35^a	10.18±0.64^a	8.75±0.76^{a,b}	8.75±0.95^a
Group II	7.87±0.78^a	7.80±0.35^a	10.17±0.42^a	11.15±0.97^b	11.52±0.70^b	12.49±0.67^b	12.75±1.06^b	12.97±0.41^b	10.73±0.38^a	10.90±0.80^a	11.44±1.01^{a,b}	10.25±0.25^a
Group III	8.10±0.27^a	8.52±0.52^a	10.72±0.45^a	10.99±1.04^b	12.20±0.58^b	13.37±0.54^b	12.46±0.48^b	11.30±1.19^b	9.14±0.90^a	10.36±0.37^a	10.91±0.78^b	9.75±0.48^a
Group IV	8.29±0.62^a	9.21±0.59^a	9.65±0.45^a	7.95±0.19^a	11.80±0.90^b	8.89±1.12^a	9.86±0.78^a	13.57±0.49^b	10.73±0.54^a	11.00±0.41^a	7.92±0.59^a	10.50±0.29^a

Values given represent the mean ± SE of 4 animals. Values lacking a common superscript in a given column differs significantly from each other ($P < 0.05$). SE = Standard error, TEC=Total erythrocyte count, TLC=Total leukocyte count

Table 3. TEffect of repeated oral administration of cadmium (0.1 mg/kg/day; 90 days), imidacloprid (0.5 mg/kg/day; 90 days), and their combination on MCV, MCH, and MCHC in buffalo

???	Treatment										Post-treatment	
Days	0	10	20	30	40	50	60	70	80	90	10	20
MCV (fL)												
Group I	48.04±3.05^{a}	50.91±4.16^{a}	44.48±2.73^{a}	50.09±3.21^{a}	52.49±1.25^{a}	44.96±1.58^{a}	41.71±1.46^{a}	48.28±1.89^{a,b}	45.67±3.75^{a}	47.88±2.02^{a}	51.09±0.94^{a}	48.55±2.79^{a}
Group II	50.25±2.63^{a}	55.95±2.54^{a}	50.71±4.37^{a}	51.41±4.21^{a}	45.89±1.23^{b}	42.83±1.61^{a}	41.57±3.56^{a}	52.87±1.82^{b}	50.82±3.21^{a}	49.39±3.56^{a}	43.03±3.74^{a}	51.84±2.11^{a}
Group III	47.62±3.28^{a}	46.44±3.29^{a}	47.16±0.98^{a}	53.27±5.30^{a}	40.08±2.90^{b}	33.20±2.50^{b}	45.89±2.29^{a}	43.93±2.98^{a}	54.17±5.78^{a}	42.53±1.36^{a}	52.56±4.26^{a}	50.01±2.16^{a}
Group IV	53.82±0.80^{a}	49.92±3.78^{a}	41.41±2.97^{a}	42.86±1.07^{a}	42.95±1.27^{b}	44.79±3.36^{a}	39.44±3.94^{a}	53.04±2.90^{b}	49.86±2.90^{a}	49.24±1.61^{a}	44.47±1.80^{a}	52.19±2.71^{a}
MCH (g/dL)												
Group I	19.05±1.60^{a}	17.53±0.88^{a}	16.85±1.36^{a}	14.20±0.91^{a}	17.04±0.73^{a}	15.10±0.25^{a}	15.91±1.35^{a}	17.08±0.45^{a}	16.58±0.21^{a}	17.84±1.17^{a}	16.75±0.83^{a}	17.17±1.48^{a}
Group II	17.01±0.22^{a}	18.43±0.54^{a}	17.14±1.39^{a}	12.18±1.26^{a}	11.16±0.55^{c}	15.89±1.52^{a}	15.16±0.95^{a}	15.20±0.67^{a}	13.33±0.73^{b}	17.50±0.90^{a}	15.67±0.81^{a}	19.11±1.30^{a}
Group III	18.78±1.36^{a}	17.34±0.89^{a}	18.77±0.81^{a}	15.02±1.46^{a}	14.42±0.95^{b}	17.35±1.76^{a}	18.14±1.36^{a}	15.73±0.31^{a}	15.55±1.11^{a,b}	14.32±0.37^{b}	18.17±1.87^{a}	17.82±1.12^{a}
Group IV	18.38±0.28^{a}	17.15±1.32^{a}	17.49±1.31^{a}	15.67±0.93^{a}	16.71±0.65^{a}	15.17±1.47^{a}	16.98±0.80^{a}	18.03±1.63^{a}	17.13±1.34^{a}	16.49±0.69^{a,b}	15.57±0.54^{a}	20.72±0.32^{a}
MCHC (pg/dL)												
Group I	40.11±3.08^{a}	35.99±2.90^{a}	37.61±2.61^{a}	32.20±0.92^{a}	36.44±1.04^{a}	33.75±1.67^{a}	37.98±2.08^{a}	37.41±2.55^{a}	37.74±2.65^{a}	37.58±3.17^{a}	36.55±2.91^{a}	35.67±1.94^{a}
Group II	34.07±1.44^{a}	32.65±1.14^{a}	33.98±1.55^{a}	23.56±1.23^{c}	24.32±1.08^{b}	37.25±3.77^{a}	36.77±1.81^{a}	29.27±2.37^{b}	27.83±2.20^{b}	35.66±1.58^{a}	36.79±1.44^{a}	36.75±1.17^{a}
Group III	40.68±3.67^{a}	37.62±1.88^{a}	39.88±2.84^{a}	28.37±1.11^{b}	36.66±1.74^{a}	38.16±0.88^{a}	39.89±3.26^{a}	40.64±1.16^{a}	24.53±1.27^{b}	33.74±0.93^{a}	34.49±1.32^{a}	35.42±2.30^{a}
Group IV	34.19±0.93^{a}	34.94±1.58^{a}	42.38±3.79^{a}	33.13±1.53^{a}	37.20±0.87^{a}	39.19±1.18^{a}	43.87±3.04^{a}	27.46±3.50^{b}	28.69±1.58^{b}	33.56±1.47^{a}	35.10±1.23^{a}	37.79±1.20^{a}

Values given represent the mean ± SE of 4 animals. Values lacking a common superscript in a given column differs significantly from each other ($P < 0.05$). MCV = Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, SE = Standard error

heme is inhibited by cadmium involve at least two enzymes, a cytoplasm one (delta-aminolevulinic acid) at the beginning of heme synthesis and a mitochondrial ferrochelatase, at the end of heme synthesis (Moore *et al.,* 1987). The decrease in hemoglobin concentration on subchronic oral exposure to imidacloprid in the present study might have occurred because of interference with heme synthesis as reported with certain toxins and drugs (Turk and Casteel, 1997).

ESR reflects mainly changes in the plasma protein pattern (Dacie and Lewis, 1997), whereas PCV is considered a reflection of the circulating red cell mass. There could be three possible causes for the decrease of the hematocrit during the stress such as increase in the volume of the plasma, loss of water in the erythrocyte, and hemolysis of erythrocytes in the bloodstream. The elevation of TLC after imidacloprid exposure is supported in a subchronic toxicity study reporting a significant increase in TLC in buffalo calves (Livingstone *et al.,* 2006). The effect of insecticide treatment on bone marrow and/or pituitary adrenal system contributes to an increase in leukocytes count (Hassan *et al.,* 1988). This increase in leukocyte counts has been reported to indicate an activation of the animal's immune system.

Increase in MCV indicated an erythroid regenerative response with the release of immature cells of increased size, whereas microcytic anemia is indicated by decrease in MCV (Gossett, 2004). Increase in MCV indicated an erythroid regenerative response with the release of immature cells of increased size, whereas microcytic anemia is indicated by decrease in MCV (Gossett, 2004).

Therefore, on the basis of the present investigation, it can be concluded that the combined exposure of cadmium and imidacloprid is not relatively more toxic as compared to their individual exposures in buffalo calves.

4. References

1. Goyer R. Toxic effect of metal. In: Klaassen CD, editor. Casarett and Doull's Toxicology. The Basic Science of Poisons. 5th ed. New York: McGraw-Hill; 1995. p. 691–736.
2. Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure− A review of the literature and a risk estimate. Scand J Work Environ Health 1998; 24 Suppl 1:1–51.
3. Järup L, Hellström L, Alfvén T, Carlsson MD, Grubb A, Persson B, *et al.* Low level exposure to cadmium and early kidney damage: The OSCAR study. Occup Environ Med 2000; 57:668–72.
4. Llobet JM, Granero S, Torres A, Schuhmacher M, Domingo JL. Biological monitoring of environmental pollution and human exposure to metals in Tarragona, Spain III. Blood levels. *Trace Elem* Electrolytes. 1998; 15:76–80.
5. Lafuente A, Márquez N, Pérez-Lorenzo M, Pazo D, Esquifino AI. Pubertal and postpubertal cadmium exposure differentially affects the hypothalamic-pituitary-testicular axis function in the rat. Food Chem Toxicol. 2000; 38:913–23.
6. Quintas G, Armenta S, Garrigues S, Guardia MD. Fourier transform infrared determination of imidacloprid in pesticide formulations. J Braz Chem Soc. 2004; 15:307–12.
7. Kidd H, James D. Agrochemicals Handbook. 3rd ed. Cambridge, England: Royal Society of Chemistry; 1994.
8. Shimomura M, Okuda H, Matsuda K, Komai K, Akamatsu M, Sattelle DB. Effects of mutations of a glutamine residue in loop D of the alpha7 nicotinic acetylcholine receptor on agonist profiles for neonicotinoid insecticides and related ligands. Br J Pharmacol. 2002; 137:162–9.
9. Benjamin MM. Outline of veterinary clinical pathology. 3rd ed. Ludhiana, India: Kalyani Publisher; 1985. p. 25, 48, 60.
10. Kostic MM, Ognjanovic B, Dimitrijevic S, Zikic RV, Stajn A, Rosic GL, *et al.* Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rats: *In vivo* effects. Eur J Haematol. 1993; 51:86–92.
11. Hiratsuka H, Katsuta O, Toyota N, Tsuchitani M, Umemura T, Marumo F. Chronic cadmium exposure-induced renal anemia in ovariectomized rats. Toxicol Appl Pharmacol. 1996; 137:228–36.
12. Chun YS, Choi E, Kim GT, Choi H, Kim CH, Lee MJ, *et al.* Cadmium blocks Hypoxia-Inducible Factor (HIF)-1-mediated response to hypoxia by stimulating the proteasome-dependent degradation of HIF-1alpha. Eur J Biochem. 2000; 267:4198–204.
13. Moore MR, Goldberg A, Yeung-Laiwah AA. Lead effects on the heme biosynthetic pathway. Relationship to toxicity. Ann N Y Acad Sci. 1987; 514:191–203.
14. Turk JR, Casteel SW. Clinical biochemistry in toxicology. In: Kaneko JJ, Harvey JW, Bruss ML, editors. Clinical Biochemistry of Domestic Animals. 5th ed. San Diego: Academic Press; 1997. p. 829–44.
15. Dacie JV, Lewis SM. Practical Haematology. 5th ed. Edinburgh: Churchill Livingstone; 1997. p. 580–2.
16. Livingstone C, Kaur B, Sandhu HS, Kaur R. Toxic effects of subacute oral exposure of imidacloprid on biochemical parameters in crossbred cow calves. Toxicol Int. 2006; 13:43–7.
17. Hassan GA, Salem MH, Aba-Allah GA, Shahar N, Tohraa AE. Effect of organophosphorus (dimethoat) and pyrithroid pesticide on plasma level of cortisol and thyronine and on some haematological characteristics in growing male rabbits. Indian J Anim Sci. 1988; 58:1395–401.
18. Gossett KA. Anemias associated with drugs and chemicals. Schalm's Veterinary Haematology. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2004. p. 185–9.

Source of Support: Nil. **Conflicts of Interest:** Nil.