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Exploring Hepatotoxicity of Benomyl: Histological and Histochemical Study on Albino Rats

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Benomyl, a fungicide of the benzimidazole group applied against a wide range of fungal diseases of crops and vegetables. The effects of benomyl on the hepatic tissue of albino rats were investigated. Treating rats with benomyl at a dose level of $^{1}/_{10}$ LD_{50} three times/week for three weeks induced various histopathological changes in the liver, such as hepatic tissue impairment, cytoplasmic vacuolization of the hepatocytes, leucocytic infiltrations, congestion of blood vessels and fatty degeneration. Histochemical investigations revealed reduction in total carbohydrates, total proteins and nucleic acids (DNA, RNA) in the hepatic cells. These alterations were time-dependent and were obvious in animals treated with benomyl for 3 weeks. It is speculated that one or more metabolites of benomyl may be responsible for the hepatotoxicity recorded in the present work.

Key words: Benomyl, rats, histopathology, histochemistry

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INTRODUCTION

Owing to the significant role played by the liver cells in the detoxification of various xenobiotics, liver injury gained wide spread of scientific and public attractions. Liver is among the first organs to be affected by different chemicals gained entry into animal body regardless the route of administration. Intoxication with fungicides constitutes a major problem, especially because this could be happened by ingestion of contaminated crops like fruits and vegetables. Benomyl is a systemic benzimidiazole fungicide that is applied against wide range of fungal diseases of field crops, fruits, nuts, ornamentals and mushrooms[1]. Although, benomyl proved useful, it does not excluded from expressing its hazardous actions in mammalian animals. It has been observed by Kavlock *et al.*[2] that Wister rats showed teratogenic effects after treatment with benomyl. Cummings *et al.*[3] indicated that rats exposed to benomyl during the first week of pregnancy induced embryotoxicity, resulting in embryogenic death, growth retardation and developmental abnormalities. It has been observed by McLean *et al.*[4] that benomyl inhibited neuronal cell differentiation as well as produced high incidence of fetal brain anomalies in rats[5]. Urani *et al.*[6] reported that benomyl is a glutathione-depleting agent. Added to the forementioned effects, benomyl induced hispathological changes in the liver, kidney and spleen of rats[7] and also in male reproductive system where it causes testicular dysfunction[8]. This work was planned essentially to clarify the histological and histochemical alterations induced by benomyl in the liver of albino rats.

MATERIALS AND METHODS

Male white albino rats *(Rattus norvegicus* of the inbred strain) of weight 90 ± 5 g and two months of age were selected for the present study. Animals were obtained from the Egyptian Organization for Serology and Vaccination and kept for at least one week before initiation of the experiment and fed on standard rodent diet obtained from Tanta company for oils and soap and water was supplied *ad libitum*. The experiment was conducted at the end of summer where the temprature may reach $28 \pm 2°C$ and with light-dark cycle was 12 hours. Animals were kept in standard plastic rodent cages till the end of the experimental period. They were divided into two groups, one group was served as control and the other one treated with $^1/_{10}$ (LD_{50}: 360 mg kg^{-1} body weight) benomyl orally three times/ week for three weeks. Animals were killed by cervical disslocation, quickly dissected and small pieces of liver were fixed in Bouin's fluid for histological examination, dehydrated, embedded in wax and 5 μm thick sections were stained with haematoxylin and counterstained with eosin. For histochemical purposes other pieces of the liver were fixed in 10% neutral formalin and processed in the same manner as for the histological method. Sections were stained with the PAS-technique for the demonstration of general carbohydrates[9], mercuy bromophenal blue method for the identification of total proteins[10], Feulgen reaction for detection of DNA[11] as well as Feulgen-methylene blue method for both DNA and RNA[12].

RESULTS

Histology: The normal histological structure of liver is shown in Fig. 1. Application of the pesticide under investigution induced many pathological alterations in the hepatic tissues. Such altrations were time-dependent (Table 1). Materials taken for inspection one week from the begining of the experimental exposure manifested disorganized structure. Such sections showed disruption of normal cords arrangements, leading almost to disappearance of the sinusoids. The nuclei of the hepatocytes appeared small-sized compared that of normal animals as well as condensation of their chromatin. Leucocytic infiltration and congestion of blood vessels were also evident (Fig. 2). As time passed the condition became worse. Therefore, examination of sections obtained from liver of animals maintained in the same condition for two weeks revealed cytoplasmic vacuolization of the hepatocytes as was clearly demonstrated in Fig. 3. Besides, the sinusoidal spaces were disappeared, most cells lost their nuclei, that might be a preparatory step before cellular degeneration takes place. Nuclear chromatin and nuclei were condensed and were difficult to recognize. Pyknosis was also evident in such cells. Time is still a critical factor in determining the degree of alterations observed in animals kept exposed to such route of treatment. In this concern, liver sections examined after three weeks showed loss of staining characteristics of normal liver cells. The hepatocytes became completely disrupted, cell membranes were ruptured, nuclei showed no sign of having nucleoli, or chromatin. In other liver sections large fat droplets were observed. These droplets displace the nuclei and occupied almost the entire space of the cells (Fig. 4). Hyperblastic bile ductules and widened blood vessels were found also among the numerous damaging effects induced in these animals.

Histochemistry

Total proteins: Total protein contents of the liver cells of control rats are positively reflected by the appearance of blue colour after staining with bromophenol blue. Generally, hepatic tissue cytoplasm contains excessive

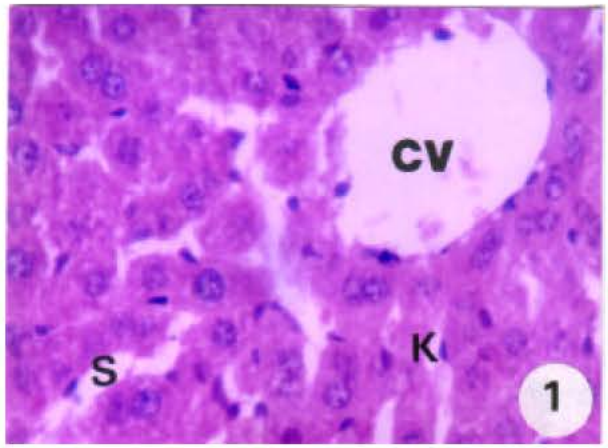

Fig. 1: Liver of a control rat showing central vein (CV), Kupffer cell (K) and sinusoid (S) X 400.

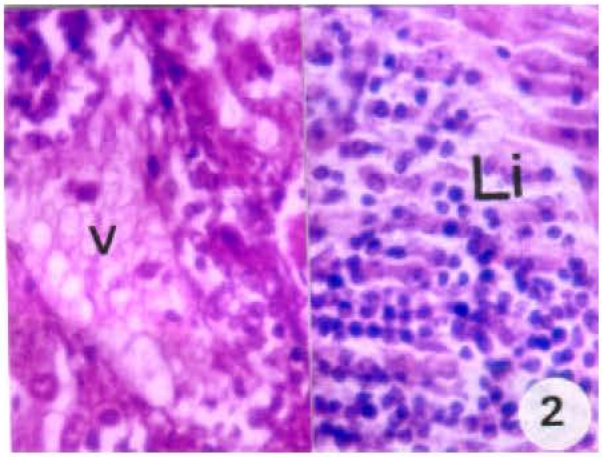

Fig. 2: Section of liver from a rat treated with benomyl for one week showing leucocytic infiltration (Li) and congested central vein (V), X 400.

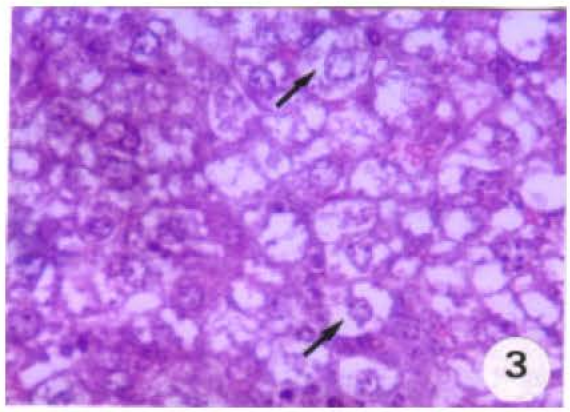

Fig. 3: Liver section of a rat examined 2 weeks of treatment with benomyl showing cytoplasmic vacoulization of the hepatocytes(arrows), X 400.

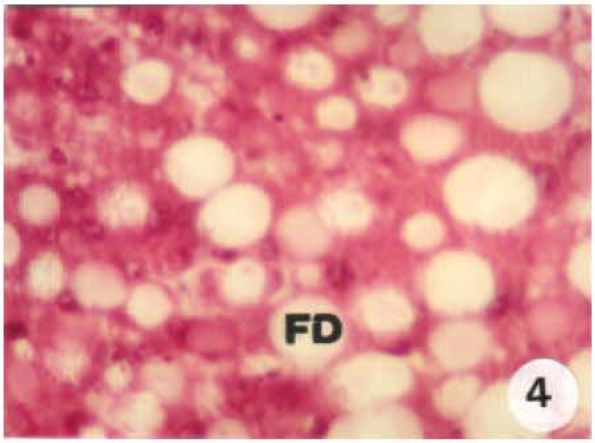

Fig. 4: Liver section obtained from a treated rat showing fat droplets (FD), X 120.

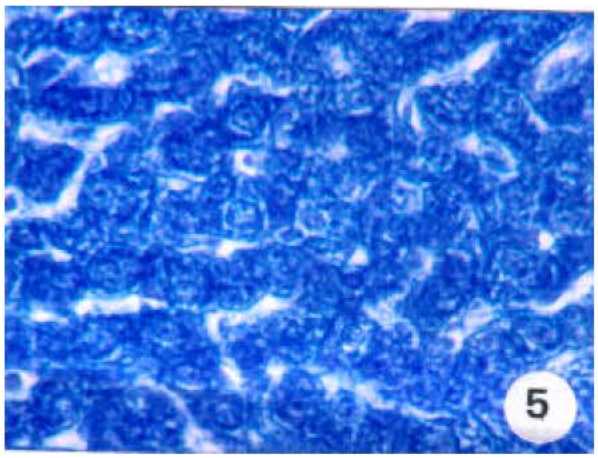

Fig. 5: Normal proteinic content in the liver of a control animal , X 400.

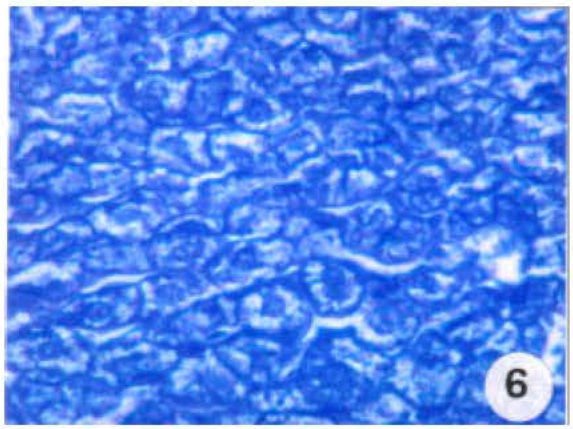

Fig. 6: Marked reduction of proteins in hepatocytes of a rat treated with benomyl for 3 weeks, X 400.

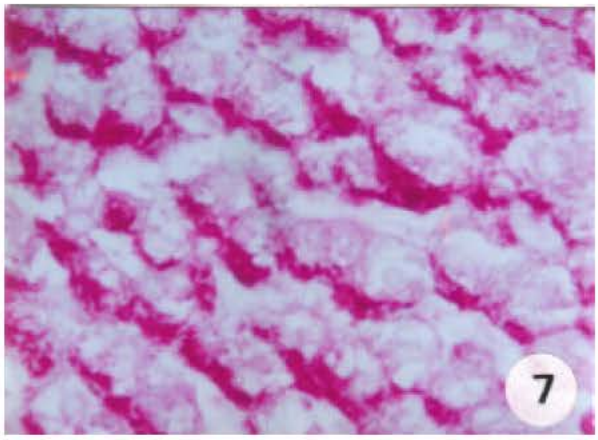

Fig. 7: Liver section of a control rat showing distribution of glycogen in the cytoplasm of the hepatocytes, X 400.

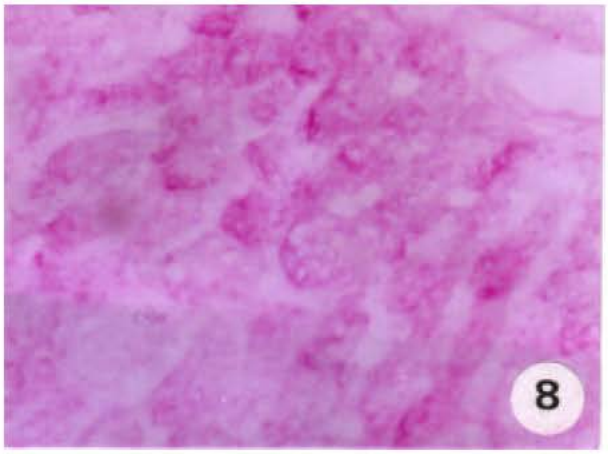

Fig. 8: Marked reduction of glycogen in hepatocytes of a rat treated with benomyl and examined after 3 weeks, X 400.

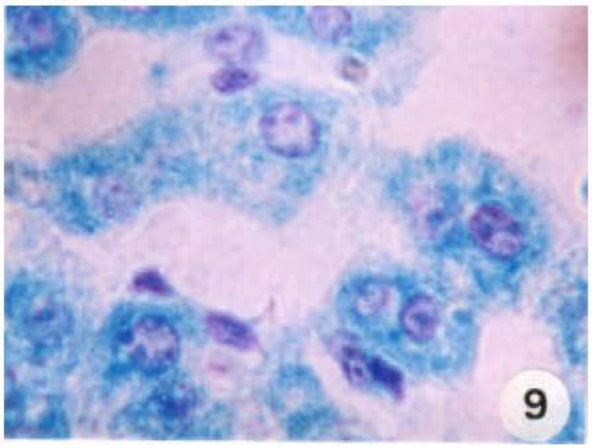

Fig. 9: Normal content of RNA-containing particles in the cytoplasm and nucleoli of the hepatocytes, X 1000.

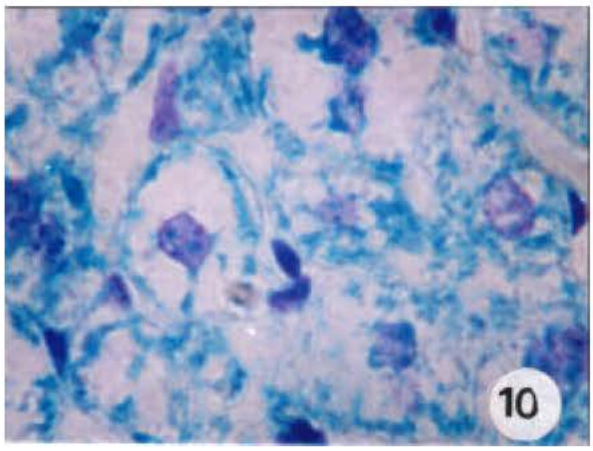

Fig. 10: Marked loss of RNA-containing particles in the hepatocytes of an animal examined 3 weeks after treatment with benomyl, X 1000.

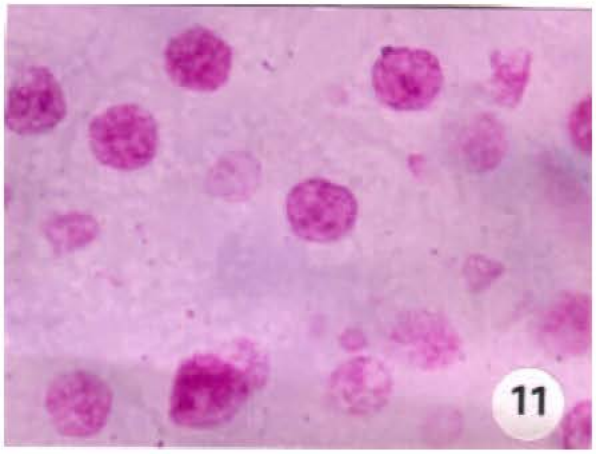

Fig. 11: Normal distribution of DNA-containing particles in the nuclei of hepatocytes of a control animal, X 1000.

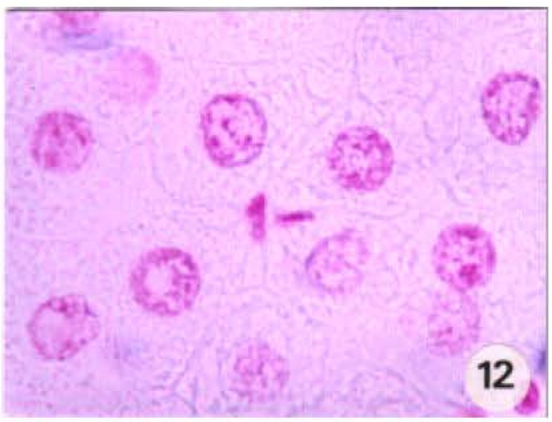

Fig. 12: Diminution of DNA-containing particles in the nuclei of hepatocytes of an animal treated with benomyl and examined 3 weeks, X 1000.

Table 1: Histopathological changes induced in liver during the experimental periods

Time elapsed after initiation of treatment (weeks)	Number of animals	Histopathological alterations			
		Lymphocytic infiltration	Cytoplasmic vacuolation	Fatty infiltration	congestion of blood vessels
1	6	++	-	-	++
2	5	+++	++	-	++
3	5	++	+++	++	++

amount of total proteins in the form of fine granules as was obvious in Fig. 5. Positive reactivity observed in cell membrane and nuclear membrane acquiring an intense stainability denoting their protein richness. In addition, both chromatin bodies and nucleoli exhibiting deep colouration. Kupffer cells and endothelial lining cells of sinusoids give moderate reactivity with bromophenol blue. Also, the walls of blood vessels exhibited strong stainability. Application of the fungicide induced changes in the total protein contents in the liver cells as clearly demonstrated in cells obtained from animals exposed for one week. At this time, only minor reduction in the total protein content could be detected. As time proceeds, sections examined after two weeks showed noticeable reduction in the total protein contents . Sections obtained from animals following this course of treatment for three weeks indicated marked decrease in the protein content of hepatocytes in conjunction with the cytoplasmic vacuolization (Fig. 6).

General carbohydrates: A considerable amount of carbohydrates in the cytoplasm of liver cells of control animals was detected by PAS-technique. These carbohydrates are in the form of glycogen and give red or magenta colour with Schiff's reagent (Fig. 7). It is noted that glycogen is not uniformly distributed in the cytoplasm of the hepatocytes, but occurred concentrated at one pole of the cells, this is termed glycogen flight. The nuclei appeared entirely PAS-negative indicating absolute lack of glycogen. Examination of sections obtained from liver of animals treated with benomyl for a period of one week exhibited slight diminution in their glycogen content. Still, after two weeks in the same condition, inspection of liver sections indicated reduction in the glycogen content more than the previous stage and to a noticeable effect in comparison to control animals. After three weeks the hepatic cells revealed more reduction of glycogen compared to the previous two stages and to a marked degree to controls (Fig. 8).

Ribonucleic acid (RNA): The RNA content of liver cells of normal animals appeared after identification by Feulgen-methylene blue method as small blue patches randomly scattered in the cytoplasm. Also, similar behavior was exhibited in the nucleoli denoting their RNA-contents as clearly seen in figure 9. Figure 9 also clearly denoting the presence of red-stained nuclei which is referred to their DNA-content. Kupffer cells exhibiting weak Frelgen- methylene blue reactivity. Estimating RNA-contents of liver cells of benomyl-treated animals indicated gradual decrease in such contents which move in the same direction as the obligatory decreasing attitude induced in total protein contents. Referring to this statement, liver cells of animals exposed to the treatment for one week showed slight reduction in RNA-containing particles. After two weeks, examination of hepatocytes revealed noticeable decrease in RNA contents. Such decrease became more pronounced in sections obtained from the liver of animals treated for three weeks (Fig. 10).

Deoxyribonucleic acid (DNA): DNA-containing particles (chromatin) in the nuclei of hepatocytes of normal rats demonstrated by Feulgen reaction as red densely stained particles in the nucleoplasm (Fig. 11). These particles are either distributed equally in the nucleoplasm or restricted to the peripheral rims of the nuclei. Sometimes, few granules of DNA appeared attached to the nucleoli. These granules called nucleoli-associated chromatin. The nuclei of Kupffer cells give strong reactivity reflecting their DNA richness. Keeping the animals on the prescribed treatment induced gradual reduction in the DNA-containing particles ranging from minor change in animals exposed for one week to greater effects in the following stages. After two weeks, examination of liver sections indicated faintly stained preparations reflecting noticeable decrease in DNA content. Still, after three weeks of treatment, liver sections displayed marked diminution in DNA-containing particles in the nuclei of both the hepatic cells and those of Kupffer cells (Fig. 12).

DISCUSSION

Benomyl is a fungicide widely applied against wide variety of fungi. The present study indicated that benomyl induced marked histopathological alterations in the liver tissue of rats such as, tissue impairment, cytoplasmic vacuolization, leucocytic infiltration, congestion of blood vessels as well as fatty infiltration. Proposed mechanism for the cytoplasmic vacuolization has been given by Robbins and Angell[13] as one of the important responses to all forms of cell injury. This implies increased permeability of cell membranes, leading to an increase of intracellular water. Other mechanism to

account such phenomenon was declared by Sherlock and Doely[14] that the vacuolar degeneration changes with the marked disturbances took place in lipid inclusions as a result of injurous treatment. The small highly condensed nuclei (might be remnant of the original nucleus, displaced to one pole of the cell adjacent to cell membrane which is the same way by which fat cells are formed in the adipose tissues, meaning that the liver cells are transformed into permanent fatty tissue.

Leucocytic inflammatory infiltration induced by benomyl treatment in liver of rats was also observed in liver of mice by the fungicide mancozeb[15] and such leucocytic infiltrations were accounted as prominent response of the body tissue facing this toxicant. Among the changes observed in the liver tissue by benomyl treatment was the congestion of blood vessels. These lesions were also found in liver of animals exposed to various fungicides[16,17].

Many authors had elucidated that hepatocellular damage could be correlated with the disturbance in enzyme activities. Martin *et al.*[18] announced that hepatic tissues lose their enzymes (e.g transaminases) in case of liver damage. This ultimately leads to their raised levels in the sera of those animals. Hence they suggested that the higher value of these enzymes, whenever they are detected in the blood sera, should be taken as an indicator of various causes of liver damage. Benomyl was found to affect liver enzymes[19] and inhibit hepatic mitochondrial aldehyde dehydrogenase[20]. These results together with the histopathological observations indicated that benomyl caused liver injury in rats.

Regarding the histochemical changes observed in this work under benomyl intoxication, results clearly indicated reduction in total proteins, general carbohydrates, DNA and RNA. These changes were consistent with those induced histopathologically. A similar decrease in these materials were induced by mancozeb in the liver, uterus and ovary of albino rats[21]. Baligar and Kaliwal[22] further demonstrated reduction in glycogen and protein contents in the liver and ovary of rats. The decrease in carbohydrate contents was attributed by some investigators to be due to increase stress on the organs leading to consuming high energy in attempt to light or equalize the pressure exerted upon them.

Total proteins decreased in liver of benomyl-treated animals. This result is in agreement with that of Igbedioh and Akinyele[7] who proved that proteins decreased in liver of benomyl-fed rats. Marinovich *et al.*[23] indicated inhibition of protein synthesis in human luekemic cell line (HL-60 cell) by a dose of 50 μg ml^{-1} of benomyl. Oral administration of the fungicide maneb inhibited protein synthesis in liver and testis of rats[24]. Reduction in protein content in liver of benomyl-treated animals might be due to either arrested metabolism in the liver or to use it to build up new cells or enzymes to reduce the stress. Alternatively, the reduction in nucleic acids could be a factor determining the decrease in protein content.

Reduction of nucleic acids was observed in the hepatocytes of rats under the influence of benomyl. In this concern, Hellman and Laryea[25] reported that benomyl inhibited DNA turnover in liver and kidney of mice by measuring the incorporation of [3H] thymidine 24hr after oral administration of different doses. Nicolau[26] reported that exposure to mancozeb affected RNA, DNA and protein content in the thyroid and adrenal. It has been speculated that the decrease in DNA and RNA could be attributed to disruption of lysosomal membranes under the effect of various toxicants leading to freeing their hydrolytic enzymes (DNase & RNase) in the cytoplasm and resulted in marked lysis and dissolution of the target materials, DNA and RNA. This result confirmed that of Awasthi *et al.*[27] who found elevated lysosomal enzymatic activity accompanied by a decrease in protein and nucleic acids contents in response to organophosphate insecticide with release of nucleases and proteases affecting RNA, DNA and protein metabolism.

It is concluded from the present work that benomyl induced hepatotoxicity in the albino rats.

REFERENCES

1. Maloy, O.C., 1993. Fungicide development and use. In: plant disease control. John Wily and Sons, Inc. New York, pp: 163-180.
2. Kavlock, R.J., N. Chernoff, L.E. Gray, Jr, J.A. Gray and D. Whitehouse, 1982. Teratogenic effects of benomyl in the Wister rat and CD-1 mouse with the emphasis on the route of administration. Toxicol. Appl. Pharmacol., 62: 44-54.
3. Cummings, A.M., M.T. Ebron-McCoy, J.M. Roger, B.D. Barbee and S.T. Harris, 1992. Developmental effects of methyl benzimidazole carbamate following exposure during early pregnancy. Fundam. Appl. Toxicol., 18: 288-293.
4. Mclean, W. G., A.D. Holme, O. Janneb, A. Southgate, C.V. Howard and M.G. Reed, 1998. The effect of benomyl on neurite outgrowth in mouse NB2A and human SH-SY5Y neuroblastoma cells in vitro. Neurotoxicology, 19: 629–632.
5. Zeman, F.J., E.R. Hoogenboom, R.J. Kavlock and J.L. Semple, 1986. Effects on the fetus of maternal benomyl exposure in the protein-deprived rat. J. Toxicol. Environm. Health, 17: 405-417.

6. Urani, C., E. Chiesara, P. Galvani, L. Marabini, A. Santagostino and A. Camatin, 1995. Benomyl affects the microtubule cytoskeleton and glutathion level of mammalian primary cultured hepatocytes. Toxicol. Lett., 76: 135-144.
7. Igbedioh, S.O. and I.O. Akinyele, 1992. Effect of benomyl toxicity on some liver constituents of albino rats. Arch Environ. Health, 47: 314-317.
8. Hess, R.A. and M. Nakai, 2000. Histopathology of the male reproductive system induced by the fungicide benomyl. Histol. Histopathol., 15: 207-224.
9. Hotchkiss, R.D., 1948. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem., 16: 131.
10. Mazia, D., P.A. Brewer and M. Alfert, 1953. The cytochemical staining and measurements of protein with mercuric bromophenol blue. Biol. Bull., 104: 57-67.
11. Stowel, R., 1945. Feulgen reaction for thymonucleic acid. Stain Technol., 20: 45-52.
12. Garvin, A., R. Brissic and S. Spicer, 1979. Cytochemical differentiation of nucleic acids with Schiff-methylene blue sequence. J. Histochem. Cytochem., 24: 587-590
13. Robbins, S. and D. Angell, 1976. Basic Pathology, 2nd ed, W. B Saunders Company, Philadlphia, London.
14. Sherlock, S. and J. Doely, 1993. Diseases of the liver and biliary system, 9th ed, Blackwell Sci. Publ. Cambridge, London.
15. Sakr, S.A., M.M. El-Sadany, H.A. Mahran and S.M. Abo-Elyazid, 2003. Effect of DDB on mancozeb-induced histopatholgical and histochemical changes in liver of albino mice.1st Inter Confer. Egypt. Soc. Biotechnol. Environ. Sci., Faculty of Science, Zagazig University , Egypt, 14-15 April,2003.
16. Deveci, E.C., K. Guven, M. Bashan, A. Onen and D.de-pomerai, 1997. The accumulation and histological effects of organometallic fungicides propineb and maneb in the livers of pregnant rats and their offspring. J. Toxicol. Sci., 24: 79-85.
17. Kackar, R., M.K. Srivastava and R.B. Raizada, 1999. Induction of gonadal toxicity to male rat after chronic exposure to mancozeb. Ind. Health, 35: 104-111.
18. Martin, D.W., P.A. Mayes and V.W. Rodwell, 1983. Harpers review of biochemistry. Middle East Edition, California.
19. McCarroll, N.E., A. Protzel Y. Ioannou, H.F. Frank Stack, M.A. Jackson, M.D.Waters, K.L. Dearfield, 2002. A survay of EPA/OPP and open literature on selected pesticide chemicals.III. Mutagenicity and carcinogenicity of benomyl and carbendazim. Mutat. Res., 512: 1-35.
20. Staub, R.E., G.B. Quistad and J.E. Casida, 1998. Mechansim for benomyl action as a mitochondrial aldehyde dehydrogenase inhibitor in mice. Chem. Res. Toxicol., 11: 535-543.
21. Mehadevaswami, M. P., U.C. Jardaramkunti, M.B. Hiremath and B.B. Kaliwal, 2001. Effect of mancozeb on ovarian compensatory hypertrophy and biochemical constituents in hemicastrated albino rat. Reprod. Toxicol., 14: 127-137.
22. Baligar, P. N. and B.B. Kaliwal, 2001. Induction of gonadal toxicity to female rat after chronic exposure to mancozeb. Ind. Health, 39: 235-243.
23. Marinovich, M., M. Guizzetti and C.L. Galli, 1994. Mixtures of benomyl, pirimiphos-methyl, dimethoate, diazinon and azinphos-methyl affect protein synthesis in HL-60 cells differently. Toxicology, 94: 173-185.
24. Ivanova-Chemishanska and Land V. Izmirova, 1977. 355-cysteine incorporation into protein synthesis of the liver and testes in sub acute oral poisoning with Zineb, maneb and mancozeb. Probl. Khig., 3: 19-24.
25. Hellman B. and D. Laryea, 1990. Inhibitory action of benzimidazole fungicides on the in vivo incorporation of [3H] thymidine in various organs of the mouse. Food Chem. Toxicol., 28: 701-706.
26. Nicolau, E., 1982. Circadian rhythms of RNA, DNA and protein content in the rat thyroid, adrenal and testis in chronic pesticide exposure effects of a fungicide (mancozeb). Endocrinol., 20: 249-257.
27. Awasthi, M., P. Shah, M. Dubale and P. Gadhia, 1984. Metabolic changes induced by organophosphates in the piscine organs. Environ. Res., 35: 320-325.