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Nitenpyram-induced Hematotoxicity in Rat, *Rattus norvegicus*

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ABSTRACT

Nitenpyram is a neonicotinoid insecticide used to control the fleas and ticks in domestic animals and pets, which live in close association with humans. The main objective of this study was to evaluate the nitenpyram-induced hematotoxicity in albino rat. Sixteen albino rats in 4 groups were administered orally with LD_{10} and LD_{20} doses of nitenpyram in water for total 6 day period. Two groups, with 4 rats each, were used as control. One control and one group each from LD_{10} and LD_{20} treatments was anaesthetized and dissected for collection of blood samples at 3 and 6 day treatments. The hematotoxicity of nitenpyram was evaluated in terms of its effect on total erythrocytic count (TEC), total leukocytic count (TLC), platelet (PLT) count, hemoglobin (Hb) and haematocrit (Hct) estimation. These values were used to calculate hematological indices *i.e.,* MCV, MCH and MCHC. Nitenpyram at LD_{10} dose level produced mild haemotoxicity after 3 and 6 day treatments. During this period significant reduction (9%) in TEC was observed at 3day treatment while 5% decrease in Hb and 11% in HCT was noticed at 6 day treatment. Platelet count in LD_{10} dose however, showed 36% significant rise after 6 day treatment. LD_{20} dose of nitenpyram-fed for 3 days produced 44%, 20% and 21% decrease in Hb, TEC and Hct, respectively, with consequent decrease in MCV (6%), MCH (22%) and MCHC (20%). Platelet count showed significant increase (55%) during the same period. Similar decrease was also found when LD_{20} data was compared with LD_{10}. All these changes were statistically significant. LD_{20} dose administered to rats for 6 days also produced significant decrease in Hb (28%), TEC and Hct (22%) while alterations in index values remained statistically non-significant. On comparing LD_{20} data with LD_{10}, the significant decrease was noticed in Hb (24%), TEC (15%) and Hct (12%). The platelet count was increased significantly only in LD_{20} dose at 3 day. The TLC was increased by 43% only in LD_{20} dose after 6 day treatment. It is concluded from this study, that nitenpyram has a significant potential to induce mild to moderate hematotoxicity at LD_{10} while showed relatively high toxicity at LD_{20} doses fed to rats for 6 days. Further studies on mode of action and toxicity of nitenpyram on other systems are needed to evaluate its long and short term effects, not only in blood components but also on hepatic and renal functions in mammalian systems.

Key words: Pesticide toxicity, insecticide toxicity, blood abnormalities, hemopoietic effects.

INTRODUCTION

The use of synthetic chemicals like pesticides belonging to organochlorine, organophosphate, carbamate, pyrethroids and some new groups such as neonicotinoid and, pyrroles *etc.,* in human environment has been increased significantly since several decades, which is the major cause of environmental deterioration. The World Health Organization (WHO) estimated that about 25% of the diseases facing by mankind today occur due to prolonged vulnerability to environmental pollutants (EPA, 2006; WHO, 2008). Most of the sprayed pesticides including herbicides approach to the non-target systems and objects due to the aerial drift (Miller, 2004).

A study conducted by the Agency for Toxic

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Substances and Disease Registry (ATSDR, 2009) showed that in houses where parents use pesticides, children are twice as prone to develop brain cancer as compared to homes where pesticides are not used. Overuse of these harmful chemicals was considered as serious environmental hazard as mentioned by Carson (1962) in a book "Silent Spring" in which the author reveals about the disastrous effects of chemicals on human and animal health. Pesticides are associated with broad range of human and animal pathologies such as skin and eye irritation/damage, dizziness, nausea, fatigue, changes in immune system, systemic poisoning to chronic impacts like hepatic and liver damage, cancer, reproductive harm and endocrine disruption which may be fatal (Shakoori *et al.,* 1988; Pakington *et al.,* 2001; Alavanja *et al.,* 2004; Azmi *et al*., 2009; Shalaby *et al.,* 2012).

The unplanned use of these potentially dangerous chemicals has not only resulted in induction of variety of pathologies and abnormalities in non-target systems but also lead to the development of resistance in the target organisms (Casida, 1980; London *et al.,* 1998; Snedeker, 2001; Eljarrat and Barcelo, 2003; Tomlin, 2006; Beketov and Liess, 2008**;** Walker, 2009). Number of studies have depicted the effects of these insecticides on various components of blood and blood cells (Ali and Shakoori, 1988; Ali and Shakoori, 1997; Ali and Mir, 1998; Kazmi *et al.*, 2003; Mossa, 2004; Mansour and Mossa, 2005; Jamil *et al*., 2007; Azmi *et al.*, 2009; Emam *et al.,* 2012; Shalaby *et al.*, 2012; Shahi *et al.*, 2013; Khatun *et al*., 2014). Acute exposure to OPs leads to neurotoxic effects by inhibiting the enzyme acetylcholine esterase (Costa *et al.,* 2008). Chronic exposures to pesticides includes reduction in blood cholinesterase activity and possible subtle or subclinical neurological impacts (Pilkington *et al.,* 2001; Jamal *et al.,* 2002; Farahat *et al*., 2003; Albers *et al*., 2004; Young *et al.,* 2005; Rothlein *et al.,* 2006)

Recently a new group of insecticides *i.e.,* neonicotinoid, has been introduced (Sparks, 2013; Simon-Delso *et al*., 2014). Neonicotinoids are insecticides, which are chemically related to nicotine and belong to neuro-active class of insecticides. They were developed in 1980s by Shell (Kollmeyer *et al.,* 1999). Due to their different and systemic mode of action they are the most used insecticides now days (Jeschke *et al.,* 2011; Pollack, 2011; Krupke *et al*., 2012).

Nitenpyram is one such recently used neonicotinoid compound, which belongs to class nitromethylene (Jeschke *et al.,* 2011). It was discovered by Takeda Chemical Industries (Tinembart and Tashiro, 2000).

Nitenpyram is effective against fleas on domestic animals cats, dogs and kittens. It begins its activity within 30 minutes of application, but its efficacy lasts for 24 hours. It kills adult insects within few hours (Witte and Luempert, 2001). Like other neonicotinoids it is also an agonist of nAChR but has a systemic mode of action. It replaces the usual acetylcholine neurotransmitters present in the receptor but could not inhibit the receptor, and stop permanently because it cannot be shut off by acetyl cholinesterase. As a result of its interaction with nerve cells they are over activated that lead to paralysis and ultimate death of the organism (Schenker *et al.,* 2001).

The main objective of current study was to evaluate the effects of orally administered nitenpyram insecticide on some hematological parameters in mammalian model using albino rats as an experimental animal.

MATERIALS AND METHODS

Experimental animals and their maintenance

Twenty four healthy male albino rats (*Rattus norvegicus*) were collected and maintained in animal house of the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore. There was no significant difference in age and weight of individual rats. During the experiment the animals were provided with food and water *ad libitum.*

Insecticide used and its administration

The insecticide used during this study was a neonicotinoid, nitenpyram (10% EC) with IUPAC name(E)-N-((6-Chloropyridin-3-yl)methyl)-N-ethyl-N'-methyl-2-nitroethene-1,1-diamine; which was taken from Ali Akbar Group of Industries, Defense Road, Lahore. The insecticide was administrated to

rats orally along with water in two sub-lethal doses *i.e.,* LD_{10} and LD_{20} which were determined from its LD_{50} value as mentioned in Schenker *et al*. (2001).

Experimental procedure

Twenty four albino rats were divided randomly into six groups of 4 animals each. Two groups were used as control, one for 3 days and second for 6 days experiments. Similarly two groups were administered with LD_{10} and two with LD_{20} dose (one group each for 3 day and 6 day experiments). After the stipulated periods of 3 and 6 days, one control, one LD_{10} treated and one LD_{20} treated groups were dissected and their blood samples were collected for haematological studies.

Collection of blood samples

The blood samples were collected from inferior vena cava with 5ml syringe and immediately transferred into EDTA-coated tubes for various analyses. The samples were processed for analyzing total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin **(**Hb) and hematocrit (Hct) *i.e.,* packed cell volume, according to the hematological techniques as mentioned in Dacie and Lewis (1986).

Hematological indices

The hematological index values *i.e.*, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated using Hb and Hct concentration and TEC values according to the formulae given in Dacie and Lewis (1986).

RESULTS

The present study was conducted to determine the toxicity of two concentrations of nitenpyram on some hematological parameters of rat, *Rattus norvegicus* administered orally through water for the total period of six days.

Effects of nitenpyram administered to rats for three days

The Hb content did not show any significant change in LD_{10} dose while LD_{20} dose produced highly significant decrease (44%) when compared with control values. The Hb values also showed highly significant decrease of 43% ($p \leq 0.01$) in case of LD_{20} dose. The TEC showed gradual decrease of 9% and 21% from their respective control values which were statistically significant with $p \leq 0.05$ for LD_{10} and 0.01 for LD_{20} doses, respectively. On the other hand LD_{20} dose showed 13% highly significant decrease when compared with LD_{10} dose.

The Hct did not exhibit any significant change in LD_{10} dose while decreased 21% in case of LD_{20} dose when compared to control values (Table 1). The MCV did not show any statistically significant change both in weak (LD_{10}) and strong (LD_{20}) dose experiments.

The MCH also remained unaffected in case of LD_{10} dose while in LD_{20} dose it showed highly significant decrease of 23% with $p \leq 0.01$ when compared with control group (Table 1). Similar change was also observed in LD_{20} dose with 21% highly significant decrease as compared to LD_{10} dose.

The MCHC did not show any statistically significant change in case of LD_{10} when nitenpyram was administered for three days but decreased significantly (20%) in LD_{20} doses. On the other hand when LD_{20} dose was compared with LD_{10} dose it also exhibit highly significant decrease of 18% as shown in Tables 1-2.

Table 1: Effects of sublethal doses of nitenpyram administered to albino rats for 3 days on various haematological components in albino rats, *Rattus norvegicus*

Parameters	Control (n=4)	LD_{10} (n=4)	LD_{20} (n=4)
Hb (g/dl)	17.15±2.10	16.75±0.75	9.53±0.31***^{c}
TEC ($\times 10^{12}$/l)	8.43±0.21	7.70±0.05*	6.71±0.05**^{c}
PLT ($\times 10^{9}$/l)	530.51±5.77	594.36±21.26	789.18±23.40**^{a}
HCT (%)	42.54±1.35	39.04±0.57	33.67±2.72*
MCV (fl)	49.76±2.44	49.21±2.56	46.93±2.77
MCH (pg)	20.46±0.80	20.03±0.54	15.97±0.57**^{b}
MCHC (g/dl)	41.82±1.84	40.53±0.28	33.53±0.41*^{c}

Values in the table are given as means ± SEM; Student's "t" test; *significant at $P \leq 0.05$; **Significant at $P \leq 0.01$ and ***Significant at $P \leq 0.001$; a, b & c, represents significance b/w LD_{10} & LD_{20} doses.

Abbreviation used: Hb, hemoglobin; TEC, total erythrocytic count; PLT, platelet count; HCT, haematocrit (packed cell volume); MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Table 2: Percent increase (+) or decrease (-) in various haematological components of albino rat blood following nitenpyram administration for 3 days.

Parameters	Control vs LD_{10}	Control vs LD_{20}	LD_{10} vs LD_{20}
Hb	- 2.33	-44.26***	-42.93[c]
TEC	-8.65*	-20.42**	-12.98[c]
PLT	+12.03	+41.22**	+33.78[a]
HCT	-8.23	-20.77*	-13.66
MCV	-1.12	-5.74	-4.67
MCH	-2.1	-22.28**	-20.617[b]
MCHC	-3.03	-19.78*	-17.27[c]

For significance and abbreviations, see Table 1

Nitenpyram did not affect the PLT count in case of LD_{10} dose while LD_{20} dose produced 55% significant rise from control and 43% rise from LD_{10} value which was also statistically significant (Table 1).

The nitenpyram did not show any significant effect on TLC and differential leukocytes count (DLC). The small differences shown by different parameters were statistically non-significant as shown in Tables 5-6.

Effects of nitenpyram administered to rats for six days

The Hb content showed significant and gradual decrease in both low and high dose treatments, with $p \leq 0.05$ and 0.01 respectively when compared to control. When the effects of LD_{20} dose were compared to LD_{10} it also showed a statistically significant decrease of 24% with p value ≤ 0.01 (Table 3).

The TEC did not exhibit any significant change in case of LD_{10} dose while produced a significant decrease of 23% in case of LD_{20} dose. On the other hand LD_{20} dose showed a significant decrease (15%) with $p \leq 0.05$ when compared to LD_{10} dose.

Nitenpyram administration did not affect the Hct value in case of LD_{10} dose but shows a highly significant decrease of 22% in LD_{20} dose ($p \leq 0.001$). While LD_{20}exhibits significant change of 13% when was compared to LD_{10} dose (Tables 3-4). Administration of nitenpyram for six days did not exhibit any statistically significant change in MCV and MCH values in case of both the doses as shown in Table 3 and IV. While MCHC had not shown any prominent change in LD_{10} dose as compared to control and decrease of 12% in case LD_{20} which is statistically non-significant (Table 3).

The PLT count did not show any significant change in case of LD_{10} while the LD_{20} dose caused a significant increase of 58% with $p \leq 0.05$ when compared to control as shown in Tables 3-4).

Table 3: Effects of sublethal doses of nitenpyram on various hematological components, administered to albino albino rats for 6 days.

Parameters	Control	LD_{10}	LD_{20}
Hb (g/dl)	15.8±0.24	15.03±0.08*	11.46±0.56**[b]
TEC ($\times 10^{12}$/l)	8.76±0.27	8.00±0.34	6.8±0.23**[a]
PLT ($\times 10^{9}$/l)	611.3±98.78	833.67±144.75	964.67±67.02*
HCT (%)	49.67±0.87	44.33±1.76	39.00±0.57***[a]
MCV (fl)	57.33±0.87	56.67±0.87	55.67±0.67
MCH (pg)	19.00±0.57	18.00±0.57	17.67±0.32
MCHC (g/dl)	34.00±1.52	32.67±0.66	30.00±1.15

For significance and abbreviations, see Table 1.

Table 4: Percent increase (+) or decrease (-) in various haematological components of albino rat blood following nitenpyram administration for 6 days.

Parameters	Control vs LD_{10}	Control vs LD_{20}	LD_{10} vs LD_{20}
Hemoglobin	-4.87*	-27.46**	-23.75[b]
TEC	-8.67	-22.37**	-15.00[a]
PLT	+36.37	+57.80*	+15.71
HCT	-10.75*	-21.48***	-12.02[b]
MCV	-1.15	-2.89	-1.76
MCH	-5.26	-7.00	-1.83
MCHC	-3.91	-11.76	-8.17

For significance and other details, see Table 1.

The TLC value did not exhibit any significant change in LD_{10} dose while the LD_{20} dose shows highly significant increase of 44% with $p \leq 0.001$ when compared to control. On the other hand LD_{20} dose exhibit a rise of 39% as compared to LD_{10} dose (Tables 7-8).

Administration of nitenpyram cause rise in the DLC values which were statistically non-significant as shown in Tables 7-8.

Table 5: Effect of sublethal doses of nitenpyram administered to albino rats for 3 days on Total and differential leukocytic count (TLC and DLC).

Parameters	Control	LD_{10}	LD_{20}
TLC ($\times 10^9$/l)	7.8 ± 0.45	7.9 ± 0.05	8.13 ± 0.81
Neutrophils (%)	37.00± 0.57	39.00±0.57	40.33 ± 3.52
Lymphocytes (%)	58.00 ± 0.57	63.67±4.33	63.67 ± 4.09
Monocytes (%)	4.00 ± 0.57	4.30±0.60	5.67 ± 0.87
Eosinophils (%)	1.83 ± 0.16	2.33±0.32	2.67 ± 0.32
Basophils (%)			

Values are given as means ± SEM; Student's "t" test; *significant at P ≤ 0.05, ** significant at P ≤0.01 & ***significant at P ≤0.001; a, b & c, represents significance b/w LD_{10} & LD_{20} doses.
Abbreviations used: TLC, total leukocytic count; DLC, differential leukocytic count.

Table 6: Percent increase (+) or decrease (-) in TLC and DLC of albino rats blood following nitenpyram administration for 3 days.

Parameters	Control vs LD_{10}	Control vs LD_{20}	LD_{10} vs LD_{20}
TLC	1.28	4.23	2.91
Neutrophil	9.77	9.77	0
Lymphocytes	5.4	9	3.41
Monocytes	0	41.75	41.75
Eosinophils	27.32	45.9	14.6
Basophils (%)			

For abbreviations and other details, see Table 5

Table 7: Effects of nitenpyram administered to albino rats for six days on TLC and DLC values.

Parameters	Control	LD_{10}	LD_{20}
TLC ($\times 10^9$/L)	9.23±0.14	9.53±0.31	13.23±0.41***[b]
Neutrophils (%)	33.67±2.72	37.00±0.57	40.33±3.52
Lymphocytes (%)	76.00±4.35	88.00±8.50	89.00±4.35
Monocytes (%)	5.67±0.87	6.00±0.57	7.67±0.87
Eosinophils (%)	2.33±0.32	2.5±0.28	2.67±0.32

For abbreviations and other details, see foot note in Table 5

DISCUSSION

Nitenpyram is a new insecticide with novel systemic mode of action that is active against fleas, ticks, mites etc. It affects the AChE receptor of insects and kills the insect (Schenker *et al.,* 2001a and 2001b). Hematological parameters are extensively used in the diagnosis of various diseases and pathologies that are caused by various environmental pollutants like drugs, dyes, heavy metals and pesticides. Currently no studies are available on the hematological effects of neonicotinoid pesticides like nitenpyram however several studies on some other groups of insecticides have been conducted on non target mammalian systems (Morgan and Stockdale, 1980; Ali *et al*., 1988; Ali and Shakoori, 1988; Shakoori *et al*., 1988; Mossa, 2004; Mansour and Mossa, 2005).

Table 8: Percent increase in TLC and DLC following nitenpyram administration to albino rats for six days

Parameters	Control vs LD_{10}	Control vs LD_{20}	LD_{10} vs LD_{20}
TLC	3.25	43.33***	38.82[b]
Neutrophils	9.89	19.78	9
Lymphocytes	15.78	17.1	1.13
Monocytes	5.82	35.27	27.83
Eosinophils	7.29	14.6	6.8

For abbreviations and other details, see Table 5.

Hemoglobin concentration and TEC has direct correlation in all vertebrates including man (El-Bakary *et al*., 1995; Harris, 1972). In our study, hemoglobin content and TEC was reduced in all doses of nitenpyram. This may be due to higher breakdown rate or decreased proliferation rate of TEC. The recorded lower rate of TEC in the treated groups supported this observation during this study.

In another similar study Shakoori *et al.* (1990a) demonstrated the effects of Talstar (a pyrethroid) on the blood of rabbits with resultant decrease in TEC which may either be due to excess damage of erythrocytes or inhibition of erythropoiesis in rabbits. Furthermore, the biosynthesis of heme in hepatic tissues has also been reported to be affected by the exposure of insecticides that contributes to reduction of hemoglobin and TEC (Taljaard *et al.*, 1972). Ali and Shakoori (1990) have also reported the induction of anemia by the exposure of aldrin (OC insecticide) in experimental animals. The results in the current study are in agreement with the reported findings which showed significant decrease in TEC and hemoglobin concentration in rats provided spinosad

(a natural glycoside insecticide produced by fermentation of actinomycete) (Stebbins *et al.,* 2002; Yano *et al.*, 2002).

In the current study the Hct content was decreased in insecticide treated rats similar to the studies of Shakoori *et al.* (1990b), in which the decrease in TEC, Hct and hemoglobin content in the blood of rabbits was reported when treated with bifenthrin (a pyrethroid insecticide). Adedeji *et al.* (2009) have reported significant decrease in TEC, hemoglobin and Hct content in fishes when was exposed to diazinon (an OP insecticide). The effect of actellic (another OP insecticide) showed significant reduction in TEC, Hct and hemoglobin content in catfish, *Clarias albopunctatus* (Mgbenka *et al*., 2005). Yousef *et al.* (2003) reported the effects of cypermethrin, a synthetic pyrethroid, which caused a significant reduction in hemoglobin and TEC in rabbits. Ali and Shakoori (1981) demonstrated the decrease in TEC and hemoglobin content in rabbits when exposed to an organophosphate insecticide malathion. Khogali *et al.* (2005) have investigated the effects of dimethoate an organophosphate insecticide, on blood of mice which showed significant decrease in the hemoglobin content and Hct values in treated animals similar to the findings of current study. Fujitani *et al.* (1997) had shown the decrease in TEC, hemoglobin and Hct content in rats when was provided with a herbicide, chlorpropham.

Some studies have reported the effects of chlorpyrifos, an OP pesticide which shows a significant and transient decline in TEC, hemoglobin and Hct levels when administered to rats (Kazmi *et al.*, 2003; Akhtar *et al.*, 2009). Rahman and Siddiqui (2006) had noticed the sub chronic effects of phosphorothionate on Wistar rats. According to this study the levels of hemoglobin, Hct and TEC showed a significant reduction in treated rats. Shakoori *et al*. (1988) have also reported decrease in TEC and Hct content on blood of albino rats treated with cypermethrin. Many studies demonstrated that pesticides effects reduce the erythrocyte count, Hct and hemoglobin content. It was assumed that these changes were due to higher breakdown rate of TEC or due toxic effects of pesticides on bone-marrow decreasing the production of new blood cells (El-Sahhaf, 1995; Yousef *et al.,* 1999; Mossa, 2004; Mansour and Mossa, 2005).

Hematological indices values are sensitive indicators of various chemicals and environmental pollution in animal systems (Vosyliene, 1999; Shahi *et al*., 2013; Khatun *et al.,* 2014). Khalaf-Allah (1999) noticed significant decline in blood indices MCV, MCH and MCHC in pesticide treated *Tilapia nilotica* fishes. The decrease in MCV, MCH and MCHC values in different studies on fish exposed to lindane, an OC and diazinon, an OP has been reported (Adedeji *et al.*, 2009; Bhattacharjee and Das, 2013).Studies by Shahi *et al.* (2013) have also reported decrease in hematological indices (MCH, MCHC and MCV) values. These results are similar to the findings of present study. Fujitani *et al.* (1997) had shown the decrease in MCHC level in rats when was provided with chlorpropham. Azmi *et al.* (2009) have reported significant decrease in MCV, MCH and MCHC concentrations in farm workers.

Platelets are involved in blood coagulation. Blood coagulation requires proper size, number and function of platelet (Williams and Levine, 1982; Yakubu and Afolayan, 2009). Results from current study shows significant increase in platelet count in higher doses (LD_{20}) of nitenpyram administered to rats for 3 and six days. El-Sadek and Hassan (1999) had reported that the platelet level has increased in the treated animals. WHO has reported that several pesticides such as bentazone, caused increase in platelet count that is similar to the reported findings in current study (WHO, 2004). Some other reports have observed significantly higher levels of platelet count in farm workers exposed to pesticides (El-Saeed and Hassan, 2000; Azmi *et al.*, 2009; Emam *et al.,* 2012). Adedeji *et al.* (2009) have also reported significant increase in platelet count in fishes exposed to diazinon.

This study showed an increase in TLC and DLC values in insecticide treated rats at higher (LD_{20}) doses. The higher levels of leukocytes can occurred abnormally due to an infection, cancer, or effect of a toxic chemical and insecticide. This type of increase in TLC may be due to the activation of the animal's immune system and defense mechanism (Mansour *et al*., 2007). Yano *et al.* (2002) observed the effects of spinosad insecticide

in rats and showed increase level of TLC, lymphocyte and granulocytes in the blood which is according to them related to inflammation of lungs and thyroid. Shalaby *et al.* (2012) have noticed significant increase in TLC in pesticide spray workers. Ali and Shakoori (1990) have also observed the marked elevation in TLC as a result of aldrin, administered to rats. Ali *et al.* (1997) demonstrated the effects of another OP insecticide malathion on the blood of chicks that also showed significant increase in TLC, exposed to insecticide.

Stebbins *et al.* (2002) reported that TLC level in given spinosad treated mice were 2-2.5 times higher than the controls and increase in TLC which according to them was likely related to inflammation of the stomach. Studies by Shakoori *et al.* (1990a,b) on rabbits noticed the effect of Talstar and bifenthrin (pyrethroid insecticides) showed increase in TLC values. Fujitani *et al.* (1997) had shown significant increase in TLC level in rats when was exposed to chlorpropham. Akhtar *et al.* (2009) noticed an increase in TLC level in rats when was exposed to an OP, chlorpyrifos. Some other studies demonstrated the activation of the defense mechanism in pesticide treated animals which results in increase level of TLC in the treated animals (Yousef *et al*., 2003; Celik and Suzek, 2008; Celik *et al*., 2009). Many reports showed that treatment with pesticides influence the animal's immune system and defense mechanism, which could be the reason for bone marrow injuries due to exposure to toxic pollutant (Neuberger *et al.,* 1998; Mossa, 2004; Mansour and Mossa, 2005). Jamil *et al*. (2007) have reported increased TLC in agricultural workers who were exposed to pesticide.

The current study revealed the toxicological effects of nitenpyram on some hematological parameters of albino rats. In conclusion in three days study, LD_{20} dose produced maximum effect with decreasing almost all parameters significantly expect TLC and PLT count. In six days treatment, significant decrease was noticed in all parameters in LD_{20} dose while in LD_{10} dose decrease occurred in hemoglobin content only. The platelet count increased in both treatments at LD_{20} dose only. Defense induction occurred only at LD_{20} dose which was indicated by increasing Leukocyte count in LD_{20} dose following six day, treatment. When LD_{10} dose was compared to LD_{20} dose the pattern was almost similar as found in LD_{20} dose in both three and six day treatments.

It is supposed that this new generation of insecticide which were considered relatively less toxic to mammalian systems but it is not so. The current study has shown that this is not safe insecticide as for human health is considered. So, it is recommended that new group of pesticides like neonicotinoids also need to handle and use with care because they have shown significant hematotoxicity.

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