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TEMPORAL EFFECTS OF CARBOSULFAN ON TESTICULAR BIOCHEMICAL PARAMETERS AND ENZYME ACTIVITIES IN ALBINO MICE

RAGHAVENDRA.L. KSHEERASAGAR[1]* AND BASAPPA. B. KALIWAL[2]

[1] Department of Biotechnology Presidency College Hebbal Bangalore 560024, INDIA*
[2]P.G. Department of Studies in Biotechnology and Microbiology Karnataka University, Dharwad – 580 003, INDIA

ABSTRACT

Carbosulfan a N- methyl insecticide was administered orally with an effective dose of 48 mg/kg body weight per day to mice for 5, 10 20 and 30 days to examine the temporal effects. Mice were sacrificed on 31st day. Treatment with Carbosulfan for 20 and 30 days caused significant decrease in protein, glycogen and sialic acid where as cholesterol increased significantly in testis. The activities of succinic dehydrogenase (SDH), acid phosphatase (ACP), were decreased significantly, whereas lactate dehydrogenase (LDH) and alkaline phosphatase (AKP) activities were increased significantly in testis, 3β hydroxysteroid dehydrogenase (3βHSD) decreased significantly in the mice treated with Carbosulfan for 20 and 30 days. These biochemical changes seem to be the genotoxic action leading to metabolic or hormonal imbalance in any of the stage in the hypothalamo-hypophysial-testicular axis in mice.

KEY WORDS: Carbosulfan, Testis, Biochemical contents, Enzymes activities, Toxicity.

**Corresponding author*

RAGHAVENDRA.L. KSHEERASAGAR
Department of Biotechnology Presidency College Hebbal Bangalore 560024, INDIA

INTRODUCTION

Carbosulfan belongs to the benzofuranyl methyl carbamate group of insecticide cum-acaricide. Carbosulfan is active against pests of paddy and white aphids of chillies. Carbosulfan at i.p. dose 5 mg/kg per day caused increased sperm head abnormality without affecting total count of sperms at 24 hr and 48 hr of treatment. The same treatment caused increased chromosomal aberration observed in bone marrow cells. This suggests Carbosulfan is a potent genotoxic agent and potential germ cell mutagen also[1]. There are various testicular dysfunctions such as oligospermia, azoospermia, degeneration of germinal epithelium in testicular biopsies and elevated serum level of follicle stimulating hormone (FSH) and leutinising hormone (LH)[2]. Carbamate fungicide mancozeb has been reported to affect reproduction by causing gonadal toxicity[3]. Carbosulfan is in the priority list of compounds along with dimethoate and malathion for toxicological evaluation by the Joint FAO/ WHO meeting on pesticide residues in 2003[4]. The reports regarding Carbosulfan induced testicular toxicity and biochemical effects are scanty. Therefore, the present investigation was undertaken to know the effect of different schedules of Carbosulfan on histologic evaluation of testis, biochemical contents such as protein, glycogen, sialic acid and cholesterol and activities of enzymes such as 3βHSD, SDH, LDH, ACP and AKP in testis were carried out in albino mice.

MATERIALS AND METHODS

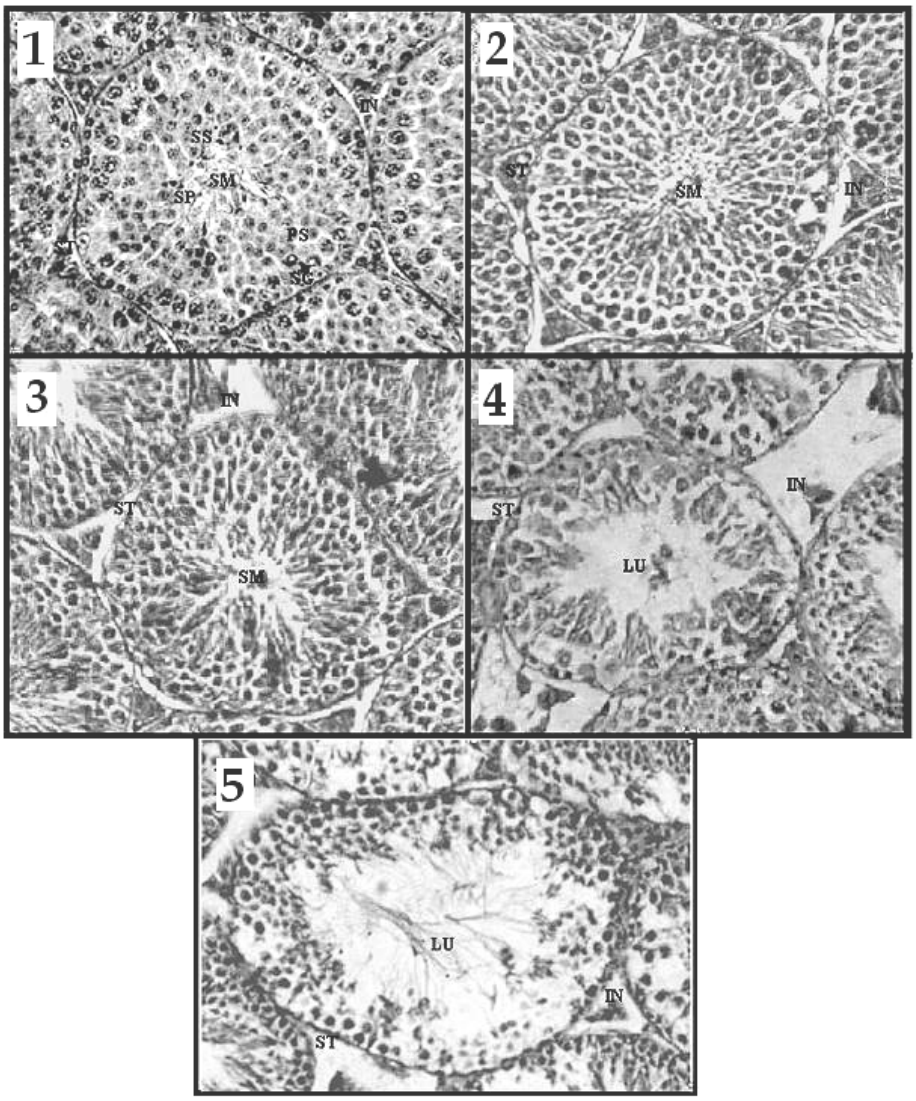
Chemical: Carbosulfan technical grade (93.33%) was obtained from Rallies India Ltd., Bangalore, had been used for the experiments. Carbosulfan was administered orally with an effective dose of 48 mg/kg body weight per day to mice for 5, 10 20 and 30 days, were given orally in olive oil vehicle below 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[5].

Animals: Laboratory bred male 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 30-35 g mice (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 dose 48 mg/kg /day for5, 10, 20 and 30 days. *Testes and accessory organs weight studies* All the animals were killed by cervical dislocation on 24 hr after the last dose treatment. The testes, epididymides, vasa deferentia, seminal vesicles, prostate, Cowper’s and coagulatory glands were dissected out and weighed. Organs weights were expressed per 100 g body weight. Histologic studies: The testes were processed, sectioned at 5 µm thickness,stained in hematoxylin - eosin and examined for histology. Biochemical estimations: Freshly removed testes weighed and biochemical studies such as estimations of protein by Lowry *et al.*[6] glycogen by Carrol *et al.,* [7], cholesterol by Abell *et al.,*[8], sialic acid by Yao *et al.,*[9] , activity of enzymes such as 3βHSD by Shivanandappa and Venkatesh [10], SDH by Nachlas *et al.,* [11], LDH by King [12], ACP and AKP by Linhardt and Walter [13], were carried out. *2.6. 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

Testis Histology: Histological examinations of the testis of the control mouse revealed the seminiferous tubules with normal

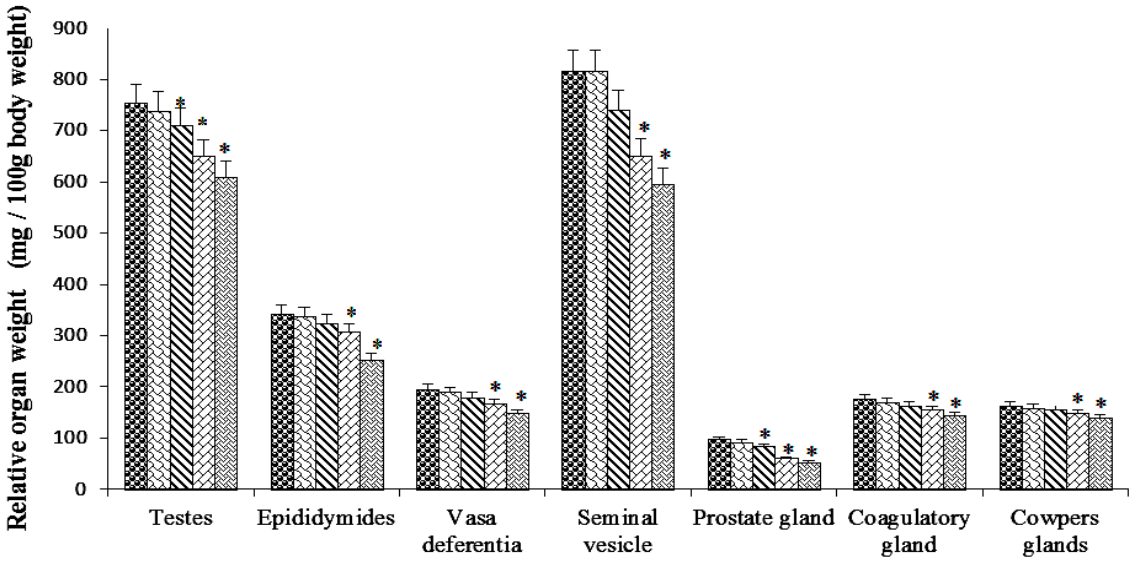
spermatogenesis with all cell types and well developed interstitial cells. (Fig.1).Testis of the mouse treated with Carbosulfan for 5 and 10 days showing stages of spermatogenesis with damaged germinal epithelium. (Fig. 2 and 3). Histologic examination of the testes of the mice treated with Carbosulfan for 20 and 30 days showed formation of giant cells, vacuoles and decreased number of spermatogenic cells and lumen with loss of sperms. Leydig cells are in deformed condition (Fig. 4, 5).



3.2. Testes and accessory sex organs weight

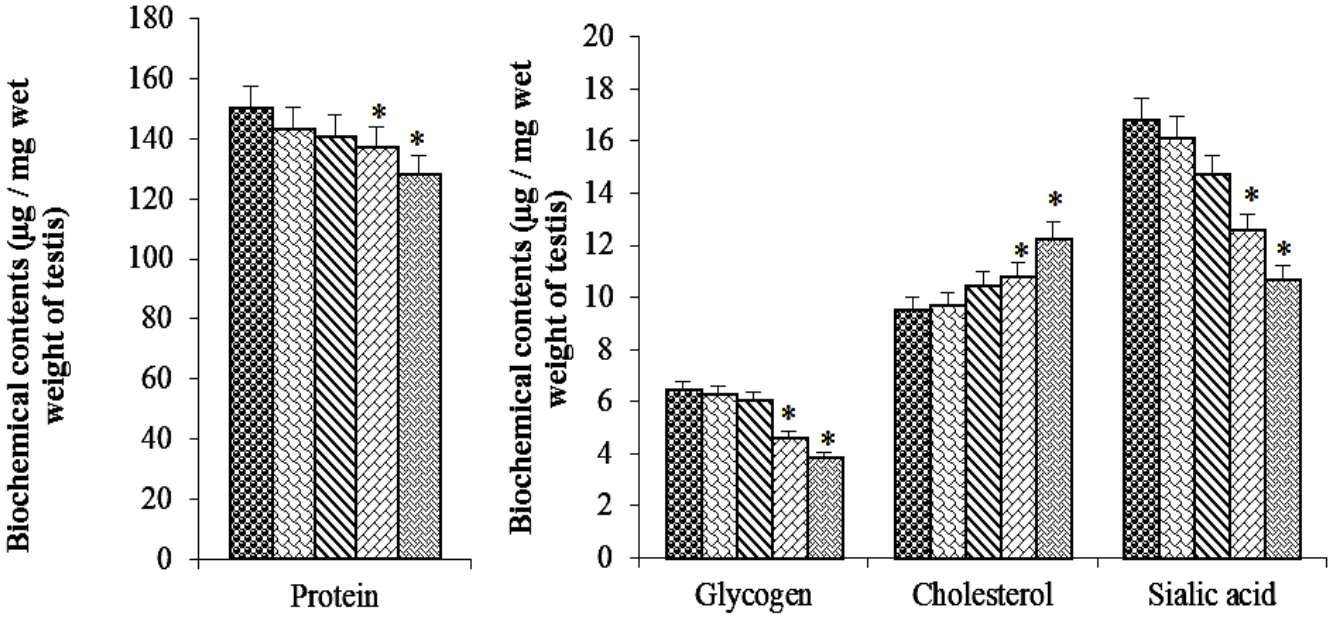
Oral administration of the Carbosulfan for 20 and 30 days caused significant decrease in the testes, epididymis, vasa deferentia, seminal vesicles, prostate, coagulatory and Cowper's glands weight. The 10 days Carbosulfan exposure caused significant decrease in testes and prostate gland weight when compared with controls (Fig.6).

Figure 6
Temporal effects of carbosulfan on testes and accessory reproductive organs.



Biochemical studies : Biochemical studies showed Carbosulfan treatment for 20 and 30 days caused significant decrease in the levels of protein, glycogen and sialic acid whereas cholesterol increased significantly in testis .Carbosulfan exposure for 10 days caused a significant increase in cholesterol in epididymis when compared with control (Fig.7).

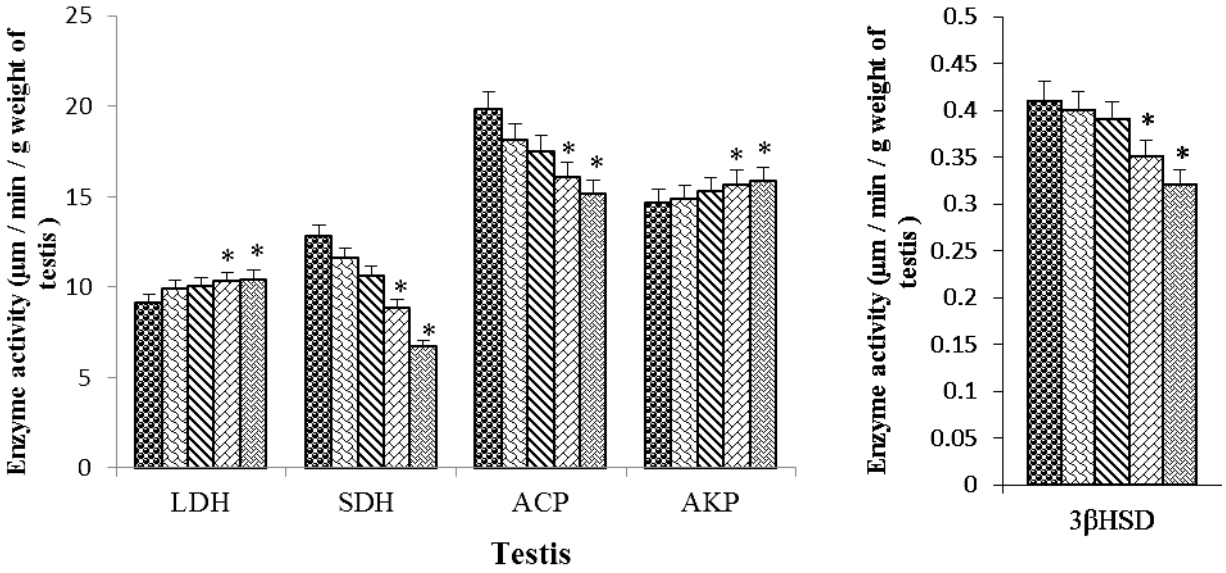
Figure.7
Temporal effects of Carbosulfan on biochemical contents in testes.



3.4. Enzyme activities Studies

Study on activity of enzymes in testes revealed that the treatment with Carbosulfan for 20 and 30 days caused significant decrease in the activities of succinic dehydrogenase (SDH), acid phosphatase (ACP), where as lactate dehydrogenase (LDH) and alkaline phosphatase (AKP) activities were increased significantly in testis. The testis 3β hydroxysteroid dehydrogenase (3βHSD) decreased significantly with Carbosulfan treatment for 20 and 30 days (Fig.8).

Figure.8
Temporal effects of Carbosulfan on testicular dehydrogenases and phosphatase enzymes activity.



4. DISCUSSION

Spermatogenesis and steroidogenesis are the major functions of testis. In the present study decrease in the testes and accessory reproductive weight may be indication of anti-androgenic activity of cabosulfan. There are several possible mechanisms for anti-gonadal actions of toxicants. Ferguson *et al.,* [14] have suggested that the treatment with carbamate pesticide carbofuran inhibits acetylcholinesterase (AChe), resulting in alterations in the pituitary gonadotropins and could influence on gonadal function directly through the effect on the pituitary AChe in rats. It has been observed that members of carbamate pesticides such as disulfiran and its metabolite dithiocarbamate can interfere with catecholamine neurotransmitter metabolism by inhibiting the activity of dopamine β-Hydroxylase (DβH) this is an enzyme that converts dopamine to norepinephrine and the norepinephorine then stimulates the release of GnRH. Thus GnRH release is affected through the inhibition of DβH[15]. The mechanism plays an important regulatory and/ or modulatory role in brain hypothalamic control of pituitary luteinizing hormone (LH) release[16]. In rats administration of N-methyl dithio carbamate causes suppression of LH surge by interfering with catecholamine activity[17].The present investigation suggests that prolonged exposure of Carbosulfan affected spermatogenesis showing anti spermatogenic and antiandrogenic property as refelected by effect on testicular histology and accessory sex organs weight. The effect may be through deprived level of androgens mediated through the gonadotropins of the pituitary due to the effect on hypothalamus[18]. The other possibility

might be due to germ cell apoptosis and chromosomal damage resulting into decreased number of germ cells and formation of giant cells[19]. Thus Carbosulfan might have caused inhibition of DβH and release of GnRH, gonadotropins there by affecting the production of gonadal steroids.

4.1. Biochemical Parameters

It has been found that Carbosulfan treatment for 20 and 30 days caused significant decrease in the level of protein in testis. This may be due to genotoxic action of Carbosulfan[20] or effect on hormones which are essential for the regulation of DNA and RNA synthesis which in turn influences protein synthesis. Observations of biochemical study revealed that Carbosulfan exposure for 20 and 30 days caused significant decrease in glycogen level in testis and epididymis. This may be due to decreased glycogen synthesis and increased catabolism to meet enhanced energy demand of animals under stress induced by pesticides[21]. In the present study increasing the duration of exposure of Carbosulfan caused rise in the cholesterol level in testis. Cholesterol being the primary substrate for androgen synthesis, an increase in testicular concentration is almost an index of reduced steroidogenesis[22].The carbamate pesticide diethyl dithiocarbamate known to inhibit Cyt P-450[23]. Thus increase in cholesterol level in testis and epididymis in the present study might be due to effect on steroidogenesis by inhibiting Cyt P-450 enzymes.

Sialic acid is a carbohydrate component attached with protein to form glycoprotein. The half-lives of LH and FSH are influenced by their oligosacharides terminated by sulfate and sialic acid respectively. The higher content of sialic acid in human FSH contributes to its half-life being longer than that of LH[24]. The synthesis and/ or secretion of sialic acid is under androgenic control[25]. The present study revealed that the increase in the duration of exposure of Carbosulfan caused decrease in sialic acid level in testis. Similar decrease in the levels of sialic acid also shows the necrotic condition of testis[26]. The altered levels of biochemical contents may be due to the genotoxic action of the Carbosulfan or imbalance in protein, carbohydrate and lipid metabolism.

4.2. Enzyme activities

In the steroid hormone biosynthesis 3β-hydroxysteroid dehydrogenase is an important enzyme for synthesis of testosterone [27]. The first and rate-limiting step in the synthesis of steroid hormones is the conversion of cholesterol to pregnenolone, catalysed by the enzyme cytochrome P_{450} SCC (Sidechain cleavage). Cytochrome P_{450} aromatase, which catalyses the conversion of androgens to estrogens [28]. Study on 3β HSD activity in testis revealed that there was significant decrease in the activity of this enzyme with Carbosulfan treatment for 20 and 30 days. Similar type of effects has been reported with organophosphate and carbamate pesticides. The Diethyl dithiocarbamate known to inhibit cyt P-450 [23]. Shivanandappa *et al.*[29] have reported the inhibition of 3βHSD activity in the adrenal cortex of rats fed with BHC. Similarly endosulfan known to induce testicular dysfunction by inhibiting 3βHSD activity and affected steroidogenesis[30]. The present study revealed that the increase in the duration of exposure of Carbosulfan caused decrease in the testicular 3βHSD activity. This could be due to inhibition of certain pathways of steroidogenesis[29,30] by affecting cytochrome P_{450}[23] by pesticide causing gonadal dysfunction as reflected by impaired biochemical contents, decreased number of spermatogenic cells in albino mice exposed to Carbosulfan.

The Succinic dehydrogenase (SDH) enzyme is associated with maturation of germ cells[31]. Succinic dehydrogenase is an enzyme associated with tissue having high metabolic activity or engaging in absorptive or secretory activity[32]. 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. In the present study it has been found that with 20 and 30 days duration of the

exposure of Carbosulfan the activity of SDH decreased whereas activity of LDH increased significantly in testis and epididymis. The change in these enzymes activity might be under the influence of Carbosulfan induced effect on metabolism of the testis and epididymis. 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[33] Similarly it has been reported that a carbamate pesticide carbofuran caused alteration in rat testicular dehydrogenase enzyme activities such as sorbitol dehydrogenase ,SDH, G-6-PDH, LDH and γ glutamyl trans peptidase[34] .

Acid phosphatase (ACP) hydrolysis the ester linkage of phosphate esters at acidic pH (5 to 6) and helps in autolysis of the degenerated cells[35]. AKP which splits phosphorus esters at alkaline pH (10) and mediates membrane transport and is intimately associated in protein synthesis[36] and glycogen metabolism[37].The alteration in the activity of phosphatase enzymes may be due to cellular leakage caused by chemical induced injury of the tissue. Several studies have been reported that carbamate pesticide such as thiram and mancozeb exposure caused decreased ACP and increased AKP activity in gonads of the rats[33,38]. In the present study, it has been found that the exposure of Carbosulfan for 20 and 30 days caused decrease in the activity of ACP and increase in the activity of AKP of testis. This could be due to 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 adaptive rise to the persistent stress [33,38,39].

It has been reported that the carbamates have high fat soluble properties and these compounds easily penetrate through cell membranes and are quickly distributed throughout the body[40].In the present study, changes in the levels of protein, glycogen and lipids of the testis with Carbosulfan treatment suggests either an increased catabolism of the biomolecules to meet the enhanced energy demand of animals under stress or their reduced function at the various biochemical enzymes[21]. These observed effects of Carbosulfan on testes and accessory reproductive organs may be due to the suppression of the production of sex steroid hormones and enzyme activity. However, further investigation is essential to understand the effect of Carbosulfan on hypothalamo-hypophysial-testis in albino mice.

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REFERENCES

1. S .Giri, A.Giri, G.D.Sharma, S.B.Prasad, Mutagenic effects of Carbosulfan, a carbamate pesticide, Mut .Res. 519**:** 75-82, **(**2002).
2. D.Whorton, R.M. Krauss ,S. Marshall ,T.H. Milby , Infertility in male pesticide workers. Lancet. 21:259-61, (1977).
3. R.L.Ksheerasagar, B.B. Kaliwal, Temporal effects of mancozeb on testes, accessory reproductive organs and biochemical constituents in albino mice, Environ. Toxicol. Pharmacol. 15: 9- 17, (2003).
4. JMPR Joint FAO/WHO Meeting on pesticideresidues,http://www.who.int/pes/jmpr/2003.htm. 2003.
5. R.T.Fukuto, Structure-activity relationship in derivatives of anticholinesterase insecticides, Pesticide chemistry human

welfare and the environment. Pergmen Press Ltd, England, pp. 203-212, (1983).
6. H.Lowry , N.I.Rosebrough , A.L.Far. R.J.Ranall, Protein measurement with folinphenol reagent, J. Biol.Chem. 193:265-275, (1951).
7. N.V.Carrol, R.W.Langely, R.H. Row, Glycogen determination in liver and mode by use of anthrone reagent,J. Biol. Chem.20:583-593, (1956).
8. L.L.Abell , B.B.Levy, B.P.Brodie, F.E.Kendal, Simplified method for estimation of total cholesterol in serum and demonstration of its specificity, J.Biol.Chem.195:357-361, (1952).
9. K.Yao ,T. Obuka, M.Mosuka,M. Kinuta, T. Ikeda, Direct determination of Bound Sialic acids in sialoglycoproteins by Acidic Ninhydrine reaction. Analytical Biochemistry.179:332-335, (1989).
10. T.Shivanandappa, S.Venkatesh, A colorimetric assay method for 3βHydrox,Δ^5-steroid dehydrogenase, Anat. Biochem. 254:57-61, (1997).
11. M.M.Nachles , S.I.Margulius,A.M.Sellirgman , Site of electron transfer to tetrazolium salts in the succinoxidase, J. Biol. Chem.235:2739-43, (1960).
12. J.King, The dehydrogenases or oxidoreductases, Lactate dehydrogenase, in: M.J.Princeton(Eds), Practical Clinical Enzymology, Van D Nostrand , Londonpp. 83-93,(1965).
13. K.Linhardt, K.Walter, Phosphatases (phosphomonoesterases) Determination in serum with p- nitrophenyl phosphate, In: H.U. Bergmeyer(Eds),Methods of Enzymatic Analysis, AcademicPress, New York,pp 783–785, (1965).
14. P.W.Ferguson , M.S.Dey, S.A.Jewell, R.L. Kriefer ,Carbofuran metabolism and toxicity in rat,Fundam.Appl.Toxicol.4:14 -21, (1984).
15. J.Maj, J.Vetulani, Effect of some N, N-disubstituted dithiocarbamates on catecholamines level in rat brain. Biochem. Pharmacol.18:2045-2047, (1969).
16. S.P.Kalra, P.S.Kalra, Neutral regulation of luteinizing hormone secretion in the rat. Endocr. Rev. 4:311-351, (1983).
17. J.M.Goldman, J.E.Stocker, R.L.Cooper, W.K. McElory, J.D.Hein. Blockade of ovulation in the rat by fungicide sodium N-methyl dithiocarbamate relationship between effects on the luteinizing hormone surge and alterations in hypothalamic catecholamines. Neurotoxicology and Teratology. 16:257-268, (1994).
18. J.M.Goldman, R.L.Cooper, S.C.Laws, Chlordimeform induced alterations in endocrine regulation within the male rat reproductive system, Toxicol. Appl. Pharmacol. 22:467-72, (1990).
19. M.A.Akabarsha, P.Sivasamy , Apoptosis in male germinal line cells of rat in vivo: caused by Phosphomidon. Cytobios. 91:33-44, (1997).
20. M.Topktas, E.Renci Zogullari, H.B.Ila, In vivo chromosomal aberrations in bone marrow cells of rats with marshal. Mut. Res.371: 259-264, (1996).
21. L.Ivanova-Chemishanska, Dithiocarbamates. In : Toxicity of pesticides, health aspects of chemical safety, Interim Document- WHO , Copenhagen (Denmark).9:158-169, (1982).
22. R.I.Dorfman , Anti-androgens in a castrated mouse test, Steroid.2:185-193, (1963).
23. I.Stott, M.Anupam , R.Alex, W.T.Norman, R.F.Jeffrey, Low dose dithiocarbamate attenuates the hepatotoxicity of 1,3-dichloro-2-propanol and selectivity inhibits CYP2E1 activity in the rat. Human Expt. Toxicol.16(5):262-266, (1997).
24. W.B.Peckham, T.Yamaji, D.J.Dierschke,E. Knobil, Gonadal function and the biological and physiochemical properties of follicle stimulating hormone. Endocrinology.92: 1660-1666, (1973).
25. T.Bohmer ,S.C.Weddington, V.Hansson, Effect of testosterone propionate on level of carnitine and testicular androgen binding

protein (ABP) in rat epididymis. Endocrinol, 100(3) : 838-8, (1977).
26. H.Levinsky,R.Singer,M.Barnet,M.Sangive, D.Alkoloaf. Sialic acid content of human spermatozoa and seminal plasma in relation of sperm counts. Arch. Androl .10:45-46, (1983).
27. W.B.Darrel,M.M.Thomas,B.M.Virendra, Female reproductions: The ovulatory cycle,in: J.WRaphael(Eds,).Reproductive Toxicology, second edition, Raven Press, New York pp.23-44, (1995).
28. B.Stoffel-Wagner , Neurosteroid metabolism in the human brain. Eur. J. Endocrinol. 145:659-679, (2001).
29. T.Shivanandappa,M.K. Krishnakumari,S.K. Majumdar, Inhibition of steroidogenic activity in the adrenal cortex of rats fed benzene hexachloride (hexachlorocydo hexane).Experentia.38:1251-1253, (1981).
30. K.C.Chitra, C.Latchoumycandane, P.P.Mathur. Chronic effects of endosulfan on the testicular function of rat. Asian J. Androl. 1(4):203-206,)(1999).
31. G.D. Hodgen, J.R.Sherins, Enzymes as markers for testicular growth and development in the rat. Endocrinology.93:985-989, (1973).
32. H.A.Padykula, The localization of succinic dehydrogenase in tissue sections of the rat. Amer. J. Anat. 91:107-145, (1952).
33. R.Kackar, M.K.Srivastava, R.B.Raizada, Induction of gonadal toxicity to male rats after chronic exposure to mancozeb, Ind. Health .35:104-111, (1997).
34. N.Pant,A.K.Prasad ,S.C.Srivastava, R.Shankar , S.P.Srivastava , Effect of oral administration of carbofuran on male reproductive system of rat, Human Expt. Toxicol.14:889-894, (1995).
35. C.DeDuve,B.G.Presman,G.Gianetto,R.Wattiau,F.Appelmans, Intra cellular distribution pattern of enzymes in rat liver tissue. Biochem. J. 60:604-617, (1955).
36. B.Pilo,M.V.Asanani,R.V.Shah, Studies on the wound healing and repair in pigeon liver III. Histochemical studies on acid and alkaline phosphatases during the process. J. Anm. Morphol. Physiol,19:205-212, (1972).
37. V.Gupta,G.Rao, Histological studies on the chloride plexes of the goat embryos III – Histochemical distribution of acid and alkaline phosphatases. Acta Histochem. 49:60-63, (1974).
38. V.K.Mishra,M.K.Srivastava,R.B.Raizada,Testicular toxicity in rat to repeated oral administration of tetramethyl thiuram disulfide (Thiram). Ind. J. Exptl. Biol.36(4):390-394, (1998).
39. S.D.Murphy,S.Porter,Effects of toxic chemicals on some adaptive liver enzymes, liver glycogen and blood glucose in faster rats. Biochem. Pharmacol. 15:1655-1676, (1968).
40. T.C.Marrs,T.Dewhurst,Toxicology of pesticides, In**:** Ballantyne B., Marrs, T.C., Syversen, T.C. (Eds) General Applied Toxicology, Stocton Press, New York .pp 1993-2012, (2000).