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Histological and Biochemical Changes in the Liver of Albino Mice on Exposure to Insecticide, Carbosulfan

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ABSTRACT
Carbosulfan (2,3-dihydro-2,2dimethyl-7-benzofuronyl [(dibutyl amino) thio] methyl] a carbamate insecticide and acaricide was administered orally at an effective dose of 48 mg/kg/day to albino mice for 5, 10, 20 and 30 days .Control mice received similar quantities of olive oil. Daily body weights were recorded and mice were sacrificed after 24 hours after the terminal exposure. The histologic examination of liver of the mice treated with carbosulfan for 10, 20 and 30 days revealed the dilation of central vein and sinusoids between hypertrophied hepatocytes. Vacuolization and hyalinization of hepatocytes with loss of radial arrangement. Treatment with carbosulfan for 20 days in female and male mice resulted in a significant decrease in protein and liver glycogen contents in female mice, whereas in male mice the glycogen was not changed significantly in the liver. The cholesterol content was increased significantly in male mice, but in female mice there was no significant change. Treatment with carbosulfan for 30 days caused significant decrease in DNA, RNA, protein, glycogen and significant increase in the level of cholesterol in male and female mice. Temporal study on liver enzymes displays treatment with carbosulfan for 20 days caused significant increase in LDH activity and significant decrease in Na^+K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and no significant change in SDH, ASAT, ALAT, ACP activity in female mice, however in male mice the activity of liver enzymes was not changed significantly. Carbosulfan treatment for 30 days caused significant decrease in SDH, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP activity, whereas LDH, ASAT, ALAT, AKP activity were increased significantly in the liver of male and female mice. The results of the present study suggests that the carbosulfan has adverse effects on liver functions leading to histologic and physiological impairment.

Key words: Carbosulfan, Enzymes, Histology, Liver, Toxicity.

INTRODUCTION

Carbosulfan (2,3-dihydro-2,2dimethyl-7-benzofuronyl [(dibutyl amino) thio] methyl carbamate is an insecticide as well as acaricide. It is active against caterpillars, green leaf hopper, white-backed plant hopper, brown plant hopper, gall midge, stem borer and leaf folder of paddy and white aphids of chillies. Sign of toxicity were generally observed when acetylcholinesterase activity was inhibited by more than 35% and tremors occurred at inhibition by more than 70% (Renzi and Kreiger, 1986). Carbosulfan is in the priority list for toxicological evaluation by Joint FAO/ WHO meeting on pesticide residues in 2003 (JMPR, 2003). The major routes of insecticide exposure to agricultural workers include dermal and respiratory (Durham and Wolfe, 1962). Accidental exposure to high level of toxic substances are known to cause liver damage. Bhaynagar *et al*., (1982) reported a significant rise in aspartate aminotransferase after chronic exposure to pesticides. Experimental studies in rats that are given intramuscular injection of propoxur a carbamate pesticide found to increase total bilurbin, alanine aminotransferase, and amylase (Kumar *et al*., 1993).

Organophosphorus insecticide parathion interfere with hepatic microsomal metabolism and affects Cyt P-450 level (Butler and Murray, 1993). Shrivastava *et al*., (1991) have reported that there was a significant increase in alkaline phosphatase in pesticide sprayers, suggests a confirmed hepatic damage.

Insecticides preliminarily act on CNS either as nerve poisons or as acetyl cholinesterase inhibitors, they also affect normal functioning of other organs, thus challenging the homeostasis of the organism.

Since liver is associated with metabolism and elimination of toxicants from the body and it's histologic and biochemical parameters are considered as key points to elucidate toxicity of the chemicals. Reports regarding carbosulfan effects on liver are scanty. Hence, the present investigation was undertaken to elucidate the effects of carbosulfan on liver histology, biochemical contents such as DNA, RNA, protein, glycogen, cholesterol and activity of enzymes such as dehydrogenases, aminotransferases and phosphatases in albino mice.

Materials and Methods

Chemical

Carbosulfan technical grade (93.33%) was obtained from Rallies India Ltd., Bangalore, had been used for the experiments. The effective dose 48 mg/kg/day given orally in olive oil vehicle for 5, 10, 20 and 30 days below their acute LD_{50} level of intoxication according to their body weight. The mouse oral LD_{50} for carbosulfan is 129 mg/kg body weight (Fukuto, 1983).

Animals and treatments

Laboratory bred adult Swiss albino mice were used in the experiments. The mice were maintained in the laboratory, Postgraduate Department of Studies in Zoology, Karnatak University, Dharwad, Karnataka, India. Mice (80- 90 day- old) each weighing 25-30 g were used. They were housed in separate polypropylene cages containing sterile paddy husk as the bedding material.

The animals were provided with standard pellet diet "Gold Mohar" (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* throughout the study. The mice were maintained under normal day/ night schedule (12L: 12 D) at room temperature 26˚C ± 1˚C. Carbosulfan administered orally in olive oil vehicle at dose 48 mg/ kg / day for 5, 10, 20 and 30 days.

Carbosulfan administered orally because the major available carbosulfan residue in the environment enters the non-target animals is by orally. All the animals were killed on 24 hours after the last dose treatment and liver taken out for histologic and biochemical studies.

Histological studies

Liver was removed, washed in saline, fixed in bouin's fluid, dehydrated in ethanol and embedded in paraffin, serial sections at 5 µm were prepared and stained with haemotoxylin eosin.

Biochemical estimations

Freshly removed liver freed from adherent tissues weighed to nearest milligram was used for biochemical studies such as estimations of DNA and RNA as per the method of Schnieider (1957), protein by Lowry *et al.,*(1951), glycogen by Carrol *et al.,*(1956) , cholesterol by Abell *et al.,* (1952), activity of enzymes such as SDH by Nachlas *et al.,*(1960), LDH by King (1965), ASAT and ALAT by Yatzidis (1960), Na^{+}-K^{+}ATPase, Ca^{++}ATPase and Mg^{++}ATPase were assayed according to the method described by Jinna *et al.,*(1989) ACP and AKP by Linhardt and Walter (1965) were carried out.

Statistical analysis

Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett's test ($P<0.05$).

Results and Discussions

Histological studies

Liver histologic observations of the control mouse showed radially arranged hepatic cords around the central vein (Fig. 1). The durational study in the mice with 48 mg /kg/day carbosulfan exposure for 5 days, liver histology revealed hypertrophy and hyalinization of hepatocytes with dilated central vein (Fig. 2). The histologic observations of the liver in the mice exposed to carbosulfan for 10, 20 and 30 days revealed vacuolization, hypertrophy, hyalinization and loss of radial arrangement of hepatocytes. Central vein and sinusoids between hepatocyts were dilated (Figs. 3-5).

Biochemical studies

Biochemical contents

Temporal study on liver biochemical contents exhibited that treatment with 48 mg/ kg / day carbosulfan for 20 days in female and male mice resulted in no significant change in the levels of DNA and RNA. However, there was significant decrease in the protein level. The same durational exposure caused significant decrease in liver glycogen in female mice, but in male mice glycogen

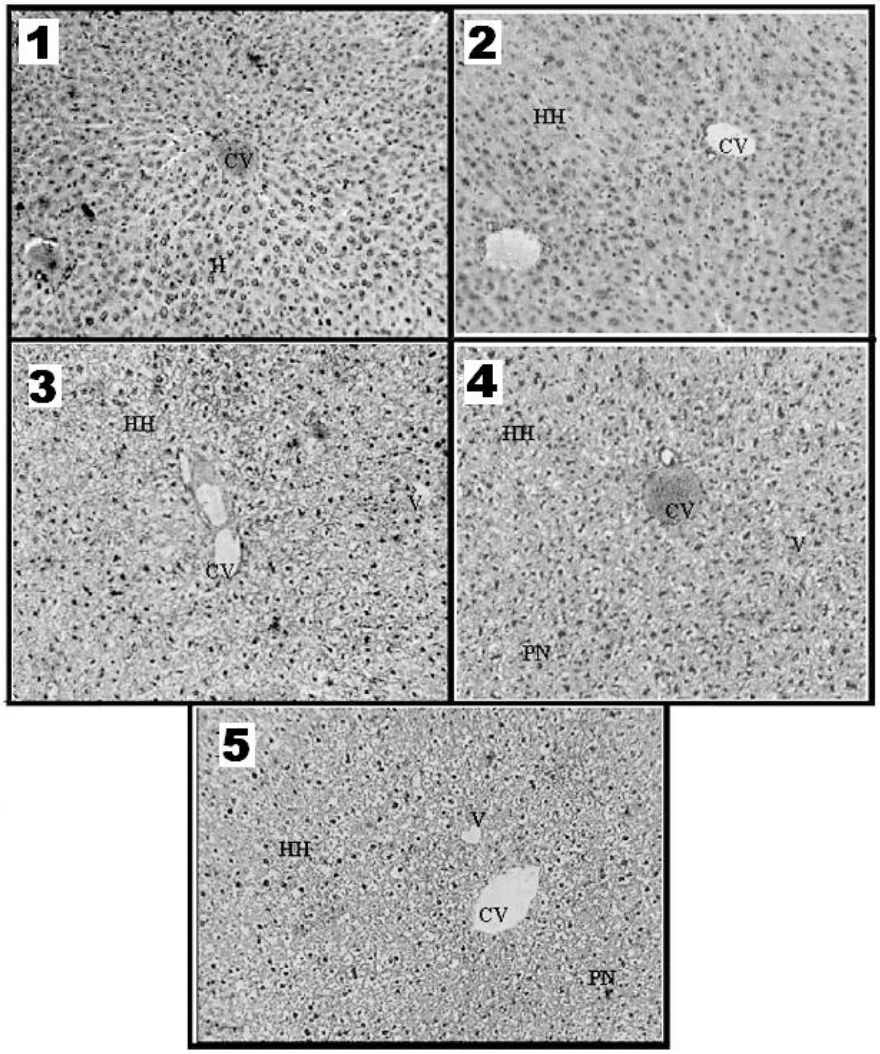

Fig 1. T. S. of the liver of the control mouse showing radially arranged hepatic cords around the central vein. Normal hepatocytes with centrally located nuclei.

Fig 2. T. S. of the liver of the mouse treated with 48 mg/ kg/ day carbosulfan for 5 days showing hypertrophy and hyalinization of hepatocytes with dilated central vein.

Fig 3. T. S. of the liver of the mouse treated with 48 mg/ kg/ day carbosuflan for 10 days showing dilation of central vein, hypertrophy of hepatocytes with pyknotic nuclei, vacuoles and hyalinization.

Fig 4. T. S. of the liver of the mouse treated with 48 mg/ kg/ day for 20 days showing vacuolization, hypertrophy, hyalinization and loss of radial arrangement of hepatocytes. Central vein and sinusoids between hepatocytes were dilated.

Fig 5. T. S. of the liver of the mouse treated with 48 mg/ kg/ day carbosulfan for 30 days showing vacuolization, hypertrophy and hyalinization of hepatocytes with dilation of central vein.

Photographs original exposure at × 100.

Abbreviations : V - Vacuoles, **CV**-Central vein , **H** –Hepatocytes , **HH** - Hypertrophied hepatocytes, **PN** - Pyknotic nuclei.

Table 1. Temporal effect on biochemical contents in Liver of female albino mice after exposure to carbosulfan.

Treatment duration (days)	Biochemical contents (µg/ mg wet weight of tissue)				
	DNA	RNA	Protein	Glycogen	Cholesterol
Control	1.78 ± 0.09	3.25 ± 0.12	188.14 ± 9.10	6.58 ± 0.47	10.77 ± 0.23
5	1.70 ± 0.06	3.22 ± 0.13	180.71 ± 7.82	6.31 ± 0.38	10.85 ± 0.12
10	1.58 ± 0.05	3.18 ± 0.14	167.12 ± 7.02	5.18 ± 0.35	10.90 ± 0.29
20	1.41 ± 0.09	3.06 ± 0.19	149.11 ± 6.51*	5.11 ± 0.31*	11.83 ± 0.34
30	1.16 ± 0.05*	2.56 ± 0.23*	139.20 ± 7.60*	4.10 ± 0.32*	13.45 ± 0.70*

Values are mean ± SEM of 5 animals.
* Significant P ≤ 0.05 compared to Olive oil control.

was not changed significantly, whereas cholesterol was increased significantly but in female mice there was no significant change in cholesterol level. Treatment with carbosulfan for 30 days caused significant decrease in DNA, RNA, protein, glycogen and significant increase in cholesterol in the liver of male and female mice. However, treatment with 48 mg carbosulfan for 5 and 10 days caused no significant change in liver biochemical contents of female and male mice (Tables 1, 2).

Enzymes activities

Treatment with 48 mg/ kg/ day carbosulfan for 20 days caused significant increase in the activity of LDH and significant decrease in the activity of Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and no change in the activity of SDH, ASAT, ALAT, ACP and AKP in female mice However there was no significant change in enzymes activities in male mice. Treatment with carbosulfan with same dose for 30 days caused significant decrease in SDH, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and ACP activity, whereas LDH, ASAT, ALAT and AKP activity were increased significantly in female and male mice. However, treatment with carbosulfan for 5 and 10 days caused no significant change in the activities of enzymes in liver of both female and male mice (Tables 3, 4).

Discussuion

In the present study liver histologic observations of the control mouse showed radially arranged hepatic cords around the central vein. The histological study of liver of the mice treated with carbosulfan for 10,20 and 30 days showed dilated central vein and sinusoids between hypertrophied hepatocytes with pyknotic nuclei, vacuoles and hyalinization. This could be due to morphological and chemical induced injury

Table 2. Temporal effect on biochemical contents in liver of male albino mice after exposure to carbosulfan.

Treatment duration (days)	Biochemical contents (µg/ mg wet weight of tissue)				
	DNA	RNA	Protein	Glycogen	Cholesterol
Control	1.85 ± 0.12	3.36 ± 0.20	186.95 ± 8.30	6.67 ± 0.41	11.61 ± 0.80
5	1.80 ± 0.08	3.28 ± 0.21	181.04 ± 7.98	6.57 ± 0.39	11.91 ± 0.77
10	1.56 ± 0.11	3.16 ± 0.23	173.29 ± 6.31	6.30 ± 0.27	12.38 ± 0.50
20	1.49 ± 0.12	2.44 ± 0.18	154.45 ± 6.40*	5.80 ± 0.36	13.89 ± 0.49*
30	1.13 ± 0.18*	2.41 ± 0.14*	147.01 ± 4.47*	4.75 ± 0.20*	14.25 ± 0.19*

Values are mean ± SEM of 5 animals. (Standard way of representation: n=5, mean±SEM)
* Significant P ≤ 0.05 compared to Olive oil control.

Table 3. Temporal effect on liver dehydrogenase, aminotransferase and phosphatase enzymes activity in female albino mice after exposure to carbosulfan

Treatment duration (days)	Enzyme activity (µmoles/ min/ g tissue weight)								
	LDH[s]	SDH[b]	ASAT[a]	ALAT[a]	Na^+-K^+ ATPase[c]	Mg^{++} ATPase[c]	Ca^{++} ATPase[c]	ACP[d]	AKP[d]
Control	12.50 ± 0.43	13.41 ± 0.46	16.03 ± 0.50	14.76 ± 0.34	3.98 ± 0.21	6.86 ± 0.22	3.50 ± 0.15	14.61 ± 0.35	16.18 ± 0.49
5	14.08 ± 0.50	13.25 ± 0.32	16.76 ± 0.29	15.10 ± 0.33	3.80 ± 0.28	6.50 ± 0.15	3.42 ± 0.19	14.48 ± 0.27	17.63 ± 0.48
10	14.32 ± 0.62	12.54 ± 0.26	17.08 ± 0.38	15.32 ± 0.29	3.65 ± 0.20	6.15 ± 0.36	3.29 ± 0.17	13.81 ± 0.29	17.74 ± 0.37
20	15.60 ± 0.41*	12.12 ± 0.35	17.77 ± 0.26	15.85 ± 0.32	2.89 ± 0.22*	5.06 ± 0.34*	3.17 ± 0.14*	13.28 ± 0.45	18.12 ± 0.31
30	15.85 ± 0.81*	11.21 ± 0.32*	18.11 ± 0.36*	16.12 ± 0.26*	2.72 ± 0.20*	4.87 ± 0.18*	2.88 ± 0.17*	11.46 ± 0.41*	18.16 ± 0.56*

Table 4. Temporal effect on liver dehydrogenase, aminotransferase and phosphatase enzymes activity in male albino mice after exposure to carbosulfan.

Treatment duration (days)	Enzyme activity (µmoles/ min/ g tissue weight)								
	LDH[s]	SDH[b]	ASAT[a]	ALAT[a]	Na^+-K^+ ATPase[c]	Mg^{++} ATPase[c]	Ca^{++} ATPase[c]	ACP[d]	AKP[d]
Control	14.61 ± 0.26	13.65 ± 0.64	15.73 ± 0.27	12.71 ± 0.08	4.38 ± 0.44	7.51 ± 0.56	3.22 ± 0.22	12.88 ± 0.49	17.06 ± 0.39
5	15.05 ± 0.51	13.40 ± 0.53	16.28 ± 0.31	13.35 ± 0.07	4.13 ± 0.27	7.42 ± 0.38	3.18 ± 0.18	13.15 ± 0.63	17.01 ± 0.55
10	15.45 ± 0.59	12.96 ± 0.27	16.32 ± 0.24	13.85 ± 0.10	3.31 ± 0.26	7.26 ± 0.34	3.05 ± 0.16	12.15 ± 0.85	18.75 ± 0.71
20	15.90 ± 0.31	12.58 ± 0.82	16.81 ± 0.18	13.94 ± 0.09	3.10 ± 0.31	7.04 ± 0.19	2.87 ± 0.23	11.32 ± 0.59	20.00 ± 0.70
30	16.00 ± 0.50*	11.73 ± 0.37*	16.98 ± 0.29*	14.06 ± 0.07*	2.75 ± 0.20*	6.82 ± 0.11*	2.75 ± 0.32*	10.90 ± 0.55*	21.56 ± 0.73*

a: µmoles of pyruvate formed/ min/ g tissue
b: µmoles formazon formed/ min/ g tissue.
c: µmoles of inorganic phosphorus formed/ min/ g tissue.
d: µmoles of p-nitrophenyl formed/ min/ g tissue.
Values are mean ± SEM of 5 animals.

that can manifest itself in different ways. The acute effect consists of accumulation of lipids (fatty liver) and the appearance of degenerative processes leading to the death of the cell. The necrotic process can affect small groups of isolated Parenchymal cells ("focal necrosis"), groups of cells located in zones ("centrilobular, mid zonal or periportal necrosis") or virtually all the cells within an hepatic lobule (massive necrosis). Altered hepatic cell membrane permeability can lead to increased enzyme activity in plasma (Plaa, 1986). Treatment with organophosphate insecticide phosphomidon alone and in combination with benzene causes hepatic changes in rabbits. The dilation and congestion of sinusoids, ballooning of hepatocytes with pycnotic nuclei and focal necrosis was found (Dikshit *et al.,* 1980). Choudhary *et al.,* (2003) have revealed that the treatment with endosulfan, 10 mg/ kg/day in rats causes liver damage which includes dilation of sinusoidal spaces with irregular nuclear shape, degenerative changes includes binucleated cells, hypertrophy of hepatocytes and lymphocytic infiltration in the central vein. In rats, treatment with permethrin 620-mg/kg/day and DDT 12 mg/kg/day separately causes liver damage. Histopathologic study revealed hepatocytes with pyknotic nuclei, acidophilic cytoplasm and cell with nuclear fragmentation induced by permethrin. Whereas DDT causes cytoplasmic vacuolization and hepatocyte necrosis (Kostka *et al.,* 2000).

Study on liver biochemical contents showed that prolonged exposure of carbosulfan caused decrease in the levels of DNA and RNA, in male and female mice. Walter *et al.,* (1980) have reported that malathion induces decreased content of RNA and DNA in the human lymphocytes in the *in vitro* studies at a concentration of 50 and 70 µg/ml. The carbamate pesticides such as benomyl and propoxur lead to formation of chromosomal breaks by breaking the phosphodiester backbone of DNA, and can induce aneuploidy and polyploidy by preventing the formation of spindles (Adhikari and Grover, 1988; Barale *et al.,* 1993; Zelesco *et al.,* 1990; Cid *et al.,* 1990; Georgieva *et al.,* 1990). Shivanandappa and Krishnakumari (1981) have also reported that in the rats treated with BHC significant reduction is caused in hepatic DNA and RNA, with an indication of cell death due to focal necrosis. In the present study the reason for decreased nucleic acids levels in liver under the influence of carbosulfan treatment in mice might caused genotoxic action by decreased mitotic index and disturbed cell division (Topaktas *et al.,* 1996) or due to inhibitory action of pesticides on DNA and RNA synthesis (Walter *et al.,* 1980) or by cell death due to focal necrosis (Shivanandappa and Krishnakumari, 1981).

In the present study it was revealed that prolonged exposure of carbosulfan caused decrease in the level of protein in the liver of female and male mice. Significant decrease in total protein level might be due to catabolism of protein and/ or malfunction of liver (Harper *et al.,* 1977). It has been reported that rapid loss in proteins of the brain during pesticide toxicity was reported (Richardson, 1981). It has been suggested that acute treatment with monocrotophos showed tissue specific inhibition of microsomal cyt-p-450 in hepatic and extrahepatic tissues resulting in the loss of haemoprotein in rats (Siddiqui *et al.,* 1987). Swamy *et al.,* (1992) have reported that the decrease in total proteins and soluble proteins indicate their metabolic utilization. The increase in the activity of proteases correlated with the decrease of soluble and total protein. The increasing duration of exposure of carbosulfan caused decrease in the level of glycogen in the liver of both female and male mice. It has been reported that there was a significant decrease in the levels of blood glucose and globulin in mancozeb treated rats, due to low thyroxin level because of impaired thyroid function (Nebbia and Ferrero, 1991). Ivanova-Chemishanska (1982) has reported that the changes in the levels of protein and glycogen suggests either an increased catabolism of the biomolecules to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function.

The present study suggested that prolonged exposure of carbosulfan caused increase in the level of cholesterol in the liver of both female and male mice. It has been reported that there was an increased serum cholesterol level in the rats exposed to BHC. Plasma cholesterol levels were considered as valuable indicator of drug-induced disruption of lipid metabolism Marked dose-dependent increase of serum cholesterol in BHC fed rats suggests increased synthesis

and accumulation of cholesterol in the liver, kidney and testis and or impaired biliary function (Shivanandappa and Krishnakumari, 1981). Similar results were also reported in rats treated with dimethoate (Siddiqui *et al.,* 1991). Diethyl dithiocarbamate inhibits hepatic cyt-p-450 dependent activity in rats (Stott *et al.,* 1997). The increase in cholesterol level indicates inhibitory action of pesticide on Cyt-p-450 enzymes (Shivanandappa and Krishnakumari , 1981; Siddiqui *et al.,* 1987 ; Stott *et al.,*1997) ,or might be due to high affinity binding (Zarh *et al.,* 2002). In the present study, cholesterol increase in the liver might be due to inhibition in the activity of enzymes involved in cholesterol break up results into deposition of cholesterol in the cell. Recently it has been reported that mancozeb and carbofuran treatments have altered the levels of protein, glycogen and total lipids in the liver, uterus and ovary in intact and hemicastrated rats and mice (Mahadevaswami *et al.,* 2000; Baligar and Kaliwal, 2001)

In the present study it has been found that increase in the duration of exposure of carbosulfan caused decrease in the activity of SDH and increase LDH activity in liver of both male and female mice. Increased activity of LDH was reported both in serum and liver of rats treated with cypermethrin (420 mg/ kg) for a period of six months (Shakoori *et al.,* 1988). Polychlorinated biphenyl (arochlor) increased the liver LDH at 50 ppm level and decreased its activity at 100 ppm in rats (Rao and Banerji, 1990). The organochlorine pesticides benzene hexachloride cyclohexane known to cause increased liver LDH activity (Shivanandappa and Krishnakumari, 1981). The methyl parathion (2 mg/ kg) treated rats showed an enhanced level of serum and liver LDH (Dikshith *et al.,* 1991). It has been reported that treatment with carbamate fungicide mancozeb caused significant decrease in SDH where as LDH increased significantly in testis and epididymis of rats (Kacker *et al*., 1997). In the present study carbosulfan caused decrease in the activity of SDH and increased LDH activity in the liver. The elevated activity of LDH indicates a compensatory mechanism by the affected tissue that requires additional energy for its maintenance and decreased SDH activity shows the pesticide-induced effect.

In the present study it has been showed that increase in the duration of exposure of carbosulfan caused increase in the activity of ASAT and ALAT in the liver of both male and female mice. Similarly Shrivastava *et al.,* (1989) have reported that ASAT and ALAT levels were increased significantly in plasma, liver, kidney, lung, brain, heart, intestine and muscle of rat treated with dichlorvos and suggested that these results might be due to cellular damage or increased permeability of plasma membrane. Similar increase in the tissues and plasma levels of these enzymes have also been reported in various species of animals given acute and sub-acute doses of other organo phosphorus (op) insecticides (Snow and Watson, 1993; Enan, 1983). The ASAT and ALAT enzymes are involved in amino acid metabolism and an increase in these enzymes in serum indicate tissue damage or toxic effects in liver (Klassen and Plaa, 1966; Worblewski and La Due, 1955). In the present study the rise in ASAT and ALAT levels in the liver and kidney of male and female mice could be due to hepatotoxicity causing permeability alterations and leakage of lysosomal enzymes causing enhanced release of enzymes (Choudhary *et al.,* 2003; Worblewski and La Due, 1955; Klassen and Plaa, 1966; Shrivastava *et al.,* 1989; Snow and Watson, 1993; Enan, 1983).

In the present study it has been recorded that increase in the duration of exposure of carbosulfan caused decrease in the activity of Na^{+}-K^{+}ATPase, Mg^{++}ATPase, Ca^{++}ATPase in the liver of female and male mice. The inhibition of ATPases by pesticides disrupt ATP utilization within the synaptic area and alter the energy metabolism of the nerve terminated by secondarily altering the activities of other enzymes for which ATP or ADP may be allosteric effects (Brown and Sharma, 1976). Organochlorine pesticides affect membrane bound ATPases involved in active transport across cell membrane (conduction of nerve impulses) in different laboratory animals (Koch, 1969a, 1969b; Desaiah, 1982; Jinna *et al.,* 1989). Although these enzymes are well-known targets of organochlorine and OP compounds but reports are also available showing inhibition of these enzymes by carbamate pesticides (Brown and Sharma, 1976; Pala *et al.,* 1991; Babu *et al.,* 1990). Thus, ATPases are very sensitive to chemical interaction and can be used as reliable biomarker for the mechanistic

toxicity studies of pesticides. In the present study, it has been found that increasing duration of exposure of carbosulfan caused decreased activity of ATPases in liver. This could be due to pesticide induced effect on cell membrane because of their strong affinity for interaction with member lipids causing inhibition of membrane bound ATPase enzymes activity by affecting enzyme complex (Kinter *et al.,* 1972; Brown and Sharma, 1976; Pala *et al*., 1991; Babu *et al*., 1990; Mishtra *et al.,* 1998).

In the present study it has been reported that increase in the duration of exposure of carbosulfan caused significant decrease in the activity of ACP and significant increase in the activity of AKP in the liver of both male and female mice. Increased serum and tissue ACP and AKP are symptoms of chemical induced tissue injury along with hepatocellular necrosis. Shrivastava *et al.,* (1989) have reported the elevated levels of ACP and AKP in plasma, liver, kidney, lung, brain, testis, heart, intestine and muscle of rat treated with dichlorvos. In contrast to this Nagoha *et al*., (1989) reported decreased ACP and AKP in liver and kidney but elevated in serum in rats treated with chlorquine. Similarly decreased AKP activity has been found in serum and liver in the rats administered with HCH and methyl parathion, except AKP elevated in liver of rats treated with methyl parathion (Dikshith *et al*., 1991). The change in ACP and AKP activity in the present study suggests the effect on absorptive or secretory surface of the cell membrane causing cellular leakage as indicated by decreased ACP activity and elevated AKP activity as an adaptive rise in enzyme activity to the persistent stress (Murphy and Porter 1966 ; Kackar *et al*.,1997; Mishra *et al*., 1998).The study reveals that carbosulfan might have affected cell metabolism and active transport of ions across cell membrane, cellular defence mechanism and detoxification system in liver. The results of the present study suggest carbosulfan has adverse effects on liver functions leading to physiological impairment.

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