Journal of Research in Ecology

An International Scientific Research Journal

ISSN No: Print: 2319 –1546; Online: 2319– 1554

Original Research

Study of changes using biochemical markers in albino mice after acute exposure to acetamiprid

Authors:
Salah M. Al-Chalabi[1], Duha Zeki Al-Swefee[2] and Ahmed Jasim Mohammed[2]

Institution:
1. Biotechnology Researches Center, Al-Nahrain University, Baghdad, Iraq.

2. Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Corresponding author:
Salah M. Al-Chalabi

ABSTRACT:

The present study aimed to investigate the acetamiprid effects on biochemical aspects in albino mice. Thirty albino mice at the age of 6-8 weeks and average weight 25±5 g were divided into three groups each having ten (10) healthy mice. The first group was orally administrated with distilled water while the second and third groups were orally administrated with 50 mg/mL and 100 mg/mL respectively of acetamprid (0.1 mL) daily for one week. LD_{50} of acetamiprid was measured and found to be 200 mg/kg. The parameters of evaluations included liver function using Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP). Lipid profile was analysed using Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL). Antioxidant factors such as Superoxide Dismutase (SOD), Malondialdehyde (MDA), Catalase (CAT) and Glutathione Peroxidase (GPx), Calcium ion (ca^{2+}) and Aacetylcholine Esterase (AchE) were also measured. The study suggested that acetamprid 100 mg\mL significantly affected the biochemical component and it was considered to be a toxic dose of acetamiprid in albino mice.

Keywords:

Acetamprid, Albino mice, Biochemical markers, Acute toxicity.

Article Citation:
Salah M. Al-Chalabi, Duha Zeki Al-Swefee and Ahmed Jasim Mohammed
Study of changes using biochemical markers in albino mice after acute exposure to acetamiprid
Journal of Research in Ecology (2018) 6(2): 2192-2200

Dates:
Received: 19 Aug 2018 **Accepted:** 05 Sep 2018 **Published:** 26 Sep 2018

Web Address:
http://ecologyresearch.info/documents/EC0654.pdf

Journal of Research in Ecology

INTRODUCTION

Our environments as well as our health are constantly threatened by various pollutants such as xenobiotics. Pesticides are one of the most common pollutant groups in the world, and they have a major drawback such as toxicity (Speck-Planche *et al*., 2012). The continuous use of pesticide imposes hazardous effects on the plants. Since pesticides are harmful, they are possibly hazardous to living organisms and the earth (Lorenz, 2006). Besides, pesticides quickly spread around through different agents, for example, water, air and food chain (Rasgele *et al*., 2015). Insecticides are chemicals used to control insects by killing them or keeping them from engaging in practices with are undesirable or dangerous (EPA, 2016). Insecticides are used in different formulations and delivery systems (e.g., sprays, baits, goads, slow-release diffusion) that impact their transport and chemical transformation. Activation of insecticides can happen by means of runoff (either dissolved or absorbed to soil particles), atmospheric deposition (primarily spray drift), or sub-surface flow (Goring *et al*., 1972; Moore and Ramamoorthy, 1984).

Administration of the neonicotinoid insecticide may provoke alterations on the cholinergic system of rats and mice producing biochemical and behavioural effects that can be correlated to the toxicity produced by other kinds of pesticides that are linked to the development of neurodegenerative diseases such as Alzheimer‟s type dementia (Rodrigues *et al*., 2010). McCarthy and Shugart (1990) characterized biomarker as "measurements of body fluid, cells or tissues that indicate in biochemical or cellular terms the presence of contaminants or the magnitude of the host response". Furthermore there are many studies which indicated the increased use of pesticide resulted in toxicity in different species and could affect various functions like neurological, hematological, biochemical and reproductive function etc. in the body. These studies on toxicological aspect of insecticides are always useful for the rational treatment and prediction of the risk of toxicity (Mondal *et al*., 2009). Thus in the present study we used mice to focus on some biochemical changes as an indirect exposure in human and mammals in general.

MATERIALS AND METHODS

Animals

Thirty albino mice at the age of 6-8 weeks and average weight 25±5 g were obtained from Al-Razi center for occupational safety and health in Baghdad. The mice were housed in polypropylene cages under controlled conditions of temperature 25±5°C and 12±2 hours light/dark cycles. Diet and drinking water were given *ad libitum*. The animals were reared and treated in the animal house of Biotechnology research center Al-Nahrain university.

Chemical product

Commercial product of ACMP (Aster 20 SL, consisting of 200 g/L ACMP as active ingredient) is manufactured by Agrichem , Australia

Determination of the median lethal dose (LD_{50})

The method is divided into stages, with the outcome from each stage, the next step was determined and proceeded (i.e. whether to terminate or proceed to the next stage) for the next 24 h (Chinedu *et al*., 2013).

Experimental design

The intragastric ACMP doses were selected according to the acute oral LD_{50} value of ACMP (200 mg/kg) in albino mice. The body weight of controls as well as treated mice were taken weekly throughout the experiment and then on the day of sacrifice. For the preparation, dosage of each solution administrated was daily freshly prepared and adjusted weekly for body weight changes.

Treated groups

Acetamiprid was dissolved in distilled water of the pharmaceutical quality, and we have proceeded to give intragastrically 0.1 mL of insecticide every day for seven days. The test concentrations were calculated

Table 1 Effect on lipid profile parameters of albino mice after 7 days of the intragastric administration of acetamiprid

		Acetamiprid (mg/kg bw)			
S. No	Parameters (mg/dl)	Control	Group 1	Group 2	LSD P≤0.05
1		0	50	100	
2	TC	100.33±2.91[a]	123.33±2.03[b]	138.67±0.88[c]	4.47
3	TG	87.67±2.60[a]	124.00±1.73[b]	141.67±0.88[c]	3.98
4	HDL	26.67±0.88[b]	29.00±0.58[c]	23.67±0.33[a]	1.35
5	LDL	56.67±2.03[a]	70.67±0.88[b]	87.67±1.20[c]	3.08
6	VLDL	17.00±0.58[a]	24.67±0.33[b]	28.00±0.58[c]	1.08

Note: Small letters indicate to the comparison in column, similar letters are non-significant. Differences between means are significant at (p≤0.05), (LSD test). The results are expressed as mean ± SE

depending on the percentage of active ingredients of commercial formulation of acetamiprid.

Group 1: 7.5 mL of acetamiprid 20 SL was dissolved in 22.5 mL of distilled water and administered at a dose of 1/4 of LD_{50} (50 mg/kg).

Group 2: 15 mL of acetamiprid was dissolved in 15 mL of distilled water and administered at a dose of 1/2 of LD50 (100 mg/kg).

Control group: received orally an equivalent volume of distilled water as previously described for treated groups.

Blood samples

At the end of experiment period, blood samples were collected by cardiac puncture from each mice for hematological studies while plasma was extracted by centrifugation of the whole blood at 3000×g for 15 min for further biochemical analysis. Blood samples were collected in eppendorf centrifuge tube and centrifuged. For further biochemical analysis, such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphates (ALP), Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) were carried out. Antioxidant factors such as Superoxide Dismutase (SOD), Malondialdehyde (MDA), Catalase (CAT) and Glutathione Peroxidase (GPx), calcium (Ca^{+2}) and Acetylcholine Esterase (AchE) were studied (Mecdad *et al*., 2011).

Table 2. Effect on liver function parameters of albino mice after 7 days of the intragastric administration of acetamiprid

		Acetamiprid (mg/kg bw)			
S. No	Parameters (U/L)	Control	Group 1	Group 2	LSD P≤0.05
1		0	50	100	
2	AST	72.00±1.53a	87.67±2.85a	145.00±26.03b	32.09
3	ALT	18.33±0.88a	27.00±1.15a	32.67±2.33c	3.36
4	ALP	71367±3.18a	143.33±4.06c	180.67±3.76a	7.80

Note: Small letters indicate to the comparison in column, similar letters are non-significant. Differences between means are significant at (p≤ 0.05), (LSD test). The results are expressed as mean ± SE

Table 3. Effect on antioxidant factors parameters of albino mice after 7 days of the intragastric administration of acetamiprid

		Acetamiprid (mg/kg bw)			
S. No	Parameters (mg/dl)	Control	Group 1	Group 2	LSD P≤0.05
1		0	50	100	
2	MDA	1.13±0.07a	2.27±0.09b	3.67±0.09c	0.17
3	SOD	12.37±0.24c	8.73±0.20b	7.73±0.27a	0.51
4	CAT	26.33±0.62c	23.13±0.49b	11.93±0.15a	0.98
5	GPX	16.20±0.31c	13.70±0.55b	7.70±0.19a	0.87

Note: Small letters indicate to the comparison in column, similar letters are non-significant. Differences between means are significant at (p≤ 0.05), (LSD test). The results are expressed as mean ± SE

Statistical analysis

Statistical significance was determined using one-way Analysis of Variance (ANOVA) and Least Significant Differences (LSD) were used to explain the differences between means at (P≤0.05) and are expressed as mean ± SE (Gerry and Michael, 2002; Rosner, 2010).

RESULTS

Effect of treatment on lipid profile

The toxicology results of acetamiprid on lipid profile parameters are shown in Table 1. A statistically significant increase in TC, TG, LDL and VLDL while a decrease in HDL was observed in mice treated with higher doses (P≤0.05).

TC: There was a significant (P<0.01) increase in the level of serum cholesterol in animal groups treated with acetamiprid 50 and 100 mg/kg (123.33±2.03 and 138.67±0.88 mg/dl respectively) as compared with the control (100.33±2.91 mg/dl).

TG: The level of triglyceride in acute treatment obtained highly significant (P<0.01) increase in animals treated with acetamiprid 50 and 100 mg/kg (124.00±1.73 and 141.67±0.88 mg/dl respectively) as compared with control group (87.67±2.60 mg/dl). HDL, LDL and VLDL density lipoprotein: The results revealed a highly significant (P≤0.05) increase in HDL level in group treated with 50 and 100 mg/kg (29.00±0.58 and 23.67±0.33 mg/dl respectively) when compared with control group (26.67±0.88 mg/dl). In the same Table 1 the results illustrated that there was a highly significant (P<0.01) increase in LDL level in groups treated with acetamiprid 50 and 100 mg/kg (70.67±0.88 and 87.67±1.20 mg/dl) compared with control group (56.67±2.03 mg/dl). Finally, mean level of VLDL revealed a highly significant (P≤0.05) decrease in groups treated with 50 and 100 mg/kg (24.67±0.33 and 28.00±0.58 mg/dl) compared with control group (17.00±0.58 mg/dl).

Effect of treatment on liver function

The toxicology results of acetamiprid on liver function parameters are shown in Table 2. A statistically significant increase in AST, ALT and ALP observed in mice treated with higher doses (P≤0.05). AST: There was a significant (P≤0.05) increase in the level of AST in animals treated with acetamiprid at the concentration of 50 and 100 mg/kg (87.67±2.85 and 145.00±26.03 U/L) as compared with the control (72.00±1.53U/L).

ALT: The level of ALT in acute treatment obtained highly significant (P≤0.05) increase in animals treated with acetamiprid at the concentration of 50 and 100 mg/kg (27.00±1.15 and 32.67±2.33 U/L respectively) as compared with control group (18.33±0.88 U/L).

ALP: Mean level of ALP had a highly significant (P≤0.05) increase in groups treated with 50 and 40 mg/kg of acetamiprid (143.33±4.06 and 180.67±3.76 U/L) compared with control group (71.67±3.18 U/L) .

Effect of treatment on antioxidant factors

The toxicology results of acetamiprid on antioxidant factors parameters are shown in Table 3. A statistically significant increase in MDA while a decrease in SOD,CAT, GPx were observed in mice treated with higher doses (P≤0.05). MDA: There was a highly significant (P≤0.05) increase in the level of serum MDA in animals treated with acetamiprid at the concentration of 50 and 100 mg/kg (2.27± 0.09 and 3.67± 0.09 µM respectively) as compared with the control (1.13±0.07 mg/dl).

SOD: The results revealed a highly significant (P≤0.05) decrease in SOD level in groups treated with 50 and 100 mg/kg of acetamiprid (8.73±0.20 and 7.73±0.27 U/L respectively) when compared with control group (12.37±0.24U/L).

CAT: The level of CAT in acute treatment obtained significant (P≤0.05) decrease in animals treated with acetamiprid at 50 and 100 mg/kg (23.13±0.49 and 11.93±0.15 U/L respectively) as compared with control group (26.33±0.62 U/L).

GPx: Mean level of GPx showed a highly significant (P≤ 0.05) decrease in groups treated with 50 and 100 mg/kg of acetamiprid (13.70±0.55 and 7.70±0.19 U/L) when compared with control group (16.20±0.31 U/L).

Effect of treatment on Acetylcholine Esterase (AchE)

The toxicology results of acetamiprid on AchE is shown in Figure 1. A statistically significant increase in AchE was observed in mice treated with higher doses (P≤0.05). The results revealed a highly significant (P≤0.05) increase in AchE level in groups treated with acetamiprid at 50 and 100 mg/kg (3072.33± 61.11 and 3255.33±56.91 U/mL respectively) when compared with control group (2305.67±173.01 U/mL). However, there was no significant differences (P>0.05) between 50 and 100 mg/kg.

Effect of treatment on Calcium ion(Ca^{2+})

The toxicology results of acetamiprid on calcium ion is shown in Figure 1. A statistically significant decrease observed in mice treated with higher doses (P≤0.05). There was a highly significant (P≤0.05) decrease in the level of Ca^{2+} in groups treated with 50 and 100 mg/kg (7.70±0.06 and 6.90±0.06 mg/dl) as compared with control group (8.20±0.12 mg/dl).

DISCUSSION

The neonicotinoids have unique physical and toxicological properties as compared to earlier classes of organic insecticides. The mammalian toxicity of neonicotinoids is considered to be centrally mediated because the symptoms of poisoning are similar to those of nicotine (Tomizawa and Casida, 2005). However, information on its toxicity to mammalian is limited. The present study reveals statistically significant changes of some biochemical parameters of mice treated with the highest dose of acetamiprid. LD_{50} value was 200 mg/kg that correlated with Gathwan *et al.* (2016) who studied the histological and cytogenetic effects of acetamiprid on male albino mice and The LD_{50} value of acetamiprid was reported to be 198 mg/kg (approximately 200 mg/kg) in male mice and 184 mg/kg in female mice (Singh *et al*., 2012 a and b). The biochemical findings showed a significant increase in lipids levels that include TC, TG, LDL and VLDL while HDL was decreased relative to the control group. The increase in cholesterol value can also result from the inability of the organism to utilize or break it down to its derivatives or other useful products as a result of the toxicant effect (Edori *et al*., 2013). The rise in complete serum cholesterol level recorded in present examination could be because of blockage of liver bile ducts reducing of its secretion to duodenum along these lines causing cholestasis (Rai *et al*., 2009). The discoveries in the present examination concur with the reports of Mondal (2007) in oral admin-

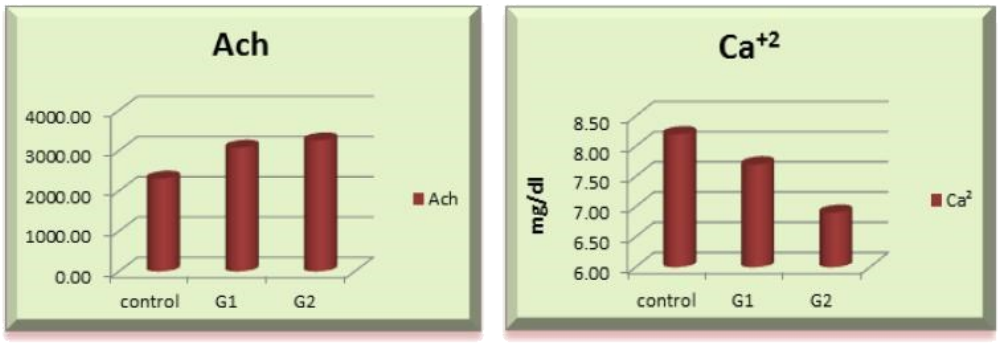

Figure 2. Means of acetylcholine esterase (Ach) and calcium ion (Ca^{+2}) with concentration exposed to acetamiprid sublethal concentrations with control sample

istration of acetamiprid to female wister rats. Zhang *et al*. (2010) affirmed noteworthy increase in serum Alanine Transaminase (ALT) of male mice in acetamiprid toxicity. Bhardwaj *et al*. (2010) revealed increase in ALT in imidacloprid toxicity in female rats. The present observations of increase in AST was in concurrence with the reports of Bhardwaj *et al*. (2010) in female rats following Imidacloprid administration and Zhang *et al*. (2010) in male mice following acetamiprid administration. Enhanced alkaline phosphatase value was likewise revealed by different researchers, for example, acetamiprid toxicity in female rats by Mondal *et al*. (2009). Elevated ALP was because of its expanded synthesis due to damaged liver conditions (Seetharam *et al*., 1986). Increase in plasma ALP may be because of acute hepatocellular damage and devastation of epithelial cells in gastrointestinal tracts (Zimmerman,1969). The results of antioxidant factors showed that increase in MDA while SOD, CAT and GPx were decreased. MDA is a biomarker that provides an indication of lipid peroxidation level (Bhale *et al*., 2014). The most deleterious impact of oxidative stress is lipid peroxidation, which has been implicated in the pathogenesis of numerous diseases including atherosclerosis, diabetes, cancer and aging (Spiteller, 2007).

Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) are the first line of defence against ROS and other free radicals (Rahaman *et al*., 1999). Whenever the amount of ROS and other radicals exceeds the capacity of such enzymes, non-detoxified radicals begin to attack cellular macromolecules. Excess production of radicals may occur in cases of diseases, as well as during the biotransformation of various xenobiotics (Mao *et al*., 2007). Kapoor *et al*. (2010) indicated that exposure to high doses of imidacloprid (20 mg/kg/day) for 90 days produced significant decreases in Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) activities in the brains of female rats.

Acetylcholine Esterase (AchE) results showed significant increase at high concentration. The insecticidal activity of neonicotinoids is primarily attributed to their action on nicotinic acetylcholine receptors (nAChRs) Acetamiprid has relatively higher affinities for rodent nicotinic acetylcholine receptors (nAChRs) than other neonicotinoids (Tomizawa and Casida, 1999). Neonicotinoids exhibited elevated Acetylcholinesterase (AChE) activity in honey bees at sublethal doses. High AChE levels may in this manner act as a biomarker of neonicotinoids exposure (Robert *et al*., 2015).

Decreased level of calcium ion (Ca^{2+}) in serum is called hypocalcemia that most commonly results when too much calcium is lost in urine or when not enough calcium is moved from bones into the blood. Causes of hypocalcemia may include a low level of par-

athyroid hormone, vitamin D deficiency, kidney dysfunction, which results in more calcium excreted in urine and makes the kidneys less able to activate vitamin D and disorders that decrease calcium absorption (Fong and Khan, 2012).

CONCLUSION

The biochemical parameters showed significant changes in mice under impact of exposure to acetamiprid after one week (acute). Measuring antioxidant enzymes in serum could be an important sign for toxicity.

REFERENCES

Bhale DV, Dhanashri SP, Roshan KM and Patil. 2014. Study of Malondialdehyde (MDA) as a marker of oxidative stress in obese male individuals. *International Journal of Recent Trends in Science And Technology*, 10(1): 51-52.

Bhardwaj S, Srivastava MK, Kapoor U and Srivastava LP. 2010. A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. *Food and Chemical Toxicology*, 48(5): 1185-1190.

Chinedu E, Arome D and Ameh FS. 2013. A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3): 224-226.

Edori OS, Nwoke IB and Edori ES. 2013. Sublethal effects of diesel on total protein levels and cholesterol in *Tympanotonus fuscatus*. *Journal of Applied Sciences and Environmental Management*, 17(1): 21-26.

EPA. 2016. Insecticides. http://www.epa.gov/caddis/ssr_ins_int.html.

Fong J and Khan A. 2012. Hypocalcemia updates in diagnosis and management for primary care. *Canadian Family Physician*, 58(2): 158-162.

Gathwan MA, Kadri ZHM, Karieb SS and Thwaini QS. 2016. Histological and cytogenetic effects of acetamiprid on male albino mice. *Al-Mustansiriyah Journal of Science*, 27(3): 17-23.

Gerry PQ and Michael JK. 2002. Experimental design and data analysis for biologists. Cambridge University press, New York. 556 p.

Goring Cleve AI and Hamaker John W. 1972. Organic chemicals in the soil environment, 2nd volume. Marcel Dekke. 968 p.

IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

Kapoor U, Mithilesh KS, Shipra B and Laxman PS. 2010). Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive it's No Observed Effect Level (NOEL). *The Journal of Toxicological Sciences*, 35(4): 577-581.

Lorenz SE. 2006. Toxicity of pesticides, pesticide safety fact sheet. College of Agricultural Sciences , Agricultural Research and Cooperative Extension Pesticide Education.

Mao H, Fang X, Floyd KM, Polcz JE, Zhang P and Liu B. 2007. Induction of microglial reactive oxygen species production by the organochlorinated pesticide dieldrin. *Brain Research*, 1186: 267-274.

McCarthy JF and Shugart LR. 1990. Biomarkers of environmental contamination. Lewis Publishers, Chelsea, Mich. 457 p.

Mecdad AA, Ahmed MH, ElHalwagy MEA and Afify MMM. 2011. A study on oxidative stress biomarkers and immunomodulatory effects of pesticides in pesticide -sprayers. *Egyptian Journal of Forensic Sciences*, 1(2): 93–98.

Mondal S, Ghosh RC, Mate M and Ghosh CK. 2009. In-vivo sub-acute oral acetamiprid toxicity on hemato-

logical indices in *Rattus norvegicus*. *Environment and Ecology*, 27(4A): 1767-1769.

Mondal S. 2007. Studies on the toxicopathology of acetamiprid in rats. M.V.Sc Thesis submitted to college of veterinary science and animal husbandry. Indira Gandhi Krishi Vishwavidyalya, Durg, India.

Moore JW and Ramamoorthy S. 1984. Heavy metals in natural waters applied monitoring and impact assessment. Book. Springer-Verlag New York. 268 p.

Rahaman Q, Abidi P, Afaq F, Shiffman D, Mossman BT, Kamp DW and Athar M. 1999. Glutathione redox system in oxidative lung injury. *Critical Reviews in Toxicology*, 29(6): 543-568.

Rai DK, Prashant KR, Aradhna G, Geeta W and Bechan S. 2009. Cartap and carbofuran induced alterations in serum lipid profile of wistar rats. *Indian Journal of Clinical Biochemistry*, 24(2): 198-201.

Rasgele PG, Okaty M, Kekecoglu M and Muranli FDG. 2015. The histopathological investigation of liver in experimental animals after short-term exposures to pesticides. *Bulgarian Journal of Agricultural Science*, 21(2): 446–453.

Robert OS, Geneviève L, Pierre-Luc M, Madeleine C, Nicolas D and Valérie F. 2015. Increased acetylcholinesterase expression in bumble bees during neonicotinoid-coated corn sowing. *Scientific Reports*, 5.

Rodrigues LA, Maschio LJ, Evangelista da Silva R and Caetano pinto da Silva ML. 2010. Adsorption of Cr (VI) from aqueous solution by hydrous zirconium oxide. *Journal of Hazardous Materials*, 173: 630–636.

Rosner B. 2010. Fundamentals of biostatistics. Brookscole /Cengage learning. 849 p.

Seetharam S, Sussman NL, Komoda T and Alpers DH. 1986. The mechanism of elevated alkaline phosphatase activity after bile duct ligation in the rats. *Hepatology*, 6(5): 374-380.

Singh TB, Mukhopadhayay SK, Sar TK and Ganguly S. 2012a. Acetamiprid induces toxicity in mice under experimental conditions with prominent effect on the hematobiochemical parameters. *Journal of Drug Metabolism and Toxicology*, 3: 134.

Singh TB, Mukhopadhayay SK, Sar TK and Ganguly S. 2012b. Induced acetamiprid toxicity in mice: a review. *Journal of Drug Metabolism and Toxicology*, 3: e115. doi: 10.4172/2157-7609.1000e115 6.

Speck-Planche A, Kleandrova VV, Luan F and Cordeiro MN. 2012. Predicting multiple ecotoxicological profiles in agrochemical fungicides: a multi-species chemoinformatic approach. *Ecotoxicology and Environmental Safety*, 80: 308–313.

Spiteller G. 2007. The important role of lipid peroxidation processes in aging and age dependent diseases. *Molecular Biotechnology*, 37(1): 5-12.

Tomizawa M and Casida JE. 1999. Minor structural changes in nicotinoid insecticides confer differential subtype selectivity for mammalian nicotinic acetylcholine receptors. *British Journal of Pharmacology*, 127(1): 115–122.

Tomizawa M and Casida JE. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annual Review of Pharmacology and Toxicology*, 45: 247–68.

Zhang JJ, Wang Y, Xiang H, Xue M, Li WH, Kai-ge MA, Wang XZ and Zhang JH. 2010. Oxidative stress: role in acetamiprid induced impairment of the male mice reproductive system. *Agricultural Sciences in China,* 10(5): 786-796.

Zimmerman HJ. 1969. Serum enzymes determination as an aid to diagnosis. In: Clinical diagnosis by Labora-

tory methods. Davidson I and Henry JB ed. W B Saunders Co, Philadelphia. 719.