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Impairment of hepatic biochemical contents and enzymes activities during carbosulfan intoxication in albino mice

R.L. Ksheerasagar[1, 3*], M.B. Hiremath[2] and B.B. Kaliwal[3]

[1]Department of Biomedical Science College of Health sciences, University of Mizan –Tepi , Mizan Teferi -260 Ethiopia
[2]KLES Kidney Foundation, KLES Dr Prabhakar Kore Hospital & MRC, Belgaum- 590010, India
[3]P.G. Department of Studies in Biotechnology and Microbiology, Karnatak University, Dharwad – 580 003, India

Abstract: Carbosulfan (2,3-dihydro-2,2dimethyl-7-benzofuronyl [(dibutyl amino) thio] methyl insecticide cum - acaricide was administered orally at doses 12, 24, 36 and 48 mg/(kg day) to albino mice for 30 days .The mice orally administer with similar volume of olive oil were served as control. Daily body weights were recorded and mice were sacrificed on day 31st or 24 hours after the terminal exposure. Liver dissected out freed from adherent tissue weighed to nearest milligram. The liver histology, estimations of biochemical contents and enzyme activities were carried out. The histological examination of the liver of the mice treated with 36 and 48 mg/(kg day) carbosulfan reveals dilation of central vein and sinusoids between hypertrophied hepatocytes. Vacuolization and hyalinization of hepatocytes with loss of radial arrangement. Study on liver biochemical contents showed liver DNA, RNA, protein, glycogen were decreased significantly in male and female mice treated with 36 and 48 mg/(kg day) carbosulfan. However, level of cholesterol increased significantly, but with 36 mg/ (kg day) carbosulfan caused no significant change in liver DNA level in male mice. Study on liver enzymes displays 48 mg/(kg day) carbosulfan treatment caused significant decrease in liver SDH, Na+-K+ATPase, Mg++ATPase, Ca++ATPase, ACP and significant increase in liver LDH, ASAT, ALAT, AKP activity in male and female mice. Carbosulfan at dose 36 mg/ (kg day) in female mice caused significant decrease in SDH activity, whereas other liver enzymes in male mice not changed significantly. However, in female mice activity of liver LDH increased significantly, Na+-K+ATPase, Mg++ATPase, ACP activity decreased significantly, whereas activity of ASAT, ALAT, Ca++ATPase and AKP not changed significantly. The activity of Mg++ATPase decreased significantly in the liver of female mice with 24 mg/(kg day) carbosulfan treatment. The results of the present study suggest carbosulfan has adverse effects on liver functions leading to physiological impairment. The study reveals carbosulfan might have affected cell metabolism and active transport of ions across cell membrane, cellular defence mechanism and detoxification system in liver.

Keywords: Carbosulfan, Liver, Histology, Biochemical contents, Enzymes activities, Toxicity.

INTRODUCTION

Carbosulfan (2, 3-dihydro-2,2dimethyl-7-benzofuronyl [(dibutyl amino) thio] methyl carbamate insecticide cum-acaricide. Carbosulfan 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. The neuortransmitter acetylcholinesterase, is potentially inhibited by carbosulfan in rats. 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 of compounds along with dimethoate and malathion for toxicological evaluation by Joint FAO/ WHO meeting on pesticide residues in 2003 (JMPR, 2003).

Pesticides are the only toxic chemicals released into the environment in large amounts. Their potential to cause adverse effects to human and wild life populations has been subject of intense study. Human exposure to insecticides can occur during storage, transport, mixing, loading and application (Coutts, 1980; Lavy and Mattice, 1985). 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 known to cause liver damage. The insecticide belongs to carbamates, phosphoric esters, pyrethroids employed by the workers know to cause hepatotoxicity. Tomei et al., (1998) have reported liver damage among 37 male environmental disinfection workers as evidenced by increased serum alanine aminotransferase, alkaline phosphatase and bilurbin levels. Organophosphorus insecticides are such as parathion interfere with hepatic microsomal metabolism and affects Cyt P-450 level (Butler and Murray, 1993). Shrivastava et al., (1991) have reported a significant increase in alkaline phosphatase in pesticide sprayers suggests the confirmed hepatic damage. Bhaynagar et al., (1982) reported a significant rise in aspartate

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*Corresponding Author,
Email: raghu.lksagar@gmail.com

aminotransferase after chronic exposure to pesticides. Experimental studies in rats given intramuscular injection of propoxur a carbamate pesticide found to increase total bilurbin, alanine aminotransferase, and amylase (Kumar et al.,1993). In the workers exposed to a variety of insectidies – carbamates, phosphoric esters and pyrethroids often simultaneously and at low dose they may cause some inhibition of hepatic microsomal enzymes through competition for Cyt P-450 binding sites. This may lead to damage to one of the body's basic detoxification pathways for endogenous and exogenous metabolites resulting in potentiation of the toxic effects of each compound (Shrivastava et al.,1991).

Toxic compounds interfere with key enzymatic processes that are concerned with normal physiology of the animal. This includes carbohydrate, protein and lipid metabolism. Biochemical changes occur before tissue pathological symptoms appear. Insecticides preliminarily acts 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 and kidney are associated with metabolism and elimination of toxicants from the body their 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 SDH, LDH, ACP, AKP, ASAT, ALAT, Na^{+}-K^{+}ATPase, Ca^{++}ATPase and Mg^{++}ATPase in in albino mice.

MATERIAL AND METHODS

Chemical

Carbosulfan technical grade (93.33%) was obtained from Rallies India Ltd., Bangalore, had been used for the experiments. The doses were given orally in olive oil vehicle at doses 12,24,36 and 48 mg/(kg day), 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 used in the experiments. The mice were maintained in the laboratory, P.G. Department of Studies in Zoology, Karnatak University, Dharwad. Mice weighing 25-30 g (80-90 day old) were used. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The animals were provided with standard pellet diet "Gold Mohar" (Hindustan Lever Ltd., Mumbai) 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 doses 12, 24, 36 and 48 mg/(kg day).

Histological studies

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

Biochemical estimations

Freshly removed liver freed from adherent tissues weighed to nearest milligram and 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

Histological studies

Liver histological observations of the control mouse showed radially arranged hepatic cords around the central vein (Fig.1). The hepatocytes with centrally located nuclei. The histological study of liver of the mice treated with 12 and 24 mg/(kg day) showed dilated central vein with hypertrophy of hepatocytes with pyknotic nuclei, vacuoles and hyalinization. Radial arrangement of hepatocytes lossed (Fig. 2, 3). The histological examination of the liver of the mice treated with 36 and 48 mg/(kg day) carbosulfan reveals dilation of central vein and sinusoids between hypertrophied hepatocytes. Vacuolization and hyalinization of hepatocytes with loss of radial arrangement (Fig. 4, 5)

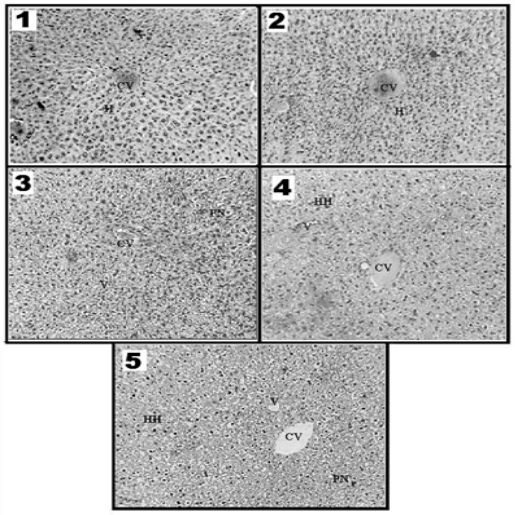

Fig. 1 : T.S. of the liver of the control mouse showing radially arranged hepatic cords around the central vein. Normal hepatocytes with centrally located nuclei.

Fig. 2 : T.S. of the liver of the mouse treated with 12 mg/ (kg day) carbosulfan for 30 days showing dilated central vein with hypertrophy of hepatocytes. Radial arrangement of hepatocytes is lossed.

Fig. 3 : T.S. of the liver of the mouse treated with 24 mg/ (kg day) carbosulfan for 30 days showing dilation of central vein and hypertrophy of hepatocytes with pyknotic nuclei, vacuoles and hyalinization. Radial arrangement of hepatopcytes is lossed.

Fig. 4 : T.S. of the liver of the mouse treated with 36 mg/ (kg day) carbosulfan for 30 days showing dilation of central vein and sinusoids between hypertrophied hypatocytes. Vacuolization and hyalinization of hepatocytes with loss of radial arrangement.

Fig. 5 : T.S. 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. Radial arrangement of hepatocytes is lossed.

Photographs original exposure at X 100.

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

Biochemical studies

Biochemical contents of liver

Study on liver biochemical contents showed liver DNA, RNA, protein, glycogen were decreased significantly in female and male mice treated with 36 and 48 mg/(kg day) carbosulfan. However, level of cholesterol increased significantly, but with 36 mg/(kg day) carbosulfan caused no significant change in liver DNA level in male mice. However, treatment with 12 and 24 mg/(kg day) carbosulfan caused no significant change in the level of liver biochemical contents in male and female mice (Table 1, 2).

Table 1. Effect on biochemical contents in liver of female albino mice after oral exposure to carbosulfan

Treatment (mg/kg/d)	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
12	1.64 ± 0.04	3.20 ± 0.11	170.82 ± 6.22	6.22 ± 0.36	10.90 ± 0.19
24	1.59 ± 0.07	3.17 ± 0.12	162.65 ± 5.65	5.13 ± 0.45	11.51 ± 0.33
36	1.32 ± 0.07*	2.76 ± 0.10*	146.21 ± 5.56*	4.20 ± 0.29*	12.37 ± 0.43*
48	1.16 ± 0.05*	2.56 ± 0.23*	139.20 ± 7.60*	4.15 ± 0.32*	13.45 ± 0.70*

Values are mean ± SEM of 5 animals

* Significant $P \leq 0.05$ compared to Olive oil control

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

Treatment (mg/kg/d)	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
12	1.76 ± 0.16	3.13 ± 0.13	178.11 ± 5.94	6.40 ± 0.33	12.11 ± 0.50
24	1.60 ± 0.13	2.76 ± 0.12	161.28 ± 6.58	5.38 ± 0.35	12.96 ± 0.26
36	1.32 ± 0.10	2.59 ± 0.27*	149.15 ± 3.84*	4.94 ± 0.15*	14.30 ± 0.20*
48	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
* Significant $P \leq 0.05$ compared to Olive oil control

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

Treatment (mg/kg/d)	Enzyme activity (µmoles / min/ g tissue weight)								
	LDH[a]	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
12	13.15 ± 0.52	12.50 ± 0.39	16.45 ± 0.31	14.97 ± 0.27	3.70 ± 0.18	5.96 ± 0.32	3.33 ± 0.19	13.98 ± 0.22	16.39 ± 0.45
24	13.70 ± 0.41	12.24 ± 0.32	17.01 ± 0.38	15.32 ± 0.29	3.35 ± 0.19	5.45 ± 0.22*	3.20 ± 0.16	13.67 ± 0.30	15.56 ± 0.58
36	14.45 ± 0.50*	11.27 ± 0.30*	17.77 ± 0.26	15.82 ± 0.39	2.81 ± 0.17*	4.91 ± 0.17*	3.12 ± 0.15	12.76 ± 0.50*	17.46 ± 0.41
48	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 ± 0.18*	11.46 ± 0.41*	18.16 ± 0.56*

[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.
* Significant $P \leq 0.05$ compared to Olive oil control

Enzyme activities in liver

Study on liver enzymes displays 48 mg/(kg day) carbosulfan treatment caused significant decrease in liver SDH, Na^{+}-K^{+}ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and significant increase in liver LDH, ASAT, ALAT, AKP activity in male and female mice. Carbosulfan at dose 36 mg/(kg day) in female mice caused significant decrease in SDH activity, whereas other liver enzymes in male mice not changed significantly. However, in female mice activity of liver LDH increased significantly, Na^{+}-K^{+}ATPase, Mg^{++}ATPase, ACP activity decreased significantly, whereas activity of ASAT, ALAT, Ca^{++}ATPase and AKP not changed significantly. However, treatment with 12 and 24 mg/(kg day) carbosulfan caused no significant change in liver enzymes activity in male and female mice, except Mg^{++}ATPase activity decreased significantly in the liver of female mice with 24 mg/(kg day) carbosulfan treatment (Table 3,4).

DISCUSSION

The extensive use of pesticide has resulted in environmental pollution by contaminating nearby living area. Owing to their myriad advantage and at the same time cannot be promoted due to the abundant disadvantage. Increase in the incidence of many diseases and impairment of normal physiology in human being have been associated with pesticide intake. Insecticides affect nervous system, gastro-intestinal irritation, vomiting and reproduction (Larson et al.,1961; O'Brien, 1967; Hayes, 1982).

Liver is the organ of immense importance due to its peculiar anatomy and metabolic functions which are exactly meant for efficient removal of the toxins. Liver is the largest exocrine gland in the body making up about 3.5 percent of the body weight of an adult rat or 2 percent of the body weight of an adult human (Hinton and Grasso, 1993).

Shivanandappa and Krishnakumari (1981) have revealed the histopathologic changes in the liver of rats treated with benzene hexachloride cyclohexane (BHC) an organochlorine insecticide, the hepatic histopathologic signs are hypertrophy, hyperplasia, vacuolization in peripheral and centrilobular areas and focal necrotic areas also found. The histochemical observations reveals accumulation of cholesterol positive lipids in the periportal hepatocytes as there is increase of total lipids content per liver. Methyl demeton one of organophosphate insecticide known to cause degenerative changes in hepatocytes includes necrosis and cytoplasmolysis in rats (Dikshit et al.,1980a). In rabbits treatment with organophosphate insecticide phosphomidon alone and in combination with benzene causes hepatic changes. The dilation and congestion of sinusoids, ballooning of hepatocytes with pycnotic nuclei and focal necrosis was found (Dikshit et al.,1980b). Althani et al. (1997) have reported oral dosing of a carbamate insecticide carbosulfan at a range 100 to 400 mg/(kg day) for 6 weeks caused liver damage in chickens. Histopathological features showed central veins, arterioles, capillaries and hepatic sinusoids were dilated, large focal areas of necrosis and hemorrhages in the liver parenchyma, increase in binucleated cells. Choudhary et al. (2003) have revealed that treatment with endosulfan ,10 mg/(kg day) in rats causes liver damage includes dilation of sinusoidal spaces with irregular nuclear shape, degenerative changes includes binucleated cells, hypertrophy of hepatocytes and lymphocytic infiltration in 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).

Liver histological observations of the control mouse showed radially arranged hepatic cords around the central vein. The hepatocytes with centrally located nuclei. The histological study of liver of the mice treated with 12 and 24 mg/(kg day) showed dilated central vein with hypertrophy of hepatocytes with pyknotic nuclei, vacuoles and hyalinization. Radial arrangement of hepatocytes lossed. The histological examination of the liver of the mice treated with 36 and 48 mg/(kg day) carbosulfan reveals dilation of central vein and sinusoids between hypertrophied hepatocytes. Vacuolization and hyalinization of hepatocytes with loss of radial arrangement.

This could be due to morphologicaly chemical induced injury can manifest itself in different ways. The acute effects can consists of an accumulation of lipids (fatty liver) and the appearance of degenerative processes leading to 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).

Nucleic acids are the most important macromolecules in the cells. DNA is known to be the genetic material and is most important of all macromolecules of cell. DNA carries all kinds of necessary biological information and is involved in gene action. RNA being the other important nucleic acid synthesized in nucleus but mainly found in cytoplasm to carry out protein synthesis. Proteins function in different ways in the cell as enzymes, structural proteins and some antibodies. Structural proteins are major constituents of skeletal and muscular tissue and also all membranous structures contain a structural component (Nelson et al.,1989). Glycogen is the polymer of glucose and is known as animal starch in muscle. Glucose is the most readily available source of energy in the animal tissues (Mayer, 1977). Cholesterol is the precursor for steroid hormones and also for vitamin D, which is essential for regulation of calcium and phosphorus metabolism and bone growth. Cholesterol is essential for membrane synthesis.

Flessel et al. (1993) have revealed malathion and organophosphate insecticide affects activity of esterases and other enzymes and possibly causes a disturbance in cell division machinery. Walter et al. (1980) reported 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. Shivanandappa and Krishnakumari (1981) have reported that in the rats treated with BHC caused significant reduction in hepatic DNA and RNA, with an indication of cell death due to focal necrosis. Topaktas et al. (1996) have reported that the carbamate insecticide carbosulfan causes chromatid breaks, fragments, sister chromatid exchange, chromosome breaks and reduction in mitotic index in bone marrow cells of rats. Similarly as carbosulfan the other 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).

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 suggested that there was a significant decrease in the microsomal protein Cyt-p-450 content of the liver, lungs, brain and kidneys of rats treated with pesticide vapacid (Mohd, 1993). Rapid loss in proteins of the brain during pesticide toxicity was reported (Richardson, 1981). 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 (Swamy et al.,1992).

It has been reported that increase in the levels of phosphoinositides and phosphotidic acid in the liver suggests the likely involvement of phospholipase in the toxicity of mancozeb in different tissues at varying levels (Subramanian et al.,1991). It has been reported that there is 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).

It has been reported that there was an increased serum cholesterol level in the rats exposed to BHC. Plasma cholesterol levels are considered valuable indicator of drug-induced disruption of lipid metabolism and development of fatty liver and altered cholesterol levels are implicated in impaired biliary excretion. The increased cholesterol levels and also suggestive of the inhibition of steroidogenesis in the testis and adrenal. 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). In the present study, cholesterol increase in liver and kidneys might be to inhibition in the activity of enzymes involved in cholesterol break up results into deposition of cholesterol into the cell.

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). Recently it has been reported that mancozeb and carbofuran treatments have altered levels of protein, glycogen and total lipids in liver, uterus and ovary in intact and hemicastrated rats and mice (Mahadevaswami et al.,2000; Baligar and Kaliwal, 2001). The 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).

Study on liver biochemical contents showed increasing dose exposure of carbosulfan caused decrease in level of DNA, RNA, protein, glycogen , where as cholesterol increased significantly in male and female mice. In the present study the reason for decreased nucleic acids levels in liver and kidneys under the influence of carbosulfan treatment in mice might have caused genotoxic action by decreased mitotic index and disturbed cell division (Topaktas et al.,1996) or may be due to effect on protein Cyt-p-450 (Mohd, 1993) or due to inhibitory action of pesticides on esterase and other enzymes causing disturbance in cell division machinery (Flessel et al.,1993) and affecting DNA, RNA synthesis (Walter et al.,1980) or by cell death due to focal necrosis (Shivanandappa and Krishnakumari, 1981).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). The changes in the levels of protein, glycogen and lipids with carbosulfan treatment 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 (Ivanova-Chemishanska, 1982).

Succinic dehydrogenase (SDH) is an important enzyme in citric acid cycle This enzyme bound to inner surface of the inner mitochondria membrane. Succinic dehydrogenase is an enzyme associated with tissue having high metabolic activity or engaging in absorptive or secretory activity (Padykula, 1952). Lactate dehydrogenase (LDH) is involved in glucose metabolism. Chemically induced stress causes elevated LDH activity and can be used as a good diagnostic tool in toxicology. Aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) are more sensitive measures of hepatotoxicity and can be assessed within a shorter time (Williams, 1980). These enzymes are known to act as an important link between carbohydrate and protein metabolism providing source of keto acids for Kreb's cycle and gluconeogenesis. ATPases are membrane bound enzymes concerned with immediate release of energy and are responsible for a large part of basic metabolic and physiological activities. Its role in the maintenance of trans membrane ionic gradients is well established (Skou, 1957), they have a vital role in the release and uptake of the biogenic amines in CNS (Banks, 1965; Patan et al., 1971) ATPase activity can be taken as meaningful tool. ATPase enzymes associated with lipoprotein in the form of a complex (Nakao et al., 1974). Acid phosphatase (ACP) and Alkaline phosphatase (AKP) are lysosomal enzymes, which catalyse the splitting of phosphoric acid from certain phosphoric esters and commonly found in most tissues of the body. They are generally located on absorptive or secretory surface of cells as membrane bound enzymes. ACP which hydrolysis the ester linkage of phosphate esters at acidic pH (between 5 to 6) and helps in autolysis of the degenerated cells (De Duve et al., 1955). AKP which splits phosphorus esters at alkaline pH (10) and mediates membrane transport and is intimately associated in protein synthesis (Pilo et al.,1972), and glycogen metabolism (Gupta and Rao, 1974).

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 pesticide 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).

Shrivastava et al., (1989) 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). ASAT and ALAT enzymes are involved in amino acid metabolism and an increase in these enzymes together with AKP in serum indicate tissue damage or toxic effects in liver (Klassen and Plaa, 1966; Worblewski and La Due, 1955).

Barlas (1996) revealed malathion induces serum enzyme alterations in mice exposed for 15 weeks. AKP activity increased in females but decreased in males significantly. ALAT activity decreased whereas ASAT activity increased. The increased AKP activity suggests possibility of lysosomal membrane releasing the enzyme from the liver damage following the treatment of an organophosphorus pesticide. Increasing levels of ASAT and ALAT are usually due to leakage of damaged membranes.

Similarly, Subramanian et al., (1991) reported that organometallic carbamate fungicide, namely maneb and mancozeb, interfere with lipid metabolism by increasing the levels of phospholipids in the liver. Kackar et al., (1999) have reported oral administration of fungicide mancozeb at doses 500, 1000 and 1500 mg/(kg day) for 98, 180 and 360 days caused alteration in hepatic enzymes, such as ASAT (aspartate amino transferase) ALAT (alanine amino transferase) activity and protein levels were decreased whereas alkaline phosphtase (ALP) was increased dose and duration dependent manner. Sunder and Rao (1998) have reported administration

of mancozeb along with metalaxyl to male rats for 90 days caused significant increase in the serum alanine amino transferase (ALAT) and aspartate amino transferase (ASAT) and liver glycogen whereas liver proteins decreased significantly.

In the present study it has been found that increasing dose exposure of carbosulfan caused decrease in SDH activity and increased LDH, ASAT, ALAT activity in liver. The decreased SDH activity shows the effect and elevated activity of LDH, ASAT, and ALAT indicates a compensatory mechanism by the affected tissue which requires additional energy for its maintenance. 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; Barlas, 1996; Worblewski and La Due, 1955 ; Klassen and Plaa, 1966; Shrivastava et al.,1989; Snow and Watson, 1993; Enan, 1983).

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, ATPase are very sensitive to chemical interaction and can be used as reliable biomarker for the mechanistic toxicity studies of pesticides.

The intentionally introduced environmental xenobiotics specially pesticides are known to have a strong affinity for interaction with membrane lipids (Antunes-Maderia and Maderia, 1987). Cell membrane is believed to be the site of action of insecticides by altering structural and functional integrity of cell membrane and also affects membrane bound enzymes such as total ATPase, Na^{+}-K^{+}ATPase and Mg^{++}ATPase (Shao et al., 1995; Rauchova et al., 1995). Insecticides such as methyl parathion and parathion are reported to inhibit activities of ATPases (Basha and Nayeemunnisa, 1993; Blasiak, 1995). Recently Archana et al., (2001) have reported an OP herbicide anilofos causes inhibition of total ATPase, Na^{+}-K^{+}-ATPase, Mg^{2+} ATPase in RBCs, brain and liver of rats. And suggested the ATPase inhibition may cause neuronal/ cellular dysfunction by affecting ionic transport across cell membrane.

In the present study, it has been found that increasing dose 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 (Antuner-Maderia and Maderia, 1987) causing inhibition of membrane bound ATPase enzymes activity by affecting enzyme complex (Kinter et al., 1972; Basha and nayeemunnisa, 1993; Shao et al., 1995; Rauchova et al., 1995; Blasiak, 1995; Mishtra et al., 1998).

In the present study, inhibition in the ATPase enzymes activity in liver and kidney of female and male mice reveals pesticide effect on cell metabolism and active transport of ions across cell membrane. Thus, ATPase are very sensitive to chemical interaction and can be used as reliable biomarker for the mechanistic toxicity studies of pesticides.

The results of the present study suggest carbosulfan has adverse effects on liver functions leading to physiological impairment. The study reveals carbosulfan might have affected cell metabolism and active transport of ions across cell membrane, cellular defence mechanism and detoxification system in liver.

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