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Effect of imidacloprid (insecticide) on Serum Biochemical Parameters and degenerative Lesions In Male Rat's Liver

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Abstract: Imidacloprid is one of the most widely used insecticides in the world. Present study is indicating the effects of orally administered imidacloprid on biochemical blood profile and histological parameters on male rat's liver. Dose response with respect to different time intervals was evaluated on different groups of rats. Signs of acute/sub-acute poisoning were observed and severity of these findings in the imidacloprid treated rats was found to be dose dependent. Higher concentrations of imidacloprid increased the amount of cholesterol in all the treated groups, the HDL level decreased and the Level of LDL in treated group was higher when these were compared with control rats. The toxicity of the pesticide leads to higher levels of AST and ALT in time and dose dependent manner as compared to the control rats. Moreover, a high concentration of imidacloprid dose transiently increased and then slightly decreased the concentration of total serum proteins, globulins and serum albumin. Additionally, histopathological examination of liver revealed bile duct hyperplasia along with degenerative changes in hepatocytes and the severity was found to be dose dependent.

Keywords: Imidacloprid; Pesticide; Blood Parameters; Amino transferases; Lipid Profile; Histopathology of liver.

1. INTRODUCTION

Pesticides are being extensively used worldwide (Chang *et al.*, 2017). Pesticides have played a vital role to meet the ever increasing demands of food, cotton fiber and tobacco etc. for the continuously increasing population and control over vector-borne diseases. Identification of pesticide hazardous and the development of safer methods of pesticide handling have withdrawn attention of scientific community. Misuse of pesticides in various sectors of the agriculture and other areas is associated with health hazards and environmental contaminations worldwide (Rome and Moro, 2003: Remor, 2009). In the under-developed world, poisoning by pesticides has caused more causalities than common infectious diseases, and the level of pesticide poisoning among occupational workers and farmers is alarming (Murthy *et al*., 1986).

Imidacloprid (IC) was the first of the neonicotinoid chemicals to come on the market in the US and has been in wide use since (1994) on crops from corn to vegetables. Imidacloprid is a neonicotinoid insecticide in the chloronicotinylnitroguanidine chemical family. Neonicotinoids are considered as a new class of insecticides that is believed to affect the central nervous system of insects, causing paralysis and ultimately leading to death. Imidacloprid binds to and activates nicotininc acetylcholine receptor, affecting synaptic transmission and causes disruptions in nervous system (Baligar and Kaliwal, 2001). It is commonly used in agriculture as foliar, as a treatment of seed, for outdoor and indoor control over insects. Its toxicity is reported as moderate level and is linked to mutagenic effects, neurotoxic, reproductive effects, generally persistent in soils and can leach to groundwater.

The primary organs affected by longer term low-dose rate of imidacloprid are liver, thyroid and this can also result in reduction of body weight. Reproductive toxicity, neurobehavioral deficits, and developmental retardation in rats and rabbits are exhibited on low to mid dose rate. Imidacloprid has neither shown any carcinogenic effects in laboratory animals nor mutagenic in standard laboratory assays. Different organs are the prone to cytotoxic effects of imidacloprid and this has been demonstrated by elevated serum transaminase, glutamate dehydrogenase and alkaline phosphatase activities and alteration of other clinical chemistry parameters, such as Cholesterol, uric acid, Glucose, albumin and total protein after oral intake (Khogali *et al*., 2005).

Imidacloprid is a relatively newer systemic insecticide which is related to the nicotine (tobacco toxin). Since liver has a fundamental role in metabolism and eliminating toxicants from the body therefore, its biochemical and histological parameters are the most important parameters in elucidating toxicity of various chemicals. The aim of our studies was to checked the IC effects of physicochemical behavior, acute toxicity of

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IC on different blood bio-parameters (Lipid profile, AST, ALT, ALP, blood protein and to assess the histopathologic consequences of liver organ) so that IC have adverse effects on rat's health so proper precautions needed be taken in use of this pesticide to care about human's health.

2. MATERIALS AND METHODS

All experiments were completed as per all the national and global enactments and research protocols placed down by the Ethics Committee about the animal well-being as well as the strategies of the University of Sargodha, Sargodha. The study was conducted in (2014-15).

Experimental protocol

Apparently healthy Swiss albino adult male rats (n = 52) almost of the same age (7-8weeks) and weight (180±10g), were obtained from Department of Pharmacy, Islamia University Bahawalpur. Animals were housed in a temperature-controlled environment (at 25-27°C temperature with 45-70% humidity) with 12-h light/dark cycles. The special rat's food prepared and offered in three times a day. After five days' acclimatization, the rats were arbitrarily divided into 4 equal groups i.e., group A served as control group while the B-D group marked as treated group administrated with Imidacloprid. IC was purchased from local grain market of Sargodha. Oral LD50 of Imidacloprid in male rats is 450 mg/kg body weight. Since imidacloprid is insoluble in water, therefore, its solution in acetone was made for oral administration to rats. Dose to be administrated was adjusted with their body weight. Groups (B, C and D) received low, medium and high (450, 500 & 550 mg/kg of body weight doses, respectively) doses for 48 hours, 8 days and 31 days once time in a day. About 20% of mortality rate of rats observed during the course of studies. Doses administrated to the rats by oral route so during the administration it was keep in mind that slow & steady and expert handling ensured the prescribed dose given. The body weight was recorded for each animal every week. Consumption of food and rectal temperature was recorded on daily basis. Animals were monitored twice a day to observe numerous clinical signs of toxicity. The clinical signs in each group animal were recorded on the basis of severity (------ to ++++++).

Sample collection and analyses

Afterevery treatment blood samples were collected from all animals and subjected to extraction of serum. The serum samples were stowed in aliquots at -20°C for biochemical studies. Triglycerides (TG), cholesterol (CHOL), HDL and LDL were measured by enzymatic colorimetric method at wavelength of 546 nm with a Chemistry analyzer of microlab 300. Commercially available colorimetrickits Analyticon Biotechnologies company, AG/Germany were used to measure Serum aspartate aminotransferase (AST), (alanine aminotransferase) and alkaline phosphatase activities. The concentration of enzymes AST, ALT and ALP was determined by using commercially available kits with a Chemistry analyzer of microlab 300. The STP (serum total proteins) and serum albumin were estimated by colorimetric assay, end point method and serum albumin by bromocresol green (BCG) dye binding method using Chemistry analyzer of microlab 300. The serum globulin was calculated by subtracting albumin from STP.

Histological Investigation

At the end of each experiment after the last doses of 31st day i have observed some changes and took the blood sample and then euthanized each rats. The liver from each animal was carefully dissected and processed for histopathology. Briefly, about five millimeter dense pieces of the organ were fixed in ten percent buffered formalin and later processed for histopathological studies using monotonous methods of dehydration, paraffin embedding, sectioning (4-5µm) and staining.(Awaad*et al*., 2010). The microscopic examination of slides of each group at a particular period, histological lesions were scored on a scale of ------ to ++++++. From this a cumulative lesion score was derived for the overall severity of pathology in a particular group.

Data analysis

Biochemical analyses are shown as the mean ± standard error of means. Comparisons between treated and control groups were made on computer using ANOVA (Analysis of one Variance) by using SPSS software. A probability (P) value of 0.05 was selected as a standard for statistically significant differences (Zar, 1996).

3. RESULTS

Manifestations of acute/sub-acute toxicity induced by imidacloprid

Severity of clinical signs of acute and/or sub-acute toxicity in the imidacloprid treated rats was dose and time dependent. Rats were administered different doses of imidacloprid as described in materials and methods section. The different parameters such as increase in response to external stimuli, dullness, lacrimation, less attraction for food and water, ptyalism and gasping are recorded (Ahmad *et al*., 2011) in Table1. These effects were found to be more prominent in the time range of 15 mins to 90 mins after each oral dose and these effects disappeared in 12-24 hours in all treated rats. Only one rat from group D (550mg.kg^{-1}) did not survive on receiving 3rd dose of imidacloprid. There were no clinical sign/symptom and behavioral changes noticed in rats of group A (control). Frequency signs indicated severity of doses and incidence showed how many animals were suffered due to treatments.

Table1. Frequency and incidence of clinical signs in rats administrated with different doses of Imidacloprid

Parameter	Group (imidacloprid dose:mg.kg^{-1})							
	B (450)			C (500)			D (550)	
	F*	I**		F		I		
Skin irritation		++--	50		+++-	70	++++	80
Coarse tremors	++--	20		+++-	30		++++	60
Dullness	++--	40		+++-	60		++++	70
Low body Temperature		++--	50		+++-	60	++++	80
Lacrimation		++--	60		+++-	70	++++	80
Dilation of pupil	++--	30		+++-	40		++++	60
Movement								
Ptyalism	++--	50		++--	60		++++	80

*F= Frequency; **I = Incidence (%). Various concentrations of imidacloprid mixed in acetone were administration to the rats at different interval. The manifestations mentioned above are recorded accordingly.

Effect of Long term administration of Imidacloprid on body weight

Continuous administration of Imidacloprid for 8days did not affect the body weight of the Swiss albino rats. Whereas as continuous administration of Imidacloprid for 31 days also didn't affects the body weight at dose rate of 450mg/kg but significant effects on the body weight of the rats were seen for both the dose rate of 500mg/kg and 550mg/kg, as presented in **(Fig. 1A).**

Effect of administration of Imidacloprid on Lipid profile

A different pattern in the levels of serum cholesterol was observed in all treated groups as compared to CG (control group). Significant (P<0.05) rise was observed in treated group D (550mg/kg) at for all the experimental day 2, 8, and 31 as shown in figure 2. While in case of group C (500mg/kg) there was a significant decrease in total cholesterol as compared to control at day 31. In case of group B (450mg/kg) there was an increase in total cholesterol level only at day 31 shown in **(Fig.1B).**

Triglycerides level was significantly raised in all the treated groups of rats compared to control group (P≤0.050). It was noticed that both the parameters (dose and time) affected the value of TGL in the same manner. Group D showed higher difference compared to control as well as B and C group as shown in **(Fig 1C).**

The serum of the rats at specific time and dose were tested for lipid profile and significantly (P≤0.05) low value of HDL were recorded in the treatment group C (500mg/kg) and D (550mg/kg) but not a much pronounced effect was observed in group B as compared to control group. Paradoxically, significantly high level of LDL was recorded in treated group C and D as compared to control group **(Fig.5).** All the data shows that high dose of imidacloprid adversely affect the level of lipoproteins the detail result are given in **(Fig.1D and E).**

Effect of administration of Imidacloprid on Proteins levels

Total serum protein was quantified and very slight but significant lower STP levels were observed in group D (550mg/kg) as shown in **(Fig.1)**. In case of serum albumin and globulin no significant changes were observed in treated groups of rats in comparison to controls (data not shown).

Effect of administration of Imidacloprid on various Hepatic enzymes

The levels of different hepatic enzymes such as AST, ALT & ALP (Alkaline phosphatase) were found high at all the days 02, 08 and 31 as compared to control group (Fig. 2). The dose dependent pattern was observed in all treated groups. Significantly higher ALT, AST and ALP were found at the highest dose (550mg/kg) irrespective of the days of the exposure.

Effect of administration of Imidacloprid on Hepatic Histology

At the end of experiment histological slides were made from tissues derived from liver and lesion was scored in each group of rats based on severity (---- to ++++). The overall lesion intensity in a particular group of rats was scored from this cumulative lesion (Table 2). Rats of group D (550mg/kg) showed condensation of hepatic nuclei and vacuolization **(Fig. 3A)** in 50% animals. Rats from control group A) have not shown any histological alterations in the liver **(Fig. 3B).** These changes were found to become more severe and extensive, in addition to cellular swelling and disorganized hepatic cord pattern at higher dose rate **(Table 2).**

Moderate bile duct hyperplasia as well as vacuolar degeneration in the parenchyma was observed in groups administered with high dose **(Fig.3C).**

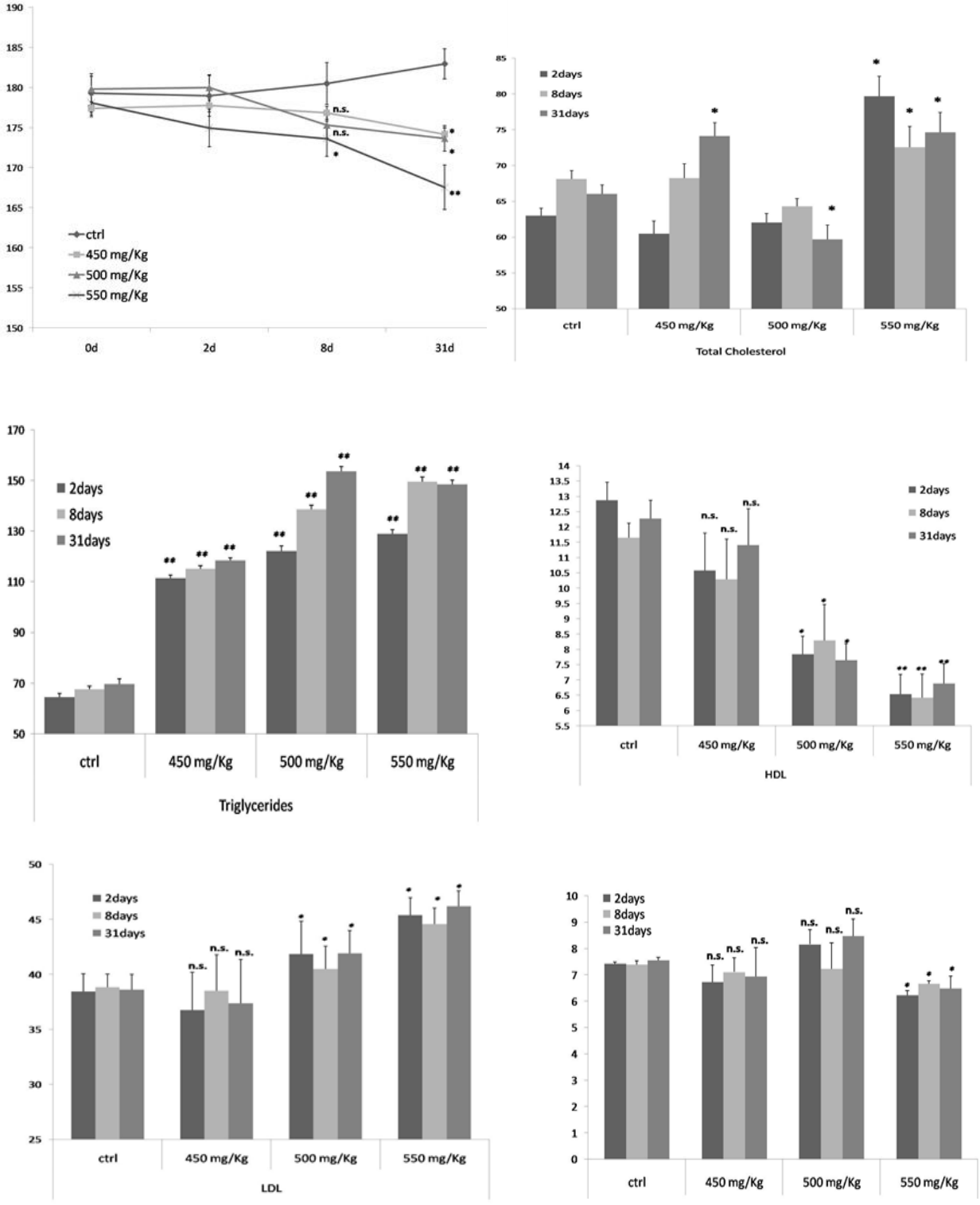

Fig. 1. A Long-term administration of Imidacloprid (at a dose mentioned) affecting body weight B. The serum total cholesterol in rats administrated with different dose of Imidacloprid. C.The serum triglycerides in rats administrated with different dose of Imidacloprid. D. The serum HDL level in rats injected with dissimilar dose of Imidacloprid. E. The serum LDL level in rats injected with dissimilar dose of imidacloprid. F. Serum total proteins and albumin in rats at various dose rate of Imidacloprid.

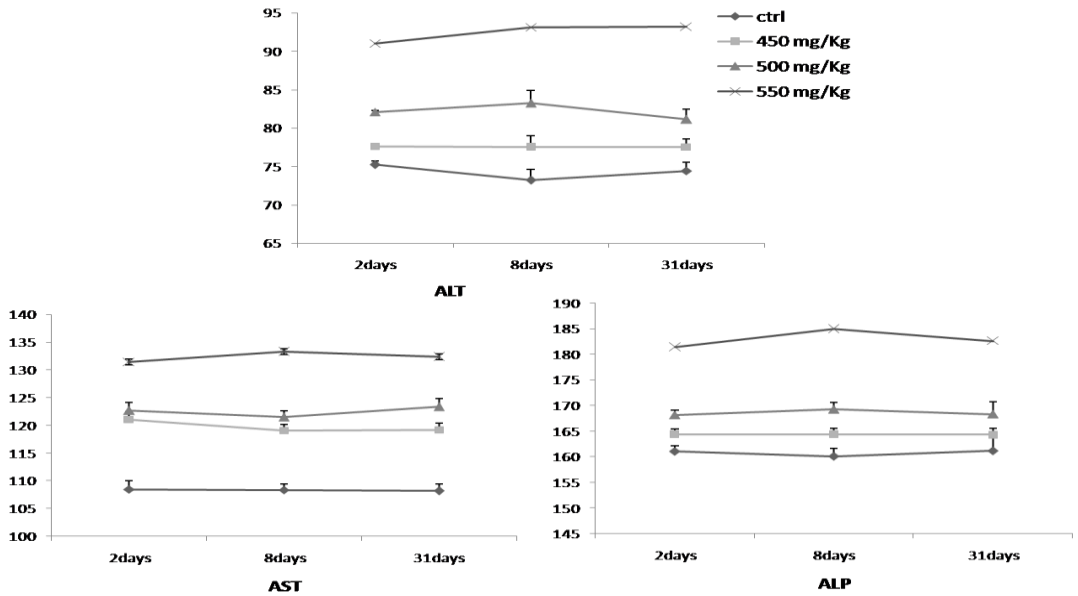

Fig..2. Serum ALT, AST and ALP in rats administrated withdifferent dose of imidacloprid

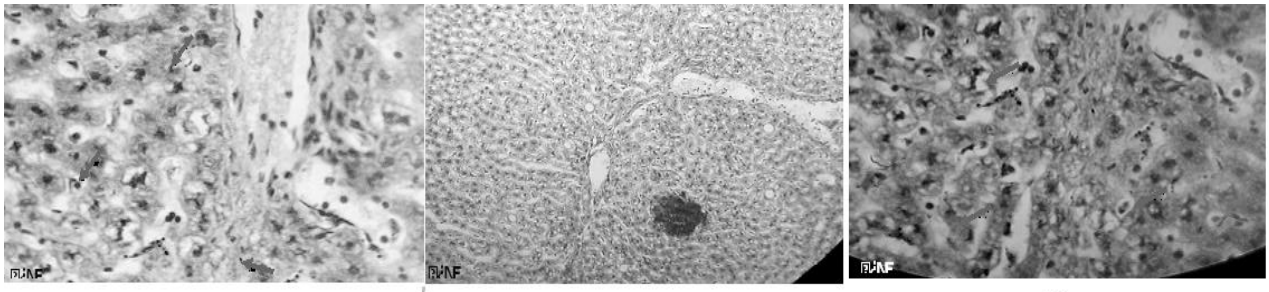

Fig. 3 (A).Photomicrograph of treated rats Liver after 31 days at 550mg.kg^{-1} dose rate showing condensed nuclei of hepatocytes and necrosed hepatocytes. (B). Photomicrograph of liver of rat treated as control after 31 days. (C). Photomicrograph of liver after 31 days for rat treated at different intervals with 550mg dose showing reasonable bile duct hyperplasia along with degenerative changes in hepatocytes

Table2. Overall frequency and incidence of histological lesions in liver of rats administrated with different doses of imidacloprid

Lesion	Group (imidacloprid dose:mg.kg^{-1})					
	B (450		C (500)		D(550)	
	F*	I**	F	I	F	I
Hyperplasia of bile duct	+---	10	++--	30	++++	70
Degeneration	+---	20	++--	50	+++-	80
Cytoplasm vacuolation	++--	50	++--	70	+++-	100

***F= Frequency; **I = Incidence (%). Various concentrations of imidacloprid mixed in acetone were administration to the rats at different interval. At the end of experiment 31 days dissected. Tissue sample of liver were conserved and managed to prepare histological slides and lesion in each group were scored on the basis of severity (------ to ++++++). From this an increasing lesion score was derived for the overall intensity of lesions in a particular group. Control A group did not exhibit any lesion.**

4. DISCUSSION

It was observed that average feed intake and gain in net body weight in rats are well related to each other and both were found to decrease significantly at higher dose rate of Imidacloprid. Bhardwaj*et al*. (Chao and Casida, 1997) have also showed a similar decrease in rats at high dose rate of imidacloprid and similar results with different dose patterns are reproduced here in our study. Here we demonstrated a decrease of weight in a dose dependent manner on treatment with imidacloprid. The growth rate index can be assessed by weight gain in animals (Rahman *et al*., 2002) and conversely reduction in weight refers to the toxicity of the drug. Drug toxicity in animals is assessed on the basis of loss of organ weight (Anatra and Cordone, 2005). The decrease in the level of food consumption and weight of liver at high dose rate in treated animals seemed to be due to toxicity of imidacloprid (Barros and Saliba, 1978).

The high level of cholesterol was observed in group D (550mg) and the low level was noticed in C (500mg). Some earlier reports (12-14) also exhibited similar results. These changes are probably because of accumulation of imidacloprid metabolites in the principal target organ responsible for any mechanism of detoxification i.e. liver.

A significant increase in concentration of Triglycerides in serum was detected in all rat groups. These results are in agreement with an earlier report (Georgieva*et al*., 1990). The significant point is the generation of free radicals which are responsible to induce oxidative stress upon treatment with imidacloprid, and this is considered for direct utilization of triglycerides and cholesterol as an antioxidant which will eventually lead to termination of free radical response and exhaustion of the triglycerides (TGs) during oxidative stress, which is clear from significant alterations in hepatic biomarkers and corresponding histological and ultra-structural changes in the observed rats.

The low values of HDL were obtained in group C (550mg) and D (550mg). All the three groups of rats have lower concentrations of HDL than that of group A (control). These results were not in conflict with the findings of FAO, 1999. These results are in agreement with previous studies, where on supplementation with various biochemical enzymes, an improvement in the histoarchitechture of the liver in Japanese quail (Coronado, 2004) was observed as well as GSH levels were restored to normal on administration of imidacloprid in mice (Baligar and Kaliwal, 2001).

A significant decreased in total protein levels was observed in group D of rats compared to the control group. David *et al*. (Duzguner and Erdogan, 2010) also marked similar observation. The reduction in total protein can be explained by hepatotoxicity caused by imidacloprid. Compared to control group, a significant increase was observed in serum ALT and AST levels. These results are in good agreement with the earlier reports (Tomizawa and Casida, 2005: Tomlin, 2006). This might be due to degeneration and necrosis of hepatocytes, which causes an increase in permeability of cell membrane resulting in release of transaminases into blood stream.

The high contents of Alkaline Phosphates were observed in group D (550mg) and the low level was found in B (450mg). All the three groups of rates have greater values of alkaline phosphates as compared to group A (control). Changes were observed in the levels of enzyme ALP and AST in liver tissues on treatment of rats with imidacloprid. It might be explained on the basis of fact that on prolonged exposure of pesticides an increase in enzymes level is required for detoxification of pesticides, and genes mutation is responsible for the production of these enzymes. Inflammation and oxidative stress in the central nervous system and liver of rats on chronic exposure to imidacloprid has also been reported (FAO, 1999). An increase in the level enzyme ALT, ALP and AST to significant extent were also recorded in blood plasma of rats on treatment with high dose imidacloprid. An increased enzymes level in serum of rats treated at various dose rate of imidacloprid was also observed in other studies of (Chao and Casida, 1997:Muthviveganandavel, 2008). Balani*et al*. (Barros and Saliba, 1978) obtained similar results on treating male white leghorn with different concentrations of imidacloprid. Present investigations reveal that an enhanced level of specific enzymes is in good correlation with the gross and histopathological changes in the liver. Increased activity of serum AST, ALT, and ALP might be attributed to enzyme loss in liver tissues The hepatocellular damage at higher dose rate in treated animals also confirms this in our study. In an earlier study increased activity of transaminase was noticed in alpha-cypermethrin treated rats which were thought to induce pathological changes in liver and these increased levels might be considered responsible for hepatic damage causing pathological alterations in liver and other visceral organs. Functional status of liver is represented by activities of serum enzymes like AKP, AST, and ALT (Mahadevaswami, 2000).

Histopathological analysis of liver tissues treated at higher dose rate of imidacloprid revealed blood sinusoids, dilation of central vein, and infiltration of wide spread pycnotic nuclei and leucocyte into the hepatic tissue. Similar histopathological lesions were found in livers of male rats and Japanese quail on

treatment with imidacloprid for 6 weeks. Inflammation and oxidative stress in liver and CNS (central nervous system) in non-target organisms on treatment with IC is also known in rats (Bolognesi and Morasso, 2000).

5. CONCLUSIONS

Imidacloprid (IC) is a widely used new systemic insecticide, caused changes in physicochemical behavior of rats after administering different doses. Taking in account the different biochemical parameters such as liver enzyme like AST, ALP, phospholipids, total lipids, glycerides and the cholesterol level observed. It is observed that higher concentration of IC increased the level of TGLs. In addition, our results also revealed the low level of HDL and high level of LDL observed in treated groups especially in groups C and D. A decreasing trend observed in STP with reference to normal group, while the serum globulin did not show much variation. Moreover, the higher AST and ALT level was recorded with respect to dose concentration as dose increased. In albino's rats it observed that physiochemical parameters were distressed considerably subsequent imidacloprid toxic effect in dose and time dependent manner. Furthermore, the study described condensation of hepatic nuclei cytoplasmic and vacuolization were observed in higher doses treated groups with respect to the normal group. Generally, our findings are providing a gate way to observe that IC have adverse effects on rat's health and extrapolation of the results on humans indicates for the use of proper precautions in the use of this pesticide.

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