Haryana Vet. (Dec., 2017) **56 (2),** 167-172

Research Article

EFFECT OF SUBCHRONIC EXPOSURE OF THIAMETHOXAM ON GROWTH AND HAEMATOLOGICAL PARAMETERS IN MALE WISTAR RAT AND ITS AMELIORATION BY QUERCETIN

M.S. AUWAL*, A. BHIDHASRA and V. KUMAR
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, India

ABSTRACT

The present study was conducted to ascertain the toxic effects of thiamethoxam following subchronic exposure and amelioration properties of quercetin in male Wistar rats. A total of 96 male Wistar rats; six groups of 8 rats each were exposed to both thiamethoxam (2.5% and 5.0% of maximum tolerated dose; 4200mg/kg b. wt.) and quercetin for 60 and 90 days. The gap of 12 h was maintained between thiamethoxam and quercetin administration. The vital organs viz. liver, heart, spleen, brain, kidney and testis were weighed individually and relative body and organ weight gain (per 100g body weight) were calculated. Blood samples were taken directly from the heart in heparinized vials for the determination of hematological parameters. ANOVA by Bonferroni test was applied to statistically analyze the results. Administration of 2.5 and 5.0% maximum tolerated dose (MTD) of thiamethoxam in rats resulted in a significant decrease (pd"0.05) in relative body weight, organ weight, and haematological parameters while differential leucocytic count indicated percent increase in neutrophils, eosinophils, basophils and monocytes. Groups treated with quercetin alone and with thiamethoxam and quercetin (50mg/kg) at 60 and 90 days did not show a significant difference in these parameters with that in the control group, which was suggestive of ameliorative properties of quercetin. Conclusively the subchronic exposure of 2.5 and 5.0% MTD of thiamethoxam exhibited significant (pd"0.05) decrease in body weight, organ weight and hematological parameters in male Wistar rats.

Key words: Subchronic exposure, thiamethoxam, quercetin, body weight, organ weight, haematological parameters

Neonicotinoids represent the fastest and newest major class of insecticides introduced to market since the launch of pyrethroids and has an outstanding potency and systemic action for crop protection against piercing and sucking pests. These are also highly effective for flea control on cats and dogs and other animals. The neonicotinoid class is divided into first (acetamiprid, thiacloprid, imidacloprid and nitenpyram) and second generation (thiamethoxam and clothianidin) chemical agents (Roberts and Hutson, 1999; Nauen *et al*., 2001). Due to the agricultural use of thiamethoxam, human and animal exposure could be possible; therefore authentication of safety of this compound is absolutely necessary.

Thiamethoxam 3-(2-chloro-thiazol-5-ylmethyl) -5-methyl-[1, 3, 5] oxadiazinan-4-yllidine-N-nitroamine) causes low acute oral toxicity in rat (LD_{50}=1563 mg/kg b wt.) (European Commission, 2006). Maximum single application rate of thiamethoxam ranges between 20 and 150 g/ha, and the total dose on any one crop can reach a maximum of 200 g/ha in one year (Martin *et al*., 2016). It selectively acts on insect nicotinic acetylcholine receptors (nAChR) with only a little action on mammalian nAChR. Liver, kidneys and reproductive organs are identified as target organs (European Commission, 2006). Various symptoms of acute toxicity in mice are ataxia, straub tail, tremors, convulsions on touch and sound, changes in body posture with tilted head, decreased grip strength and decreased spontaneous motor activity (Sole *et al*., 2008).

Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavon) is a plant bioflavonoid found in leafy vegetables, fruits, beverages and onions. They have been reported to share a wide spectrum of pharmacological and biological properties including anti-inflammatory, antiallergic, anti-apoptotic, antitumor and antioxidant abilities (Morikawa *et al*., 2012; Dong *et al*., 2014). Quercetin (Qu) directly scavenges the superoxide anion and inhibits several superoxide generating enzymes such as xanthine oxidase or the neutrophil membrane NADPH oxidase complex. In addition, the qu has the ability to chelate metal ion. Antithrombotic, hepatoprotective, antifibrogenic, free radical scavenger and anti-lipoperoxidative effects have also been reported (Renugadevi and Prabu, 2009). It is a more potent antioxidant than other antioxidant nutrients such as vitamin C, vitamin E and β-carotene (Renugadevi and

*Corresponding author: auwal1971@gmail.com

Prabu, 2009). Quercetin pretreatment followed by fenvalerate administration showed better recovery as evidenced by the significant reduction of liver specific markers in serum and elevated levels of antioxidants with the depletion of malondialdehyde (MDA) compared to fenvalerate treated group (Waheed and Mohammed, 2012). Farombi *et al*. (2012) revealed that Qu ameliorated quinine sulphate induced testicular toxicity and oxidative stress. However, detailed research has not been done in rats in relation to the sub-chronic toxicity of thiamethoxam and its amelioration in animals and human population at risk of being exposed to this compound. Therefore, the present study was taken to investigate the subchronic toxicity of thiamethoxam alone and in presence of quercetin in male Wistar rats.

MATERIALS AND METHODS

Animals: A total of 96 male Wistar rats weighing between 120-140 g were procured from the Disease Free Small Animal House of the university. The study was undertaken after due approval by Institutional Animal Ethical Committee of the University. Rats were housed in polyacrylic cages, 8 rats per cage in the departmental animal house. Bedding material (rice husk) was changed on alternate days. The animals were provided with feed and water *ad libitum* and maintained at room temperature (22 to 27^{o}C) with a natural light-dark cycle. The rats were acclimatized to laboratory conditions for 2-3 days before the experiment.

Drugs and Chemicals: Thiamethoxam (ACTARA®) 25G (Syngenta India, Ltd) and quercetin dihydrate were used in this experiment. Gum acacia was used as a vehicle for oral administration of the compounds. All glasswares were cleaned by dipping in 5% HCl, washed by detergent and rinsed in distilled water.

Experimental Design: Thiamethoxam at two dose levels (2.5% and 5.0% of MTD; 4200mg/kg b. wt.) was orally administered daily for 60 and 90 days in adult male rats for subchronic toxicity study. An ameliorative effect of Qu at dose rate of 50 mg/kg orally was also studied in rats administered thiamethoxam for 60 and 90 days. Details of each group are as follows**:** Group I: Vehicle control (16 rats), 2% gum acacia was given twice daily orally for 60 and 90 days; Group II: Thiamethoxam (2.5% of MTD) (16 rats), thiamethoxam suspension in 2% gum acacia was administered once daily orally for 60 and 90 days; Group III: Thiamethoxam (5.0% of MTD) (16 rats), thiamethoxam suspension in 2% gum acacia was administered once daily orally for 60 and 90 days; Group IV: Qu (50 mg/kg) (16 rats), Qu suspension in 2% gum acacia was administered once daily orally for 60 and 90 days; Group V: Thiamethoxam (2.5% of MTD) and Qu (50 mg/kg) (16 rats), Qu and thiamethoxam suspension in 2% gum acacia was administered once daily orally for 60 and 90 days. A gap of 12 h was maintained between thiamethoxam and Qu administration; and Group VI: Thiamethoxam (5.0% of MTD) and Qu (50 mg/kg) (16 rats), Qu and thiamethoxam suspension in 2% gum acacia was administered once daily orally for 60 and 90 days. Eight rats from each group were sacrificed on next day of completion of subchronic exposure to collect blood and different organs viz. brain, liver, testis and kidney for further studies.

Body Weight: Body weight (b.wt.) of rats was recorded on day one and at every alternate day, till the completion of experiment and relative weight gain was expressed as gram/100g b.wt.

Relative weight gain (gram/100g b.wt.) = [Final body weight (g) – Initial b.wt. (g)]/ Initial b.wt. (g) X 100.

Relative Organ Weight: After sacrificing the animals, vital organs viz. liver, heart, spleen, kidney and testis were collected and weighed individually. Later on, relative organ weights (per 100 g b.wt.) were calculated. Relative organ weight (per 100g b.wt.) =[Organ weight (g)/ Body weight (g)] x 100.

Sampling and Analysis: After euthanasia, blood samples were collected from the heart in heparinized vials for hematological parameter determination using standard procedure (Cole, 1986; Dacie and Lewis, 1994).

Statistical Analysis: The results obtained were presented as Mean$\pm$SE of mean (S.E.M). Difference between means was assessed using one way analysis of variance (ANOVA) and post-test using Bonferroni multiple comparison test (Mead and Curnow, 1982) using Graph Pad Prism Version 4.0 software. $P \leq 0.05$ was the critical criterion for the statistically significant differences.

RESULTS AND DISCUSSION

Pesticides are widely used to enhance crop production. Derivatives of these pesticides are highly toxic to a number of non-target organisms *viz*. humans,

birds, bees, fresh water fishes and other aquatic organisms (Chantelli-Forti *et al.,* 1993; Chaudhuri *et al.,* 1999; Oudou *et al.,* 2004; Begum, 2005; El-Sayed *et al.,* 2007). Increase in resistance to pesticides and their implication in public health leads to gradual replacement of the older insecticide families by newer pesticides groups such as neonicotinoids and phenylpyrazoles though very few toxicological studies have been conducted on these chemicals (Tomizawa *et al*., 2001; Tingle *et al*., 2003).

There was a significant ($p<0.05$) decrease in relative body weight and organ weight especially liver and kidneys of the male Wistar rats in groups II and III, treated with toxic doses of 2.5 and 5.0% MTD of thiamethoxam particularly in 60 days study. Rats in group II in 90 days study administered 2.5% MTD thiamethoxam also exhibited significant weight loss. Sole (2008) observed similar decrease in body weight gain in rats exposed to subchronic toxicity study. Pesticides generally affect body weight gain of mammals especially rats as was reported in the study of cypermethrin exposure by Hussain *et al. (*2009) *and* Lakkawar *et al.* (2004). The decrease in relative body and organ weight gain of these groups could be a result of toxicity of thiamethoxam on these organs which usually serve as a metabolizing and excretory organ to the compound, although the rats exposed to the oral doses of thiamethoxam exhibited some degree of anorexia which could also be the reason for weight loss.

The ameliorative effect of quercetin was exhibited in relative body weight gain at 2.5% MTD+ Qu treatment in 90 days treatment schedule. The Qu showed non-significant ameliorative effect on relative weight of liver at both doses and treatment schedules. The ameliorative effect of Qu on WBC count was observed at 2.5% MTD + Qu treatment in 60 days treatment schedule. Ameliorative effect of quercetin was seen on Hb at 2.5% MTD (TMX)+Qu dose at 60 days and at 2.5% MTD (TMX)+Qu and 5.0% MTD (TMX)+Qu dose in 60 and 90 days schedules, respectively. Amelioration in RDW was observed at higher dose in 60 days schedule. The ameliorative effect on PCV was seen at 2.5% MTD (TMX)+Qu and 5.0% MTD (TMX)+Qu in 60 days and 5.0% MTD (TMX)+Qu in 90 days treatment schedule. Amelioration in MCV was seen at 2.5% MTD (TMX)+Qu in 90 days schedule and in MCHC at 2.5% MTD (TMX)+Qu in 60 days schedule. Amelioration effect of quercetin is seen for MCH at 2.5% MTD (TMX)+Qu dose at 60 and 90 days schedule. The ameliorative effect of Qu on WBC count was observed at 2.5% MTD+Qu treatment in 60 days treatment schedule. The ameliorative effect was not observed in differential leucocytic counts in any of the treatment groups.

Evaluation of hematological indices plays a significant role in assessing the toxicity of compounds. Decrease or increase in the level of these parameters indicates problem with production, maturation and release of these cells into circulation which directly affects transportation of oxygen, food, drugs and immunological capacity of the animal thus affecting its physiological status significantly (Olson *et al*., 2000).

The results of hematological studies are presented in Table 3. Significant ($p<0.05$) decrease in Hb, RBC, PCV, WBC, MCV, MCH and MCHC were observed in 2.5 and 5.0% MTD thiamethoxam treated animals when compared to control group. The effect of thiamethoxam on these parameters could be as a result of inhibition of erythropoietin release by the kidney, which serves as a

Table 1
Effect of subchronic oral exposure of thiamethoxam, quercetin and their combination on relative body weight gain (g/100g bwt.)

	Relative body weight gain (g/100g b.wt.) in groups					
Days	I	II	III	IV	V	VI
60	64.02±1.20	53.53[a]±6.67	53.01[a]±4.85	46.89[a]±0.89	56.44[b]±0.86	45.52[a]1.11
90	68.38±1.91	57.39[a]±2.32	64.45±1.66	67.22[b]±0.44	65.17[b]±1.23	70.97[b]2.29

Values are expressed as Mean±SEM of eight animals in each group. a, b, c, d, e ($p<0.05$) vs. control. Group I=control; Group II=2.5% TMX, Group III= 5.0% TMX; Group IV= Quercetin alone; Group V=2.5% TMX+Qu; Group VI=5% TMX+Qu. TMX=Thiamethoxam, Qu=Quercetin.

Table 2
Effect of subchronic oral exposure of thiamethoxam, quercetin and their combination on relative organ weight (g/100g bwt.)

		Relative body weight (g/100g bwt.) in groups					
Organ	Days	I	II	III	IV	V	VI
Liver	60	3.44±0.07	2.49[a]±0.15	2.27[a]±0.33	3.16±0.17	3.63±0.16	4.07[d]±0.19
	90	2.77±0.19	1.98[a]±0.16	1.78[a]±0.18	2.61±0.19	2.63±0.21	2.89±0.06
Heart	60	0.29±0.06	0.28±0.03	0.29±0.02	0.29±0.34	0.27±0.01	0.26±0.01
	90	0.29±0.02	0.29±0.01	0.31±0.01	0.27±0.02	0.27±0.02	0.27±0.01
Spleen	60	0.22±0.06	0.24±0.02	0.23±0.01	0.19±0.06	0.18±0.09	0.17±0.02
	90	0.19±0.01	0.29±0.14	0.17±0.01	0.15±0.01	0.15±0.02	0.18±0.01
Brain	00	0.66±0.08	0.62±0.03	0.59±0.03	0.66±0.05	0.74±0.05	0.67±0.06
	90	0.56±0.08	0.52±0.03	0.59±0.03	0.56±0.05	0.54±0.05	0.57±0.06
Right kidney	60	0.32±0.03	0.23±0.01	0.22±0.01	0.27±0.01	0.25±0.06	0.26±0.02
	90	0.27±0.02	0.20[a]±0.01	0.19[a]±0.01	0.23±0.02	0.23±0.02	0.25±0.01
Left kidney	60	0.31±0.03	0.21[a]±0.01	0.20[a]±0.02	0.26±0.01	0.26±0.01	0.24[a]±0.01
	90	0.26±0.02	0.19 [a]±0.02	0.17 [a]±0.01	0.22±0.02	0.21±0.01	0.22±0.01
Right testis	60	0.52±0.02	0.56±0.04	0.55±0.03	0.52±0.04	0.53±0.02	0.50±0.03
	90	0.50±0.04	0.51±0.02	0.48±0.01	0.48±0.01	0.45±0.03	0.45±0.02
Left testis	60	0.56±0.02	0.59±0.04	0.58±0.03	0.54±0.04	0.57±0.02	0.56±0.03
	90	0.51±0.04	0.53±0.03	0.52±0.02	0.50±0.04	0.48±0.03	0.48±0.02

Values are expressed as Mean±SEM of eight animals in each group. a, d (p = 0.05) vs. control. Group I=control; Group II=2.5% TMX, Group III=5.0%TMX; Group IV= Quercetin alone; Group V=2.5% TMX+Qu; Group VI=5% TMX+Qu. TMX=Thiamethoxam, Qu=Quercetin.

precursor for erythrocytes production, maturation and release or interference of thiamethoxam with the absorption of folic acid, vitamin B complex or iron in the GIT since its orally administered as reported by earlier researchers (Polenakovic and Sikole, 1996; Sanchez-Elsner *et al*., 2004) which is reflected by significant ($p<0.05$) decrease in relative liver and kidneys weights as shown in Table 2. The level of these parameters is not significantly reduced when compared to control group. The Qu (Group IV) alone and thiamethoxam plus Qu treated groups (Groups V and VI) signify the amelioration properties of Qu (Renugadevi and Prabu, 2009).

The blood indices (MCV, MCH, MCHC) are of valuable importance in diagnosis of anaemia in mammals (Cole, 1986) therefore significant ($p<0.05$) decrease in these parameters suggests that thiamethoxam causes anaemia. There was a significant ($p<0.05$) increase in leucocytes such as neutrophils, eosinophils, basophils and monocytes whereas lymphocytopenia was observed 60 and 90 days post thiamethoxam administration. Groups administered Qu alone (Group IV) and combination of thiamethoxam and Qu (Group V and VI) at 60 and 90 days did not show significant difference in leucocytic values when compared with rats in the control group which signifies ameliorative properties of Qu.

Lymphocytopenia (lymphopenia) is a pathological condition where low level of lymphocytes in the blood is observed usually as a result of chemotherapy with cytotoxic agents or immunosuppressive drugs (Faguet, 1975; Weiss *et al*., 1975). Significant ($p<0.05$) level of lymphocytopenia observed post administration of 2.5 and 5.0% MTD thiamethoxam is suggestive of immunosuppressive effect of thiamethoxam in rats; similar characteristics was observed in honey bees that were exposed to clothianidin (Annely *et al*., 2016). These results suggest that thiamethoxam had immunosuppressive characteristics.

Neutrophilia was observed in groups II and III treatment where significant ($p<0.05$) neutrophil leukocytosis was observed after administration of 2.5 – 5.0% thiamethoxam (MTD=4200 mg/kg); though

Table 3

Effect of subchronic oral exposure of thiamethoxam, quercetin and their combination on hematological parameters

Parameters	Days	Haematological parameters in groups					
		I	II	III	IV	V	VI
HB (g/dL)	60	12.50±0.11	9.55^{a}±0.11	10.56^{a}±0.08	11.68^{abc}±0.15	10.59^{abd}±0.15	10.74^{abd}±0.25
	90	12.90±0.11	6.55^{a}±0.11	5.60^{a}±0.08	10.68^{bc}±0.15	10.60^{bc}±0.15	10.00^{bc}±0.23
RBC (x10^{6} /µL)	60	6.55±0.10	3.54^{a}±0.03	2.47^{ab}±0.11	3.59^{ac}±0.09	3.49^{ac}±0.11	3.65^{ac}±0.08
	90	6.55±0.11	3.54^{a}±0.03	2.47^{ab}±0.11	4.31^{abc}±0.09	3.52^{acd}±0.11	2.57^{abde}±0.08
PCV (%)	60	28.70±0.07	22.30^{a}±0.27	16.60^{ab}±0.08	24.10^{ac}±0.31	26.80^{ac}±1.09	24.2^{ac}±1.04
	90	16.60±0.08	22.90^{a}±0.17	16.64^{b}±0.08	25.05^{ac}±0.30	23.60^{ac}±1.37	25.4^{ac}±0.69
WBC (x10^{3} / µL)	60	8.50±0.13	5.50^{a}±0.11	7.50^{ab}±0.13	6.60^{abc}±0.08	8.60^{bcd}±0.09	7.70^{abde}±0.12
	90	8.50±0.12	5.47^{a}±0.11	7.50^{b}±0.13	3.26^{abc}±0.23	4.58^{acd}±0.28	4.35^{ac}±0.56
MCV (fL)	60	64.76±0.24	65.00±0.26	63.85±1.22	64.60±0.99	65.63±1.52	64.90±0.54
	90	64.74±0.24	55.06^{a}±0.29	64.58±1.26	65.80±0.23	58.93^{abcd}±1.17	64.89^{e}±2.58
MCH (pg)	60	34.23±0.74	26.25^{a}±0.80	36.04^{b}±0.96	45.29^{abc}±0.99	36.81^{bd}±0.89	35.14^{bd}±1.09
	90	34.24±0.75	25.78^{a}±1.05	36.06^{b}±0.96	24.98^{ac}±1.68	35.28^{bd}±2.41	28.33±3.17
MCHC (%)	60	55.65±1.14	45.55^{a}±0.99	55.64^{b}±0.90	76.08^{abc}±1.04	60.59^{bd}±2.38	44.30^{acde}±1.04
	90	55.65±1.14	45.55±0.99	55.64±0.90	34.65^{ac}±0.64	60.65^{d}±5.08	55.56±6.82
Lymphocytes (%)	60	88.48±0.81	89.48±0.57	79.01^{a}±1.07	85.33±0.81	80.76^{ab}±0.80	83.06^{b}±2.13
	90	90.91±0.81	82.4^{a}±0.57	82.10^{a}±1.07	88.80±0.80	87.10±0.80	86.76±2.13
Neutrophils (%)	60	7.41±0.08	4.52±0.09	12.19^{ab}±1.08	9.55^{abc}±0.09	12.65^{abcd}±0.48	10.45^{ab}±0.84
	90	4.80±0.08	9.50^{a}±0.11	10.5^{ab}±1.08	3.60^{abc}±0.39	4.10^{ad}±1.07	4.80^{b}±1.50
Eosinophils (%)	60	2.39±0.09	2.28±0.06	5.70^{ab}±0.09	1.35^{abc}±0.08	2.53^{c}±0.09	2.74^{cd}±0.21
	90	2.50±0.09	4.20^{a}±0.09	4.40^{a}±0.09	2.30^{bc}±0.64	4.10^{ad}±1.11	4.80^{ad}±0.54
Basophils (%)	60	0.28±0.05	0.36^{a}±0.02	0.40^{a}±0.05	0.48^{a}±0.10	0.30±0.02	0.34±0.02
	90	0.28±0.05	0.54^{a}±0.02	0.56^{a}±0.05	0.28^{a}±0.10	0.30±0.02	0.32±0.02
Monocytes (%)	60	1.44±0.09	3.36^{a}±0.09	2.70^{ab}±0.18	3.29^{ac}±0.08	3.76^{ac}±0.16	3.41^{ac}±0.09
	90	1.51±0.06	3.36^{a}±0.09	2.44^{a}±0.18	5.02^{abc}±0.39	4.40^{ac}±0.27	3.32^{ad}±0.51

Values are expressed as Mean±SEM of eight animals in each group. a, b, c, d, e ($p<0.05$) vs. control. Group I=control; Group II=2.5% TMX, Group III=5.0%TMX; Group IV=Quercetin alone; Group V=2.5% TMX+Qu; Group VI=5% TMX+Qu. TMX=Thiamethoxam, Qu=Quercetin.

Mahmoud and Magda (2010) reported that thiamethoxam @25, 50, 100 mg/kg in female rats produced no significant effect on hematological parameters. The reason for the variation in these results could be duration of exposure, where male rats were exposed to thiamethoxam for the period of period of 60 – 90 days at a higher dose in this study. Though eosinophils are leucocytes usually seen in parasitic infestation, basophils in inflammation and monocytes in inflammatory and myeloproliferative conditions, significant ($p<0.05$) levels of these cells was seen in rats administered 2.5–5.0% MTD of thiamethoxam when compared to the rats in the control group. Rats exposed to Qu alone, thiamethoxam and Qu did not manifest significant change in leucogram when compared to rats in control group indicating ameliorative property of Qu (Renugadevi and Prabu, 2009). The possible mechanism of Qu leading to its amelioration effect could be as a result of reducing the production of oxidative stress parameters such superoxide radicals and other related oxidative stress biomarkers and its potential to increase production of antioxidant substances such as glutathione complex biomarkers.

Conclusively, the study revealed the subchronic toxic effect of various doses of thiamethoxam on relative weight gain, organ weight especially liver and kidney, hematological and differential leucocytic count parameters in male Wistar rats. Amelioration effect of quercetin was observed on relative body weight gain, hemoglobin concentration, white blood cells and neutrophils count.

REFERENCES

Annely, B., Anna, G., Reinhold, S., Marina, M. and Ralph, B. (2016). The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera L.*). *J. Insect Physiol*. **86**: 40-47.

Begum, G. (2005). *In vivo* biochemical changes in liver and gill of *Clarias battrachus* during cypermethrin exposure and following cessation of exposure. *Pestic. Biochem. Physiol.* **82**: 185-196.

Chantelli-Forti, G., Paolini, M and Hrelia, P. (1993). Multiple end point procedure to evaluate risk from pesticides. *Environ. Health Perspect*. **101**: 15-20.

Chaudhuri, K., Selvara, S. and Pal, A.K. (1999). Studies on the genotoxicity of endosulfan in bacterial systems. *Mutant Res.* **439**: 63-67.

Coles, E.H. (1986). Veterinary Clinical Pathology. 4th edn. W.B. Saunders Co. Philadelphia.

Dacie, I.V. and Lewis, S.M. (1994). Practical Hematology. 8th edn. Churchill Livingstone, London, 49-59.

Dong, Z., Yang, N., Yeo, S.Y., Chitnis, A., and Guo, S. (2012). Intralineage directional notch signaling regulates self-renewal and differentiation of asymmetrically dividing radial glia. *Neuron* **74(1)**: 65-78.

El-Sayed, Y.S., Saad, T.T. and El-Bahr, S.M. (2007). Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. *Environ. Toxicol. Pharmacol.* **24**: 212-217.

European Commission Health & Consumer Protection Directorate-General. (2006) Directorate E-Safety of the Food Chain Unit E.3-Chemicals, contaminants, pesticides Thiamethoxam. SANCO/10390/2002 - rev. final 14 July 2006.

Faguet (1975). Quantitation of immunocompetence in Hodgkin's disease. *J. Clin. Invest*. **56**: 951-957.

Farombi, E.O., Adedara, M. and Tonwe, I.A. (2012). Quercetin protects against testicular toxicity induced by chronic administration of therapeutic dose of quinine sulfate in rats. *Physiol. Pharmacol*. **23**: 39-44.

Hussain, S., Khan, M.Z., Khan, A., Javed, I. and Asi, M.R. (2009). Toxico-pathological effects in rats induced by concurrent exposure to aflatoxin and cypermethrin. *J. Toxicol*. **53**: 33-41.

Lakkawar, A.W., Chattopadhyay, S.K. and Somuanshi, R. (2004). Experimental cypermethrin toxicity in rabbits- a clinical and patho-anatomical study. *Folia Veterinaria.* **48**: 3-8.

Mahmoud, H.I. and Magda E.M. (2010). Effect of thiamethoxam and emamectin benzoate on hematological, biochemical and histopathological parameters in female rats. *J. Agric. Chem. Biotech*. **8**: 457-472.

Martin, J.H., Tim, D.J and Dean, C.R. (2016).The degradation rate of thiamethoxam in European field studies. *Pest Management Sci*. **72**: 388-397.

Mead, R. and Curnow, R.N. (1982). A Simple Statistical Method in Agricultural and Experimental Biology. Chapman Hall, London, UK.

Morikawa, T., Chaipech, S., Matsuda, H., Hamao, M., Umeda, Y., Sato, H., Tamura, H., Ninomiya, K., Yoshikawa, M., Pongpiriyadacha, Y., Hayakawa, T. and Muraoka, O. (2012). Anti-hyperlipidemic constituents from the bark of *Shorea roxburghii*. *J. Nat. Med*. **66**: 516-524.

Moser, V.C. and Padilla, S. (1998). Age and gender related differences in the time course of behavioral and biochemical effects produced oral chlorpyrifos in rats. *Toxicol. Appl. Pharmacol*. **149**: 107-119.

Nauen, R., Ebbinghaus-Kintscher, U., Elbert, A., Jeschke, P.and Tietjen, K. (2001). Acetylcholine Receptors as Sites for Developing Neonicotinoid Insecticides. In: Biochemical Sites of Insecticides Action and Resistance. Ishaaya, I. (ed.), Springer Verlag, Berlin, Heidlberg, New York.

Olson, H., Betton, G., Robinson, D., Thomas, K. and Monro, A. (2000). Concordance of toxicity of pharmaceuticals in human and in animals. *Regul. Toxicol. Pharmacol*. **32**: 56-67.

Oudou, H.C., Alonso, R.M. and Hansen, H.C.B. (2004). Voltammetric behavior of the synthetic pyrethroid lambdacyhalothrin and its determination in soil and well water. *Analytica Chimica Acta.* **523**: 69-74.

Polenakovic, M. and Sikole, A. (1996). Is erythropoietin a survival factor for red blood cell. *J. Am. Soc. Nephrol*. **7**: 1178-1182.

Renugadevi and Prabu, A. (2009). Ameliorative effect of quercetin against cadmium induced toxicity in liver of Wistar rats. *J. Cell Tissue Res*. **9**: 1665-1672.

Roberts, T. and Hutson, D. (1999). Metabolic Pathways of Agrochemicals. Part two: Insecticides and Fungicides. The Royal Society of Chemistry, Cambridge.

Sanchez-Elsner, T., Ramirez, J.R., Rodriguez- Sanz- Varela, E., Bernabew, C. and Botella, L.M. (2004). A cross talk between hypoxia and TGF-â orchestrates erythropoietin gene regulation through SPI and smads. *J. Mol. Biol*. **36**: 9-24.

Sole, S.S., Vinod, K. and Rana, R.D. (2008). Acute toxicity studies of thiamethoxam in mice and rats. *J. Vet. Pharmacol. Toxicol*. **7**: 50-52.

Tingle, C.C., Rother, J.A., Dewhurst, C.F., Lauer, S. and King, W.J. (2003). Fipronil: environmental fate, ecotoxicology, and human health concerns. *Rev. Environ. Contamin. Toxicol.* **176**: 1-66.

Tomizawa, M., Cowan, A. and Casida, J.E. (2001). Analgesic and toxic effects of neonicotinoid insecticides in mice. *Toxicol. Appl. Pharmacol.* **177**: 77-83.

Waheed, M.P. and Mohammed, H.S.M.(2012). Fenvalerate induced hepatotoxicity and its amelioration by quercetin. *Int. J. Pharm. Tech. Res*. **4**: 1391-1400.

Weiss, R.B., Brunning, R.D. and Kennedy, B.J. (1975). Hodgkin's disease in the bone marrow. *Cancer.* **36**: 2077-2083.