ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Alterations in key enzymes and micromorphology of vital organs during exposure of imidacloprid in albino rats

***Prerna Vohra, Kuldeep Singh Khera**

Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana 141004, Punjab, India

Manuscript Info

Manuscript History:

Received: 15 January 2015
Final Accepted: 22 February 2015
Published Online: March 2015

Key words:

Imidacloprid, Two generation study, Histopathology, Biochemical analysis, Wistar albino rat

****Corresponding Author***

***Prerna Vohra**

Abstract

The effect of oral administration of two doses of imidacloprid 10mg/kg/day and 20 mg/kg/day (corresponding to $1/45^{th}$ and $1/22^{th}$ LD_{50} of imidacloprid) on alterations in biochemical milieu and histopathology of female albino rats of two generations was assessed. This study was designed to evaluate the effects of imidacloprid on the female wistar rats that were treated through two generations (F0 and F1 generation). Average feed intake of F1 female rats was significantly reduced ($P<0.01$) at 20mg/kg/day dose of imidacloprid. At the end of experiment F1 rats were sacrificed, significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP) and alkaline phosphatase (AKP) enzyme activities were observed in higher dose treated groups of F1 females. There was significant decrease ($P<0.05$) in acetyl cholinesterase (AChE) activity in plasma and brain of higher dose treated groups. Tissue samples of liver, kidney and brain were subjected to histopathological examination. Based on the present physiological, biochemical and histological studies it is evident that imidacloprid did not produce any significant effects at 10 mg/kg/day dose but induced toxicological effects at 20 mg/kg/day to female rats.

INTRODUCTION

Indiscriminate usage of pesticides in agriculture is leading to contamination of environment and natural resources, and thereby producing an adverse impact on animal and human health. Imidacloprid is relatively a new pesticide which belongs to the class of the neonicotinoids compounds[1].It is one of the fastest sold insecticide across the world because of its high selective toxicity in insects and apparent safety in humans. Due to its acute toxicity, imidacloprid has become one of the most widely used insecticides in the world[2].It is one of the fastest sold insecticide across the world because of its high selective toxicity in insects and apparent safety in humans[3]. Its selective toxicity results from its high affinity to insect's nicotinic acetylcholine receptors compared to mammals[4]. It acts on nervous system by blocking post-synaptic acetylcholine receptors, which kills the insect[3]. It was demonstrated that acute exposure to imidacloprid leads to oxidative and inflammatory effects in rats.[5]It wasreported that treatment with oral 0.5, 2 or 8 mg/kg imidacloprid induced morphological changes, DNA fragmentation, antioxidant imbalance and apoptosis in the reproductive system of developing male rats[6]. Experimental studies suggest that chronic exposure to imidacloprid may induce neurobehavioral deficits in offspring rats following in utero exposure,[7]genotoxic and mutagenic alterations[8].

Biochemical alterations in response to pesticides are considered to be rapidly responding endpoints and thus most biochemical biomarkers in the laboratory studies are assessed after acute exposure to chemicals. Changes in the biochemical profile indicate alterations in metabolism of the organism resulting from the effect of the

pesticide and they make it possible to study the mechanisms of the effects of these pesticides[9]. Since liver is associated with metabolism and elimination of toxicants from the body and its histological and biochemical parameters are considered as key points to elucidate toxicity of the chemicals. Imidacloprid treated birds also showed significant increase in serum aspartate aminotransferase (AST) level at 14 and 28 days of experiment, while no significant change in serum alanine aminotransferase (ALT), serum total protein, serum total albumin, serum total globulin and serum creatinine was seen.[10]However, it has been established that on repeat administration at 20 mg/kg per day, imidacloprid produces pathomorphological changes and hormonal imbalance in female rats[11]. The availability of such information regarding the effect of imidacloprid on the female reproductive system, indicates the necessity to explore a two-generation study, to assess the outcome of imidacloprid exposure to gravid females and their subsequent progeny.

Materials and Methods

Chemical- Commercial product of imidacloprid (Confidor, 17.8% w/w, Bayer) used in this study was purchased from the local market in Ludhiana, India.

Animals- Eighteen female and eighteen male Wistar albino rats, obtained from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The animals were housed in groups of two rats per cage. The rats were acclimatized for one week before using them for experimentation. The rats were maintained under controlled conditions of temperature (22 ± 2^0C) and humidity (30–70%) with 12 h light and dark cycle. The animals were given standard diet containing pelleted food and water ad libitum. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. The Institutional Animal Ethics Committee (IAEC) approved this experimental protocol.

Experimental design and treatment- Eighteen female rats were divided into three groups. Group 1 served as Control and given corn oil through oral intubation. Group 2 treated with $1/45^{th}$ $LD_{50}$of imidacloprid. Group 3 treated with $1/22^{th}$ LD_{50} of imidacloprid. Dams and their offsprings were treated with imidacloprid during the periods of mating, gestation and lactation. The offsprings of different dams in a group of each generation were mated among themselves throughout two generations. F1generation was attained by the same procedure.

Processing of tissues for histopathology- All F1 rats were weighed and sacrificed by chloroform anaethesia. The tissues of liver, kidney and brain were removed, weighed and immediately fixed in 10% formaldehyde. After the routine procedure, the fixed tissues were embedded in paraffin and 5 µm thick tissue sections were stained with routine haemotoxylin and eosin (H&E) in order to examine under lightmicroscopy.

Sample preparation for biochemical analysis- All F1 rats were weighed and sacrificed by chloroform anaesthesia. After sacrificing, blood samples were collected directly from heart of F1 rats, drawn into heparinized tubes and plasma was separated by centrifuging at 3000rpm for 10 minutes at room temperature. The effect of imidacloprid on aspartate aminotransferase, alanine aminotransferasewas estimated by the method of Reitman and Frankel as described by Bergmeyer[11] (1974) and acid phosphatase, alkaline phosphatase was estimated by method of Besseyet al.[12] (1946),glucose 6-phosphate dehydrogenase (G6PD) by method of Deutsch[13], acetylcholine esterase (AChE) activity by the method of Vass and Sachsse[14] as modified by Morai et al. (1946) and total protein by method of Lowry[15] et al.(1951).

Statistical Analysis- Biochemical analyses were presented as the mean ± standard error of means (SEM). Comparisons were made between control and treated groups using ‘‘Analysis of Variance (ANOVA)’’ as a statgraphics statistical package.

Results

Clinical symptoms and cyclicity- No signs of toxicity were seen in clinical appearance of new borns in all the three generations. Number of offsprings in F1 and F2 generations are shown in Table 1.

Body weight and relative organ weight- There was no significant disruption in cyclicity of female rats of F1 generation. Body weight of female F1 rats are given in Table 2. The relative organ weights of vital organs of females are shown in Table 3. The relative organ weight of endocrine glands also didnot differ significantly in control and both the treated group rats as shown in Table 4.

Biochemical analysis-The plasma biochemical parameters of female rats orally administered imidaclopridare shown in Table 5.There was significant increase in activity of AST, and ACP and non-significant increase in the activity of ALT, AKP and G6PD in the plasma of$1/22^{th}$ LD_{50} of imidacloprid treated group as compared to control group.There was significant increase in the plasma proteins of$1/22^{th}$ LD_{50} of imidacloprid treated group. Plasma and brain AChE activity was significantly decreased at$1/45^{th}$ and $1/22^{th}$ LD_{50} of imidacloprid treated rats.

Histopathological examination- Histopathological examination of liver of control rat showed radially arranged hepatic cords (RHC) around the central vein (CV) and sinusoids (S). Liver section of T1 treated rat showed dilation of central vein and infilteration by large mass of leukocytic inflammatory cells (LIC). Liver section of T2 treated rat showed infilteration by large mass of leukocytes cells (LIC) in the sinusoids and hepatic cells with pyknotic nuclei.(Fig 1)

Histopathological examination of brain of control rat showed normal structure of neurons and granular layers. T.S of brain of $1/45^{th}$ LD_{50} of imidacloprid showed degenerating neurons with pyknotic nuclei and swelling in neurons. (Fig 2)

Histopathological examination of kidney of control rat showed intact capsule with well demarcated glomerular tuft (G) and normal arrangement of tubules in the cortical portion. Kidney of T1 treated rat showing blood congestion in between tubules and small area of hemorrhage in the interstitium in rats. Kidney of T2 treated rat showing enlargement in parietal layer of Bowman's capsule and tubular degenerations. (Fig 3)

Table 1

Number of male and female offsprings in Groups I-III for three generations

Groups	F0	F1			F2		
	Female	Male	Female	Total	Male	Female	Total
Group I	6	20	16	36	25	17	42
Group II	6	18	7	25	19	17	36
Group III	6	20	13	33	27	10	37

Table 2

Effect of imidacloprid on body weight of female albino rats of F1 generation as compared to control

Traits	F1		
	Control	T 1	T 2
One week before mating	149.1 ± 5.54	140.83 ± 1.53	141.6 ± 6.54
Mating	158.33 ± 3.33	152.5 ± 6.02	152.5 ± 1.11
Gestation	169.16 ± 1.53	167.5 ± 2.5	166.6 ± 3.80
After Delivery	158.33 ± 3.33	159.16 ± 4.36	145.8 ± 7.12
During Lactation	185 ± 2.88	181.66 ± 3.07	176.6 ± 4.94

Values are Mean ± SE

Table 3

Relative organ weight (g %) data of organs of female rats orally administered imidacloprid

Organs	Control	T 1	T 2
Liver	3.36 ± 0.181	3.66 ± 0.146	3.91 ± 0.149*
Stomach	1.97 ± 0.225	2.46 ± 0.308	2.51 ± 0.44
Lungs	0.53 ± 0.017	0.59 ± 0.011*	0.68 ± 0.045*
Heart	0.29 ± 0.016	0.31 ± 0.013	0.31 ± 0.008
Spleen	0.22 ± 0.010	0.22 ± 0.014	0.26 ± 0.015
Kidney	0.56 ± 0.028	0.59 ± 0.023	0.60 ± 0.033

Values are Mean ± SE of six animals in each group

*Significantly different from control at P<0.05

Table 4

Relative organ weight (g %) data of endocrine glands of female rats orally administered imidacloprid

	Control	T 1	T 2
Adrenal	0.02 ± 0.002	0.03 ± 0.002	0.03 ± 0.006
Thyroid	0.19 ± 0.004	0.21 ± 0.007	0.20 ± 0.008
Parathyroid	0.07 ± 0.002	0.07 ± 0.008	0.07 ± 0.007

Values are Mean ± SE of six animals in each group

Table 5

Plasma biochemical data of female rats orally administered imidacloprid

Parameters	**Dosage/mg/kg/d**		
	0 (Control)	**10**	**20**
ALT (U/L)	32.13 ± 4.65	37.87 ± 4.07	55.49 ± 6.23*
AST (U/L)	114.6 ± 19.92	135.4 ± 23.77*	141.8± 23.62*
AKP (U/L)	82.54 ± 15.21	115.61 ± 20.87**	146.1 ± 5.38*
ACP (U/L)	1.08 ± 0.19	1.16 ± 0.27	2.46 ± 0.36**
Total proteins(g/dl)	8.21 ± 0.32	8.43 ± 0.34	8.59 ± 0.57
G6PD (U/L)	1054 ± 66.9	1686 ± 209.46	1946 ± 198.3

Values represent the mean ± SE of 6 animals in each group

*Significantly different from control at P<0.05

**Significantly different from control at P<0.01

Table 6

Acetylcholinesterase inhibition of brain and plasma of female rats after oral administration of imidacloprid

Dosage (mg/kgbw/day)	**AChE (U/L)**	
	Plasma	**Brain**
0	988.1 ± 99.54	852.79± 76.47
10	824.1 ± 91.71	769.30 ± 111.97
20	607.5 ± 31.57*	643.5 ± 89.13*

Values represent the mean ± SE of 6 animals in each group

*Significantly different from control at P<0.05

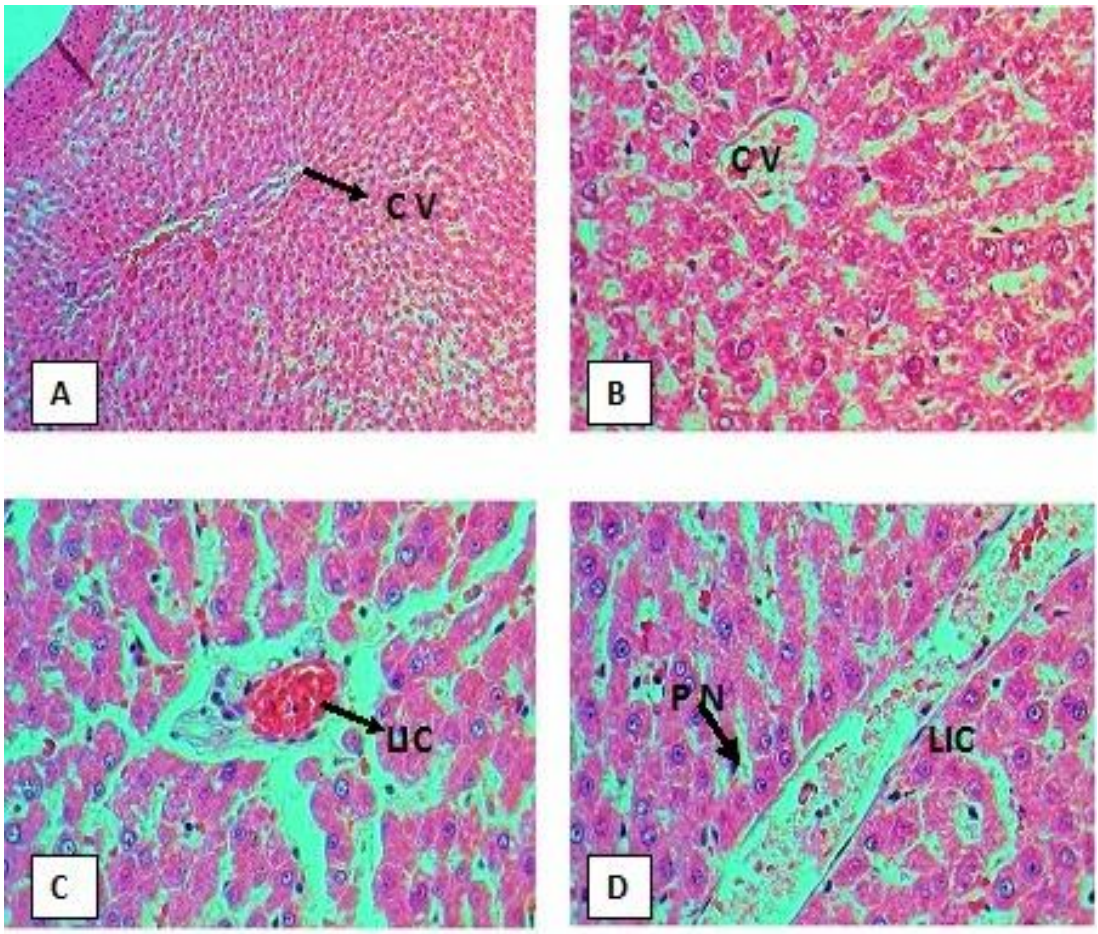

Fig 1 A. T.S of liver of control rat of F1 generation showing radially arranged hepatic cords (RHC) around the central vein (CV) and sinusoids (S)

B. Magnified view of section of liver of control rat

C. T.S of liver of T1 treated rat showing dilation of central vein and infilteration by large mass of leukocytic inflammatory cells (LIC)

D. T.S of liver of T2 treated rat showing infilteration by large mass of leukocytes cells, (LIC) in the sinusoids and hepatic cells with pyknotic nuclei

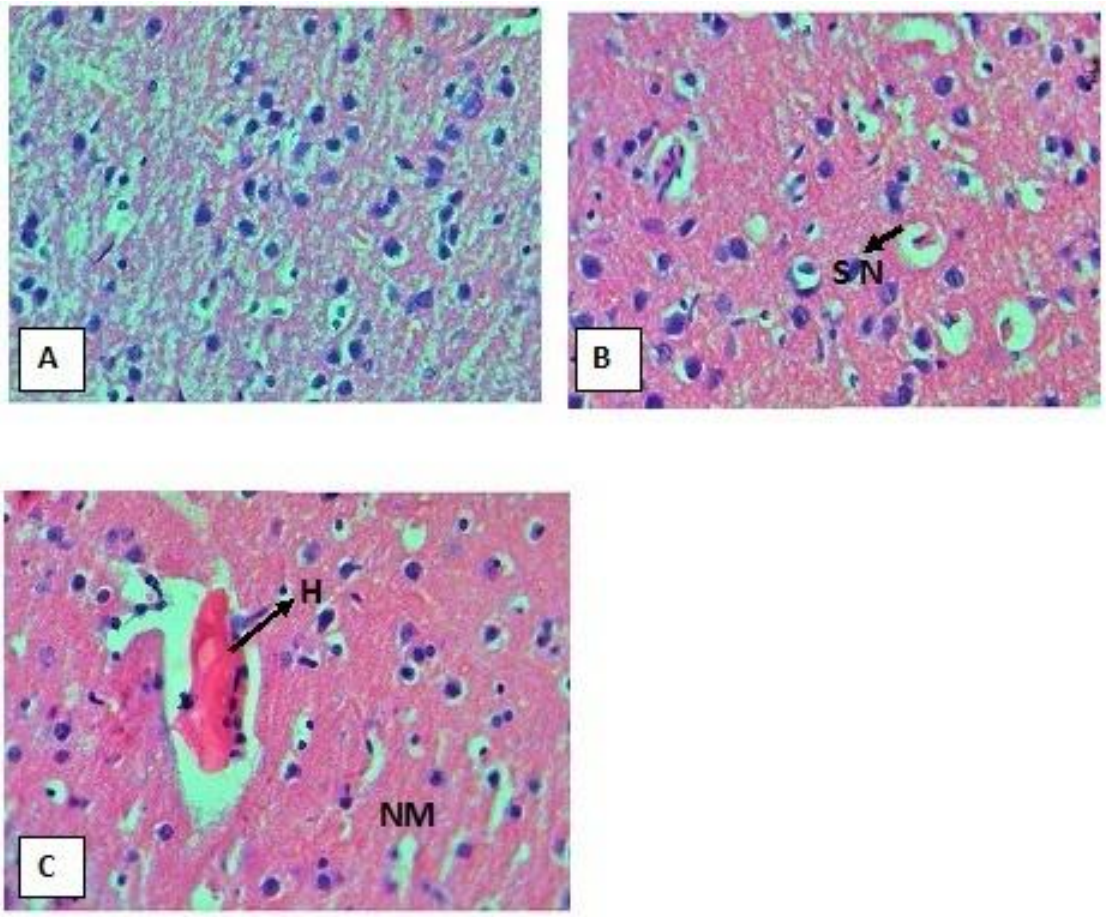

Fig 2. A. T.S of brain of control rat showing normal structure of neurons and granular layers
B. T.S of brain of T1 rats showing degenerating neurons with pyknotic nuclei and swelling in neurons
C. T.S of brain of T2 rats showing perivascular hemorrhage and nuclear migration of neurons

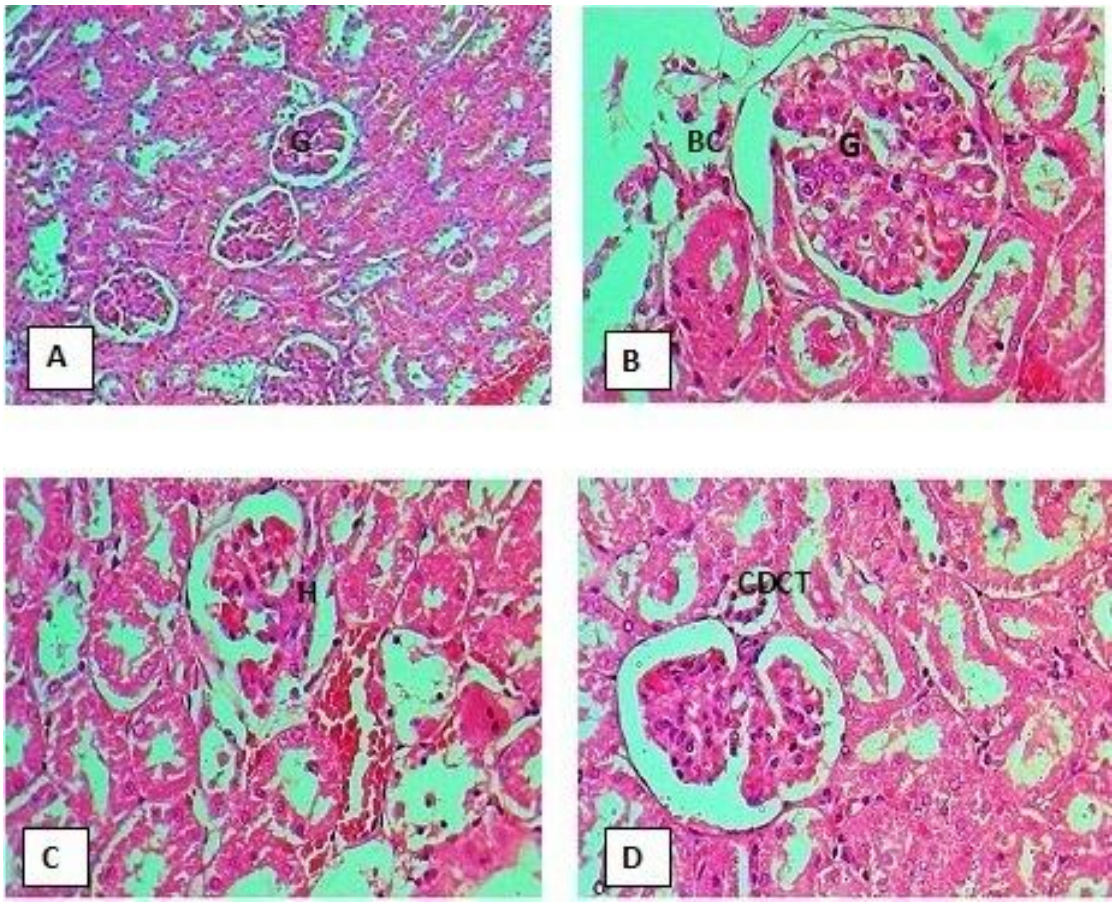

Fig.3. A. T.S of kidney of control rat showing intact capsule with well demarcated glomerular tuft (G) and normal arrangement of tubules in the cortical portion
B. Magnified view of kidney of control rat
C. T.S of kidney of T1 treated rat showing blood congestion in between tubules and small area of hemorrhage (H) in the interstitium in rats
D. T.S of kidney of T2 treated rat showing enlargement in parietal layer of Bowman's capsule, congested distal convoluted tubules (DCT) and tubular degenerations

Discussion

No signs of toxicity and mortality were seen in clinical appearance of new borns in all the three generations. There was no significant disruption in cyclicity of female rats. There was non-significant increase in body weight of female rats during mating, gestation and lactation period in both lower and higher imidacloprid treated groups. In one study it was reported that animals' digestive tract represents the functional link between foraging and the energy available for survival, growth, and reproduction. During lactation, the alimentary canal progressively increased in weight and size. It partially regressed following weaning[16].

The vital organs like brain, liver, kidney, stomach and lungs of rats did not illustrate any change in their relative weights at any doses as compared to controls. However, relative weight of heart and spleen was significantly decreased ($P<0.05$) at higher dose. Weight of liver of female F1 rats increased significantly in $1/22^{th}$ LD_{50} of imidacloprid treated group. The significant increase in weight of liver was, however, found to be associated with concomitant increase in the activity of serum GOT and GPT. It is important to note that the elevated activity of serum GOT and GPT recorded in the study maybe due to tissue of liver enzyme loss. This has been confirmed by hepatocellular damage in the higher dose treated animals. Our present knowledge on liver changes induced by imidacloprid is both limited and equivocal[17].

Biochemical alterations in response to pesticides are considered to be rapidly responding endpoints and thus most biochemical biomarkers in the laboratory studies are assessed after acute exposure to chemicals. Changes in the biochemical profile indicates alteration in metabolism of the organism resulting from the effect of the pesticide and make it possible to study the mechanisms of the effects of these pesticides[9]. The liver is rich-source of aminotransferases (ALT & AST) and alkaline phosphatase (ALP). The hepatic injury is accompanied by hepatic inflammation or necrosis (death of cells). When the hepatic injury is produced, the hepatocytic enzymes released into the circulation[18]. Biochemical analysis was carried out with the idea to screen the state of the liver during different stages of subchronic toxicity. The increases of plasma AST activity and bile acid concentration were the most specific indicators of liver disease in the racing pigeons intoxicated by ethylene glycol[19]. Earlier studies of Bhardwajet al.[20] 2010, Mohany et al.[21]2011 have also observed increased level of enzymes in serum of rats treated with different doses of imidacloprid. Results are also in accordance with those of Balani et al.[22]for male white leghorn (WLH) treated with different concentrations of imidacloprid.These changes might be due to the accumulation of imidacloprid metabolites in the liver as it is the principal target organ for any detoxification mechanism. Similar increase in levels of ALT and AST was noticed by Soujanyaet al.[23]2013 in female albino rats fed imidacloprid at the rate of 80 mg/kg body weight.

The hexokinase and G6PD are the key enzymes of glycolysis and pentose phosphate pathway, opposite changes in their activity can be regarded as a compensatory reaction of the tissue aimed at the maintenance of homeostasis during exposure to the toxic agent. The observed increase in G6PD activity may also be associated with the specific function of the enzyme as a supplier of NADPH, required for the detoxification of the insecticide entering the body [24].

The brain and plasma AChE activity was significantly reduced at $1/22^{th}$ LD_{50} of imidacloprid. According to one study the cause of AChE inhibition is unknown because imidacloprid is not choline esterase (ChE) inhibitor, since plasma AChE is synthesized in the liver, the decrease in plasma AChE activity may be related to observed changes in liver function. Earlier studies have shown that concentration of pesticides and metabolites in plasma and brain generally correlated with the severity of toxicity and symptoms of neurotoxicity which were found to increase with the pesticide concentration in brain.

There was significant increase in the plasma proteins of$1/22^{th}$ LD_{50} of imidacloprid treated group. The elevation of proteins is reported to occur in conditions when the cells are subjected to wide variety of environmental assaults including toxins, poisons and pollutants and is mainly due to stimulation of the synthesis of acute phase protein and corresponding m-RNA[25], which buffer them from harm[26]. Elevation of proteins might also be due to destruction of tissues, which causes release of proteins.

The present study revealed that treated rats by imidacloprid showed different pathological lesions in the liver tissue. The liver is the centre for detoxifying any foreign compounds entering the body. So, it uniquely exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present in food or drinking water. Microscopic changes in the liver with large areas of vacuolation, fatty degeneration, large areas of necrosis and congested sinusoidal spaces after the 14^{th} and 28^{th} days of the trial are similar to imidacloprid administration in japanese quail[27,28].Mohany et al.[21] (2011) reported that animals treated with 0.21 mg/ kg imidacloprid for 4 weeks showed heavily congested central vein and blood sinusoids, widely distributed pyknotic nuclei, and leukocyte infiltration in rat liver. Imidacloprid at $1/10^{th}$ of LD_{50} treatment resulted in dilatations of central vein and sinusoids between hepatocytes[30]. Imidacloprid produced similar histopathological lesions in liver, kidneys, and brain of japanese quail exposed to chemical for 6 weeks[30]and in layer chickens exposed to 139 mg/kg imidacloprid[31].Hepatocellular hypertrophy and fatty changes in the liver were also observed in a three-week study conducted with thiacloprid in mice[32]. The study suggested that toxic responses occur relatively more frequently in the liver compared with other organs, mainly because the liver is a predominant organ for the metabolism, and is also the first major organ to be exposed to ingested toxins, due to its portal blood supply[33].

Brain sections of rats treated with $1/22^{th}$ LD_{50} of imidacloprid showed perivascular hemorrhage and nuclear migration of neurons.It was reported by Soujanya et al.[23] 2012 that, brain sections of rats treated with 80 mg/kg body weight revealed marked congestion in cerebellum, degeneration of purkinje cells with loss of dendrites, vacuolation around neurons and shrunken neurons on 14th day of experiment. Onday 28, brain sections revealed vacuolation around neuronal cell body, chromatolysis and markedcongestion. Bhardwaj et al.[20] 2010 also reported brain damage showing degenerative changes in purkinji cells and loss of granules in granular layer. Necrosedpurkinje cells and loss of granules in the granular layer of cerebellum have also provided support to the neurobehavioral effects indicating accumulation of imidacloprid and its metabolites inthe brain. The data of the present study has indicated that imidacloprid has induced toxicological effects to female rats at 20 mg/kg/day dose level.

Kidney sections of rats treated with 1/22th LD_{50} of imidacloprid showedenlargement in parietal layer of Bowman's capsule, congested distal convoluted tubules (DCT) and tubular degenerations. Higher dose of imidacloprid (20 mg/kg/day) resulted in mild focal necrosis with swollen cellular nuclei and cytoplasmic lesions in rat liver and slight degeneration of tubules and glomeruli of kidney of the female rats (Bhardwaj et al.[20] 2010).Soujanya and Lakshman[34](2013) reported that kidney sections of male rats treated with 80 mg/kg/day imidacloprid revealed cystic dilatation of tubules, shrunken glomeruli, vacuolation, inter tubular haemorrhages and hyaline casts.

In conclusion, although the results obtained from this study showed minor histopathological, biochemical and toxicological effects in rats fed with imidacloprid at higher dose throughout two generations, it did not cause severe health concerns at lower dose.

Acknowledgements

We are thankful to the Head, Department of Zoology, PAU Ludhiana for providing facilities for conducting research.

References

1. E-L Gendy K S, Aly N M, Mahmoud F H, Kenawy A and El-Sebae A K The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid, Food ChemToxicol, 48 (2010) 215.

2. Ware GW and Whitacre DM, An Introduction to Insecticides, fourth ed., MeisterPro Information Resources, A division of Meister Media Worldwide, Willoughby, Ohio, USA. 2004.

3. Tomizawa M, Lee DL and Casida JE, Neonicotinoid insecticide toxicology: Mechanisms of selective action, Ann Revised PharmacolToxicol, 45 (2005) 247.

4. Tomizawa M and Casida J E, Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors, Ann Revised Entomol, 48(2003) 339.

5. Duzguner V and Erdogan S, Acute Oxidant and inflammatory effects of imidacloprid on the mammalian central nervous system and liver in rats, Pest Biochem and Physiol, 97 (2010) 13.

6. Bal RM, Naziroglu G, Turk O, Yilmaz T, Kuloglu and Etem E, Insecticide imidacloprid induces morphological and DNA damage through oxidative toxicity on the reproductive organs of developing male rats,Cell BiochemFunc, 30 (2012) 492.

7. Donia AMB, Goldstein LB, Bullman S, Tu T, Khan WA, Dechkovskaia AM, Abdel-Rahman AA, Imidacloprid induces neurobehavioral deficits and increases expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following in utero exposure. J Toxicol Environ Health A, 71(2008) 119.

8. Karabay NU, Oguz MG, Cytogenetic and genotoxic effects of the insecticides, imidacloprid and methamidophos, Genet Mol Res, 4 (2005) 653.

9. Luskova V, Svoboda M and Koarova J, The Effect of Diazinon on Blood Plasma Biochemistry in Carp (Cyprinuscarpio L.) Acta Vet Brno, 71(2002) 117.

10. Balani T, Agarwal S and Thaker AM, Haematological and biochemical changes due to short-term oral administration of imidacloprid,ToxicolInt, 18(2011) 2.

11. BergmeyerHU, Methods of Enzymatic Analysis, vol.3, Academic press, New York, 1974, pp. 727.

12. BesseyOA, LowryOH, BruckMJ, A method for the rapid determination of alkaline phosphatase with five millimeters of serum, J BiolChem 164 (1946) 321.

13. Deutsch J, Maleimide as an inhibitor in measurement of erythrocyte glucose-6-phosphate dehydrogenase activity, ClinChem, (1978) 24885.

14. Voss G and Sachsse K, Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylcholine/DTNB procedure,Toxicol Appl Pharmacol, 16 (1970) 742.

15. Lowry OH, Resenbrugh NJ, Farr AL and Randall AJ, Protein measurement with folin phenol reagent, J Biol Chem193 (1951) 265.

16. Cripps AW and William VJ, The effect of pregnancy and lactation on food intake, gastrointestinal anatomy and the absorptive capacity of the small intestine in the albino rat,Br J Nutr 1 (1975) 17.

17. Eiben R and Rinke M, Subchronic Toxicity on Wistar Rats: Administration in the Feed for 96 Days. Bayer AG. Department of Toxicology. FachbereichToxikologie Wuppertal, Germany. Study No. 100036. DPR Vol. 51950-0005 # 119467 (1989)

18. WalmsleyRN and WhiteGH, A guide to diagnostic clinical chemistry. 3rd Ed., Oxford Blackwell Scientific Publication, London, Edinbrugh, Boston(1994) 321.

19. LumeijJT, Avian Clinical biochemistry, In:Clinical Biochemistry of Domestic Animals, J.J Kaneko J.W.Harvey, Bruss M.L.,eds 5th ed. Academic Press, pp. 857.

20. Bhardwaj S, Srivastava MK, Upasana K and Srivastava LP, A 90 days oral toxicity of imidacloprid in female rats morphological, biochemical&histopathological evaluation. Food and ChemToxicol, 48 (2010) 1185.

21. Mohany M, BadrG, Refaat I, El-Feki M, Immunological and histological effects of exposure to imidacloprid insecticide in male albino rats, Af J Pharmacol Physiol,5 (2011)2106.

22. Balani T, Aggarwal S, Thaker AMM, Hematological and biochemical changes due to short term oral administration of imidacloprid, ToxicolInt, 18 (2011) 2.

23. Soujanya S, Lakshman M, Kumar A A and Reddy A G Histopathological and ultrastructural changes induced by imidacloprid in brain and protective role of vitamin C in rats, J ChemPharma Research, 4 (2012) 4307.

24. Kuzminskaya UA, Bersan LV, Pismennaya MV, Activity of enzymes of Glucose-6-Phosphate metabolism in the liver of rats with experimental valexon poisoning, BullenExpBiol, 84 (12) 677.

25. Puga FR, Rodrigues MA, Effects of dimethoate and propoxur on the metabolism of IB-RS-2 cells and on their susceptibility to foot and mouth disease, ArqInstBiol, (Sao Paulo) 41 (1974)141.

26. Welch WJ, How cell respond to stress, Scientific Amer, 268 (1993)34.

27. Abo-El-Ezz ZR., Abd-Allah GA, Hassieb MM, Hassan GA, Salem MH, Histopathological Changes due to the effect of organophosphorous and pyrethroid insecticides in rabbits, Alex J Vet Sci, 2 (2010) 325.

28. Eissa OS, Protective effect of vitamin C and glutathione against the histopathological changes induced by imidacloprid in the liver and testis of Japanese quail,Egypt J HospMed, 16 (2004) 39.

29. Toor HK, Sangha GK and Khera KSImidacloprid induced histological and biochemical alterations in liver of female albino rats, Pest BiochemPhysiol,(2012)http://dx.doi.org/10.1016/j.pestbp.2012.10.001.

30. Omiama SE, Protective effect of vitamin C and glutathione against the histopathological changes induced by imidacloprid in the liver and testis of japanese quail, Egyptian J Hosp Med, 16 (2004) 39.

31. Kammon AM, Brar RS, Banga HS and Sodhi S, Patho biochemical studies on hepatotoxicity and nephrotoxicity on exposure to imidacloprid in layer chickens, Veterinarskiarhiv, 80 (2010) 663.

32. Anonymous, ISSN 1443-1335, Evaluation of the new active Thiacloprid in the new product Calypos 480 SC Insecticide (2001).

33. Popp JA, CattleyRC,Hepatobiliary system, in: Handbook of Toxicologic Pathology. (Haschek, W. M., C. G. Rousseaux, Eds.) (Academic Press Inc. San Diego) (1991)279.

34. Soujanya S and Lakshman M, Imidacloprid induced nephrotoxicity in male rats, Ind J App Res, 6(2013) 415.