

EFFECT OF IMIDACLOPRID ON THE BIOCHEMICAL CONTENTS OF KIDNEYS IN MALE SWISS ALBINO MICE

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ABSTRACT

Six groups (A, B, C, D, E and F) of male Swiss albino mice (*Mus musculus albinus*) were orally administered with varied doses (0.4, 0.8, 1.6, 3.2, 4.0 and 8.0 mg/kg bw/mouse) of imidacloprid; they showed significant decrease in protein, DNA and RNA content in the kidneys of all the treated groups of mice throughout the experimental period (on day 1, 4, 8, 12, 16 and 30 of treatment) when compared with controls. It is clear from the results that the insecticide caused marked disturbance in the metabolism of protein, DNA and RNA.

INTRODUCTION

Imidacloprid is a systemic, chloro-nicotinyl insecticide. Due to its acute toxicity, imidacloprid has become one of the most widely used insecticides in the world (Ware and Whitacre, 2004). DNA and RNA are the most important macromolecules of the cell. Though both DNA and RNA are synthesized in carrying biological information, RNA is involved in the protein synthesis. Nelson (1989) suggested that proteins function as enzymes, antibodies and structural components. Biochemical changes were used as indicators of toxic reactions due to exposure of pesticides (Stegeman *et al.*, 1992). The metabolites of imidacloprid are found in the liver and kidneys of rats after a single oral dose. Toxic signs like salivation, vomiting, lethargy, diarrhea, muscle weakness and atoxia are indicative of imidacloprid action on nicotinic receptors (Hovda and Hooser, 2002; Wismer, 2004). Rats and mice treated with carbamate showed decreased level of nucleic acids and total protein in liver (Salch, 1990; Ela-Ticky *et al.*, 1992) and decreased protein level in heart, muscle and kidney (Zoahkout *et al.*, 2000). Nephrotoxicity of pesticides has been reported in mice (Khogali *et al.*, 2005; Khogali *et al.*, 2011) and rats (Benjamin *et al.*, 2006). The toxic effects are mainly due to the poisoning of metabolism. The level of biochemical constituents in various organs represent the intensity of the toxic action of the pesticides (Rathod and Kshirsagar, 2010). Kidney is the target organ for a wide variety of toxic agents as it acts as a blood filter during the excretory process. As kidneys receive high blood flow, the insecticides might be delivered to these organs in relatively high amounts through systemic

(blood) circulation. Since glomerulus reabsorb salt and water (as glomerular filtrate), the chemical which was found in the renal tubules may interrupt the biochemical constituents and histology of kidneys in test animals (Jerry and William, 1986). No information is available on the protein and nucleic acid changes in mice exposed to imidacloprid. Therefore, the present study has been carried out to describe the biochemical changes induced by the action of imidacloprid in kidneys of male Swiss albino mice.

MATERIALS AND METHOD

Male Swiss albino mice (*Mus musculus albinus*) (6-8 weeks old; 23-26g) were used as experimental animals. All the mice were fed with standard balanced diet and water *ad libitum*. All the animals were maintained and necropsied following the CPSCEA guidelines. Fresh insecticide preparations were diluted by acetone according to the design of the experiment. Experimental mice were divided into six groups (A, B, C, D, E and F) containing 12 mice in each group: they were intubated with different doses (A, 0.4 mg/kg bw/mouse; B, 0.8 mg/kg bw/mouse; C, 1.6 mg/kg bw/mouse; D, 3.2 mg/kg bw/mouse; E, 4.0 mg/kg bw/mouse; F, 8.0 mg/kg bw/mouse). Another group (a) of 12 mice served as untreated controls. Total protein, DNA and RNA content were estimated in kidneys of experimental and control mice following Lowry *et al.* (1951), Burton (1956) and Orcinol method (Endo, 1970) respectively. Two mice from each of the experimental groups were sacrificed on 1st, 4th, 8th, 16th and 30th day after imidacloprid treatment and two mice from the control group were also sacrificed on

the same designated days and kidneys were removed and used for the biochemical assays.

RESULTS

Estimated values of total protein, DNA and RNA from experimental (A, B, C, D, E and F) and control mice (a) are shown in Tables 1 to 3.

In group A (Table 1), there was a decrease in protein content from day 1 to 30 when compared to controls (group a). The decreased value was equal on day 1 (79.32 $\mu\text{g}/\text{mg}$) and 8 (79.54 $\mu\text{g}/\text{mg}$); from day 8 (78.05 $\mu\text{g}/\text{mg}$) to 30 (77.0 $\mu\text{g}/\text{mg}$) of treatment, there was a gradual decrease in protein, which was lower than the normal value (81.61 $\mu\text{g}/\text{mg}$). Lowered levels of DNA decreased gradually from day 1 to 30 of treatment when compared to controls. The lowest amount of DNA was found on day 30 (13.0 $\mu\text{g}/\text{mg}$) in the experimental mice which was lower than control value (13.95 $\mu\text{g}/\text{mg}$). The content of RNA decreased from day 1 to 30. The lowest value of RNA was found on day 30 (9.0 $\mu\text{g}/\text{mg}$) of experimental mice when compared to controls (9.63 $\mu\text{g}/\text{mg}$).

In group B (Table 2), the gradually decreased protein content

Table 1: Protein ($\mu\text{g}/\text{mg}$), DNA ($\mu\text{g}/\text{mg}$) and RNA ($\mu\text{g}/\text{mg}$) content in kidneys of control (group a, untreated) and experimental (group A, treated with 0.4 mg/kg bw/mouse) mice at different days of experiment. Values are expressed in the mean derived from 5 observations.

Days of necropsy	Experimental group – A			Control group – a		
	Protein	DNA	RNA	Protein	DNA	RNA
1	79.32	13.56	9.59	81.67	13.98	9.67
4	78.86	13.46	9.56	81.66	13.96	9.65
8	79.54	13.43	9.45	81.65	13.97	9.66
12	78.05	13.41	9.39	81.67	13.92	9.64
16	77.67	13.39	9.26	81.64	13.93	9.67
30	77.00	13.00	9.00	81.61	13.95	9.63

Table 2: Protein ($\mu\text{g}/\text{mg}$), DNA ($\mu\text{g}/\text{mg}$) and RNA ($\mu\text{g}/\text{mg}$) content in kidneys of experimental (group B, treated with 0.8 mg/kg bw/mouse; group C, treated with 1.6 mg/kg bw/mouse) mice at different days of experiment. Values are expressed in the mean derived from 5 observations

Days of necropsy	Group – B			Group – C		
	Protein	DNA	RNA	Protein	DNA	RNA
1	78.54	13.45	9.45	78.24	13.36	9.42
4	76.93	13.43	9.42	77.45	13.28	9.38
8	77.17	13.32	9.32	76.18	13.16	9.29
12	76.78	13.29	9.35	75.83	13.11	9.22
16	75.34	13.24	9.33	74.67	13.02	9.15
30	75.00	13.00	8.00	74.00	13.00	9.00

Table 3: Protein ($\mu\text{g}/\text{mg}$), DNA ($\mu\text{g}/\text{mg}$) and RNA ($\mu\text{g}/\text{mg}$) content in kidneys of experimental (group D, treated with 3.2 mg/kg bw/mouse; group E, treated with 4.0 mg/kg bw/mouse ; group F, treated with 8.0 mg/kg bw/mouse) mice at different days of experiment. Values are expressed in the mean derived from 5 observations.

Days of necropsy	Group - D			Group - E			Group - F		
	Protein	DNA	RNA	Protein	DNA	RNA	Protein	DNA	RNA
1	77.91	13.23	9.34	77.14	13.19	9.30	76.56	13.17	9.24
4	76.86	13.14	9.29	77.47	13.12	9.27	77.34	13.05	9.18
8	76.47	13.05	9.24	76.78	12.96	9.22	76.81	12.92	9.26
12	75.99	12.91	9.17	75.39	12.84	9.16	75.14	12.85	9.12
16	74.22	12.84	9.21	74.84	12.77	9.05	74.38	12.77	9.07
30	74.00	12.00	9.00	74.00	12.00	9.00	74.00	12.00	9.00

from day 1 (78.54 $\mu\text{g}/\text{mg}$) to 30 (75.0 $\mu\text{g}/\text{mg}$) was lower than the normal protein value (81.61 $\mu\text{g}/\text{mg}$). The DNA level was lowered from day 1 (13.45 $\mu\text{g}/\text{mg}$) to 30 (13.0 $\mu\text{g}/\text{mg}$) of treatment and the lowest level of DNA was found on day 30 (13.0 $\mu\text{g}/\text{mg}$) when compared to other days of treatment and to controls (13.95 $\mu\text{g}/\text{mg}$). There was a decreased level of RNA from day 1 (9.45 $\mu\text{g}/\text{mg}$) to 30 (8.0 $\mu\text{g}/\text{mg}$) in experimental mice when compared to other days of treatment and controls (9.68 $\mu\text{g}/\text{mg}$). In group C (Table 2), there was a gradual decrease in protein value from day 1 (78.24 $\mu\text{g}/\text{mg}$) to 30 (74.0 $\mu\text{g}/\text{mg}$) in comparison with controls (81.61 $\mu\text{g}/\text{mg}$). The lowest amount of protein was found on day 30 of treatment. The level of DNA was found to be below normal from day 1 (13.36 $\mu\text{g}/\text{mg}$) to 30 (13.0 $\mu\text{g}/\text{mg}$). A slight decrease in RNA content was found from day 1 (9.42 $\mu\text{g}/\text{mg}$) to 30 (9.0 $\mu\text{g}/\text{mg}$) (below normal value - 9.63 $\mu\text{g}/\text{mg}$).

In group D (Table 3), there was a slight decrease of protein from day 1 (77.91 $\mu\text{g}/\text{mg}$) to 30 (74.0 $\mu\text{g}/\text{mg}$) when compared to controls (81.65 $\mu\text{g}/\text{mg}$). The lowered values are found to be below normal value. The lowest amount of protein was found on day 30 (74.0 $\mu\text{g}/\text{mg}$) of treated mice. The level of DNA and RNA decreased markedly from day 1 to 30 in experimental mice when compared to controls. There was a gradual decrease in DNA (from day 1-13.23 $\mu\text{g}/\text{mg}$ to 30 - 12.0 $\mu\text{g}/\text{mg}$) and RNA (from day 1 - 9.34 $\mu\text{g}/\text{mg}$ to 30 - 9.60 $\mu\text{g}/\text{mg}$) content in treated mice which were below normal values (13.95 $\mu\text{g}/\text{mg}$ and 9.63 $\mu\text{g}/\text{mg}$). Experimental mice in group E showed a gradual decline in protein content from day 1 (77.14 $\mu\text{g}/\text{mg}$) to 30 (74.0 $\mu\text{g}/\text{mg}$) which was lower than control value (81.69 $\mu\text{g}/\text{mg}$). There was a slight decrease of DNA (from day 1 - 13.19 $\mu\text{g}/\text{mg}$ to 30 - 12.0 $\mu\text{g}/\text{mg}$) and RNA from day 1 - 9.30 $\mu\text{g}/\text{mg}$ to 30 - 9.0 $\mu\text{g}/\text{mg}$) when compared with that of controls (Table 3). In case of group F, there was a decrease in protein content from day 1 (76.56 $\mu\text{g}/\text{mg}$) to 30 (74.0 $\mu\text{g}/\text{mg}$) which was below the normal value (81.68 $\mu\text{g}/\text{mg}$) (Table 3). There was a slight and gradual decline in DNA (from day 1 - 13.17 $\mu\text{g}/\text{mg}$ to 30 - 12.0 $\mu\text{g}/\text{mg}$) and RNA (from day 1 - 9.26 $\mu\text{g}/\text{mg}$ to 30 - 9.0 $\mu\text{g}/\text{mg}$) content in experimental mice when compared with controls (DNA, 13.98 $\mu\text{g}/\text{mg}$; RNA, 9.69 $\mu\text{g}/\text{mg}$).

The mean values of protein, DNA and RNA from kidneys with their 't' values for 30 days experimental period are shown in Tables 4 to 6. A significant difference was found in the level of protein in all the experimental groups when compared with controls and in group A when compared with groups B, C, D, E and F. But there was no significant difference compared between the groups themselves (among groups B, C, D, E and F) (Table 4). The DNA and RNA level showed a significant

Table 4: 't' values of protein (kidney) obtained for different groups of mice treated with 0.4 mg/kg bw (group A), 0.8 mg/kg bw (group B), 1.6 mg/kg bw (group C), 3.2 mg/kg bw (group D), 4.0 mg/kg bw (group E) and 8.0 mg/kg bw (group F)

Experimental (A, B, C, D, E and F) and Control (a) groups							
Group	A	B	C	D	E	F	a
Protein:Mean	78.40	76.62	76.06	75.90	75.93	75.70	81.65
	A a	B b	C a	D a	E a	F a	
t value	_____ t=7.96* (p>0.05)	_____ t=9.50* (p>0.05)	_____ t=8.52* (p>0.05)	_____ t=9.21* (p>0.05)	_____ t=10.05* (p>0.05)	_____ t=10.54* (p>0.05)	
t value	A B _____ t=2.66*(p>0.05)	A C _____ t=3.03*(p>0.05)	A D _____ t=3.34*(p>0.05)	A E _____ t=3.52*(p>0.05)	A F _____ t=3.87*(p>0.05)		
t value	B C _____ t=0.67@ (p<0.05)	B D _____ t=0.87@ (p<0.05)	B E _____ t=0.88@ (p<0.05)	B F _____ t=1.19@ (p<0.05)			
t value	C D _____ t=0.16@ (p<0.05)	C E _____ t=0.14@ (p<0.05)	C F _____ t=0.41@ (p<0.05)				
t value	D E _____ t=0.03@ (p<0.05)	D F _____ t=0.24@ (p<.05)					
t value	E F _____ t=0.28@ (p<0.05)						

P value at 5% level of significance is 2.306; *Significant values @Non-significant values

Table 5: 't' values of DNA (kidney) obtained for different groups of mice treated with 0.4 mg/kg bw (group A), 0.8 mg/kg bw (group B), 1.6 mg/kg bw (group C), 3.2 mg/kg bw (group D), 4.0 mg/kg bw (group E) and 8.0 mg/kg bw (group F).

Experimental (A, B, C, D, E and F) and Control (a) groups							
Group	A	B	C	D	E	F	a
DNA:Mean	13.37	13.28	13.15	12.86	12.81	12.79	13.94
	A a	B a	C a	D a	E a	F a	
t value	_____ t=7.26* (p>0.05)	_____ t=9.96* (p>0.05)	_____ t=13.84* (p>0.05)	_____ t=5.90* (p>0.05)	_____ t=6.58* (p>0.05)	_____ t=6.92* (p>0.05)	
t value	A B _____ t=0.84@ (p<0.05)	A C _____ t=2.24@ (p<0.05)	A D _____ t=2.58*(p>0.05)	A E _____ t=2.92*(p>0.05)	A F _____ t=3.11*(p>0.05)		
t value	B C _____ t=1.50@ (p<0.05)	B D _____ t=2.20@ (p<0.05)	B E _____ t=2.53* (p>0.05)	B F _____ t=2.72* (p>0.05)			
t value	C D _____ t=1.53@ (p<0.05)	C E _____ t=1.84@ (p<0.05)	C F _____ t=2.02@ (p<0.05)				
t value	D E _____ t=0.19@ (p<0.05)	D F _____ t=0.27@ (p<0.05)					
t value	E F _____ t=0.08@ (p<0.05)						

P value at 5% level of significance is 2.306; *Significant values @Non-significant values

difference (except the RNA level in group B) when compared with controls and a non-significant difference when compared among themselves (Tables 5 and 6) (except the DNA level in group A compared with groups D, E and F; in group B compared with groups E and F and the RNA level in group A when compared with F).

DISCUSSION

In all the experimental groups of mice the content of protein, DNA and RNA showed a significant decrease in kidneys during 1, 4, 8, 12, 16 and 30 when compared to controls. The significant decrease of DNA and RNA in kidneys of all the treated groups of mice during the entire treatment period is

Table 6: 't' values of RNA (kidney) obtained for different groups of mice treated with 0.4 mg/kg bw (group A), 0.8 mg/kg bw (group B), 1.6 mg/kg bw (group C), 3.2 mg/kg bw (group D), 4.0 mg/kg bw (group E) and 8.0 mg/kg bw (group F).

Experimental (A, B, C, D, E and F) and Control (a) groups	A	B	C	D	E	F	a
RNA:Mean	9.37	9.14	9.24	9.20	9.16	9.16	9.37
t value	A a _____ t = 3.108* (p > 0.05)	B a _____ t = 2.27@ (p < 0.05)	C a _____ t = 6.46* (p > 0.05)	D a _____ t = 8.55* (p > 0.05)	E a _____ t = 9.89* (p > 0.05)	F a _____ t = 12.71* (p > 0.05)	
t value	AB _____ t = 0.93@ (p < 0.05)	AC _____ t = 1.30@ (p < 0.05)	AD _____ t = 1.76@ (p < 0.05)	AE _____ t = 2.13@ (p < 0.05)	AF _____ t = 2.42* (p < 0.05)		
t value	BC _____ t = 0.41@ (p < 0.05)	BD _____ t = 0.26@ (p < 0.05)	BE _____ t = 0.09@ (p < 0.05)	BF _____ t = 0.00@ (p < 0.05)			
t value	CD _____ t = 0.43@ (p < 0.05)	CE _____ t = 0.95@ (p < 0.05)	CF _____ t = 1.30@ (p < 0.05)				
t value	DE _____ t = 0.60@ (p < 0.05)	DF _____ t = 0.99@ (p < 0.05)					
t value	EF _____ t = 0.33@ (p < 0.05)						

P value at 5% level of significance is 2.306; *Significant values @Non-significant values

similar to that of Rahman *et al.* (2002) and Al-Twaty (2006) who also found DNA damage and decline in albino mice treated with chlorpyrifos, acephate and ethephon. As cellular RNA synthesis is a DNA dependent process, the decrease of DNA might effect the significant decrease of RNA in kidneys of all the experimental mice. These observations agree with that of De Hondt *et al.* (1979) who also found significant decrease in RNA content in rats exposed to Cannabis extract due to the inhibition of DNA dependent RNA polymerase. The results on decrease of DNA level are compare well with that of Walter *et al.* (1980), Adhikari and Grover (1988), Barale *et al.* (1993), Zelesco *et al.* (1990), Cid *et al.* (1990) and Georgieva *et al.* (1990) who also found decreased DNA level (in mammalian cells like mice and rats) during carbamate treatment. Shivanandappa and Krishnakumari (1981) and Topaktas *et al.* (1996) and Ksheerasagar and Kaliwal (2006) found significant decrease in hepatic DNA and RNA in rats exposed to Benzene Hexane Chloride (BHC) and carbosulfan.

Distraction in the cell metabolism and division machinery might have led to significant fall in the level of protein, DNA and RNA in kidneys of imidacloprid treated mice. These results are analogous to that of Topaktas *et al.* (1996) who reported that the chemical, carbosulfan causes fragmentation of chromosomes and chromatids leading to reduction of mitotic index in rats. The decreased level of kidney protein and DNA in the present investigations coincide with that of Zidan and Galal (2012) who also found decline in the level of protein and DNA in liver of rats treated with malathion and spinosad.

Oral administration of different doses of imidacloprid induced significant reduction in total protein, DNA and RNA levels in all the experimental groups of mice. Bhardwaj *et al.* (2010) found significant toxicological effect on biochemical changes at 20 mg/kg/day to female rats. Diminution of protein may either be due to the low level of anabolic activity of cell or higher levels of degradation activities. The decline in soluble

protein content has been reported by various authors under insecticidal stress (Barros and Saliba, 1978; Zhou *et al.*, 1985; Murthy *et al.*, 1986; Chitra *et al.*, 1999; Arshad *et al.*, 2007). The significant reduction in total protein, DNA and RNA level in kidneys of mice at varied imidacloprid dose levels indicates marked damage to vital organs and also interference with protein metabolism. The detracted value of protein in kidneys of all the experimental groups of mice reflects the dysfunction of kidneys. These findings confirm that of Sharpe *et al.* (1996) and Abdel El-Hamid and Refaie (2009) who postulated the depreciation of total protein during avermectin intoxication (in rats) reveal liver or kidney disease respectively. Loss of protein (Richardson, 1981) and decrease of total and soluble protein (Swamy *et al.*, 1992) in rat brain during pesticide toxicity was reported. Harper *et al.* (1997) studied the decrease of total protein content due to catabolism of protein and/or malfunction of liver. Mahadevaswami *et al.* (2000) and Baligar and Kaliwal (2001) found that mancozeb and carbofuran have altered protein, glycogen and total lipid levels in liver, uterus and ovary of intact and hemi castrated rats and mice.

The present findings on the toxicity of imidacloprid in mice suggest significant decrease in the level of protein, DNA and RNA in kidneys. Though six groups of the experimental mice were treated with varied concentrations of imidacloprid, the decrease of protein, DNA and RNA was not dose dependent in any of the 3 test organs. Also, there was no significant alteration with regard to day 1, 4, 8, 12, 16 and 30 of treatment. The treated kidneys in all the 6 groups of mice showed imidacloprid toxicity on all days of experimental period. The decreased level of protein, DNA and RNA has no significant correlation with the chemical dose administered and there was no significant decrease of protein, DNA and RNA when comparison was made among day 1, 4, 8, 12, 16 and 30 of experimental period. These changes reveal either a decreased catabolism of the biomolecules to meet the energy demand of

animals under stress or their reduced synthesis due to impaired tissue function as suggested by Ivanova-Chemishanksha (1982). The biochemical parameters like protein, DNA and RNA are found sensitive to imidacloprid toxicity in male mice.

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