Sci. J. Fac. Sci. Minufia Univ., 2007, Vol. XXI, 33-41

EFFECT OF MELATONIN ON FENVALERATE INDUCED HISTOLOGICAL AND HISTOCHEMICAL ALTERATIONS IN RAT LIVER

HAWAZEN A. LAMFON

Biology Department, Faculty of Applied Sciences, Umm Al-Qura University, Makka, Saudi Arabia.

ABSTRACT

Treating rats with ($\frac{1}{2}LD_{50}$) of the pyrethroid fenvalerate 3 times per week for 2 and 4 weeks induced various histopathological changes in the liver such as cytoplasmic vacuolization of the hepatocytes, congestion of blood vessels leucocytic infiltrations and fatty infiltrations. Moreover, the hepatocytes showed reduction in glycogen and total proteins. Treating animals with fenvalerate and melatonin led to an improvement, in both histopathological and histochemical pictures. These results proved that melatonin had inhibitory effect against liver injury caused by fenvalerate.

INTRODUCTION

Pyrethroids have been known as insecticides for many years and are belong to highly active insecticides. The studies conducted on animals indicate that the toxicity of pyrethroids depends on many factors, such as body construction, route of administration and period of administration The source of pyrethroids is the flowers of the pyretherum plant *Chrysanthemum cinerariafolium* . Due to the persistence of these insecticides in the environment, structures similar to pyrethroids have been synthesized and proved to be effective against different insects (Casida, 1973). On the other hand, pyrethroids were found to produce serious side effects of different types . Animals exposed to these insecticides exhibited disturbance in their physiological activities beside other histopathological features (Okuno et al. 1989, Sakr, 1999).

Melatonin (MT), an indole amine product of the pineal gland, was shown to be an endogenous hydroxyl radical (OH) scavenger and effective

antioxidant (Tan et al., 1993; Poeggeler et al.,1998). With its scavenging ability , melatonin easily passes all known morphophysiological barriers and enter all subcellular compartments (Pieri et al.,1994). It protect cells, tissues and organs from oxidative damage induced by a variety of free radical generating agents and processes (Reiter, 1998; Maestroni et al.,2001). As an antioxidant, melatonin is effective in protecting membrane lipids, nuclear DNA and protein from oxidative damage both in vivo and in vitro (Reiter et al., 1998 a,b ; Lusardi et al.,2000; Atkinson et al., 2003) . Melatonin may exert certain biologic effects such as the inhibition of tumor growth and counteraction of stress-induced immunodepression by augmenting the immune response (Drijfhout et al., 1996). The objective of the present work is to study the effect of melatonin on the histological and histochemical alterations induced in the liver of rats by the pyrethroid insecticide, fenvalerate.

MATERIALS AND METHODS

Male albino rats weighing 140 ±5g were used in the present study. Animals were fed a standard diet and water was available *ad libitium*. Animals were divided into 4 groups.

Group 1: Animals of this group (10 rats) were orally given fenvalerate at a dose level of ½ LD_{50} (10 mg/kg b.w) (Ohkawa et al. 1979) 3 times weekly for 4 weeks.

Group 2: Animals of this group (10 rats) were orally given fenvalerate followed by melatonin at a dose level of 5mg/kg/ body weight (Laurido et al. 2002) 3 times weekly for 4 weeks .Melatonin, was purchased from Sigma Company, USA.

Group 3: These animals (5 rats) were given melatonin.

Group 4: Animals of this group were considered as controls.

After 2 and 4 weeks liver was removed and fixed in alcoholic Bouin's fluid or 10% neutral formalin. Fixed materials were embedded in paraffin wax and sections of 5 micrmetre thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. For histochemical demonstration of total carbohydrates periodic acid Schiff's technique (PAS) (Hotchkiss, 1948) was used. Total proteins were detected using the mercury bromophenol blue method (Mazia et al., 1953).

RESULTS

Histological results

Control animals or animals given melatonin showed normal liver structure (Fig.1) . Examination of liver sections obtained from rats following treatment with fenvalerate for 2 weeks revealed signs of degenerative changes in some areas. In these specimens, the normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was lost .In addition ,central and portal veins were congested (Fig.2). Some of the hepatocytes appeared with cytoplasmic vacuolization. Liver sections obtained from rats 4 weeks post-treatment with fenvalerate showed that a considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization. These vacuoles were so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass cells - frequently forming a narrow peripheral rim was left (Fig.3). The nuclei of these cells were pyknotic. Congestion of the intrahepatic blood vessels and inflammatory leucocytic infiltrations were observed. Moreover, fatty infiltration was observed in large areas of the liver (Fig.4).

Animals treated with fenvalerate followed by melatonin and examined after 2 weeks revealed that few hepatocytes displayed cloudy swelling and others still showed cytoplasmic vacuolization (Fig. 5). After 4 weeks the histopathological changes were less prominent when compared with the same periods of fenvalerate group No cytoplasmic vacuolization appeared in the hepatocytes and most cells displayed a certain degree of recovery besides the appearance of some binucleated ones (Fig. 6).

Histochemical results:

Glycogen:

In control rats, the total carbohydrates exist in the form of deeply stained reddish granules in the cytoplasm of the hepatic cells as shown by PAS reaction. All these positively stained materials have been proved to be glycogen as verified by Best's carmine method with and without previous treatment with diastase. The nuclei gave a negative reaction (Fig. 7). Liver of animals treated with fenvalerate and examined after 2 weeks showed noticeable decrease in glycogen in the cytoplasm of most hepatocytes (Fig. 8). Such reduction of glycogen markedly appeared in the liver of animals examined after 4 weeks (Fig. 9). Rats treated with fenvalerate followed by melatonin revealed an improvement in glycogen contents of the hepatocytes when compared with those treated with fenvalerate alone especially after 4 weeks (Fig. 10).

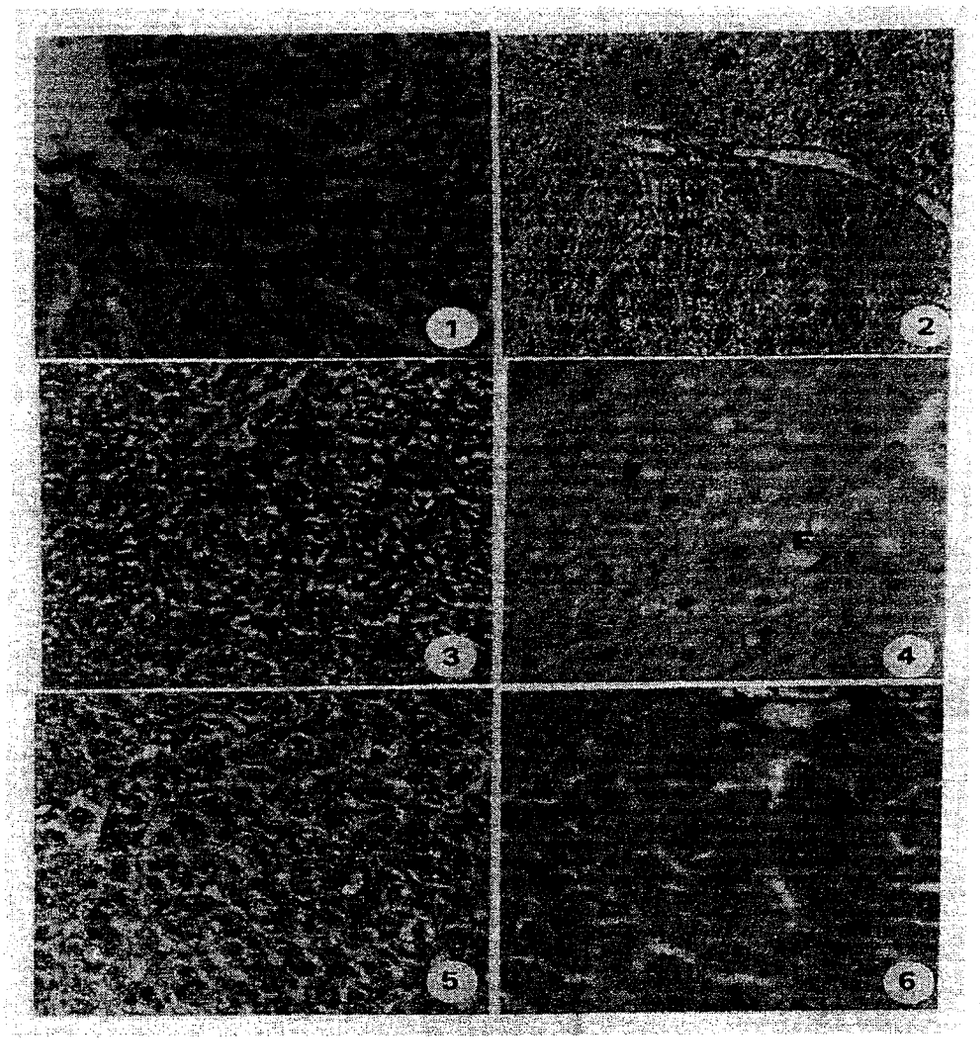

Fig.1. Liver of a control rat showing hepatocytes (H), Kupffer cell (K) and sinusoid (S) X 400.
Fig.2. Section of liver from a rat treated with fenvalerate for two weeks showing congested veins (C) X 400.
Fig.3 Liver section obtained from a rat treated with fenvalerate for 4 weeks showing cytoplasmic vacuolization of the hepatocytes (arrow heads) X 400.
Fig.4. Liver section of a rat treated with fenvalerate for 4 weeks showing fatty degeneration (F),X 400.
Fig.5. Liver section obtained from a rat treated with with fenvalerate and melatonin for 2 weeks showing few cells with cytoplasmic vacuolation (arrows),X400.
Fig.6. Liver section of a rat treated with fenvalerate and melatonin for 4 weeks showing improvement of hepatic tissue and binucleate cells (arrows), X400.

Total proteins:

The protein materials in the liver cells of control rats were displayed in the form of small bluish irregular particles which were sometimes closely packed together making blue irregular dense bodies against a weakly to moderately stained ground cytoplasm. The nuclear envelope, chromatin materials and nucleoli are positively stained (Fig. 11).

Examination of liver of rats after 2 weeks of treatment with fenvalerate showed that most of the hepatocytes appeared with cytoplasmic vacuolization and showed a reduction of their protein content (Fig. 12). After 4 weeks, a large number of cells were nearly devoid of protein(Fig. 13). Animals treated with fenvalerate followed by melatonin and examined after 2 and 4 weeks revealed improvement of proteinic content and a large number of the hepatocytes contained considerable amounts of proteins (Fig. 14).

DISCUSSION

The present results showed that fenvalerate induced many histopathologiocal changes in the liver of rats treated with ½ LD_{50} for 2 and 4 weeks. These lesions included cytoplasmic vacuolization of the hepatic cells, inflammatory leucocytic infiltrations ,congestion of blood vessels and fatty infiltrations. Similar results were obtained by some investigators using different pyrethroids. Sakr (1999) reported that tetramethrin induced leucocytic infiltrations and cytoplasmic vacuolation in liver of rats. Okuno et al. (1989) observed multifocal microgranulomas in liver of mice and rats treated with fenvalerate.El-Banhawy et al.(2000) reported that pyrethroid insecticide,Ezalo induced many histopathological changes in the liver of adult and emberyo of mice.

Treating rats with fenvalerate induced reduction of hepatic glycogen. Such observation is in close agreement with that of Abdeen et al. (1994) who found that fenvalerate decreased glycogen in liver of mice. The decrease of carbohydrates was observed in different animals under the effect of insecticides and was suggested to be achieved through modifying the activities of the enzymes of glycolytic pathway, TCA cycle, glucogenesis and the oxidative phosphorylation (Shakoori et al. 1988). It was also reported that some insecticides may affect the carbohydrate metabolism through their effects on the endocrine system, especially by modifying the secretion of glucocorticoids and insulin (Pilo and Mehan, 1987). However, one or more of such factors could be considered as the causal agent of glycogen reduction observed in the liver of fenvalerate -treated animals.

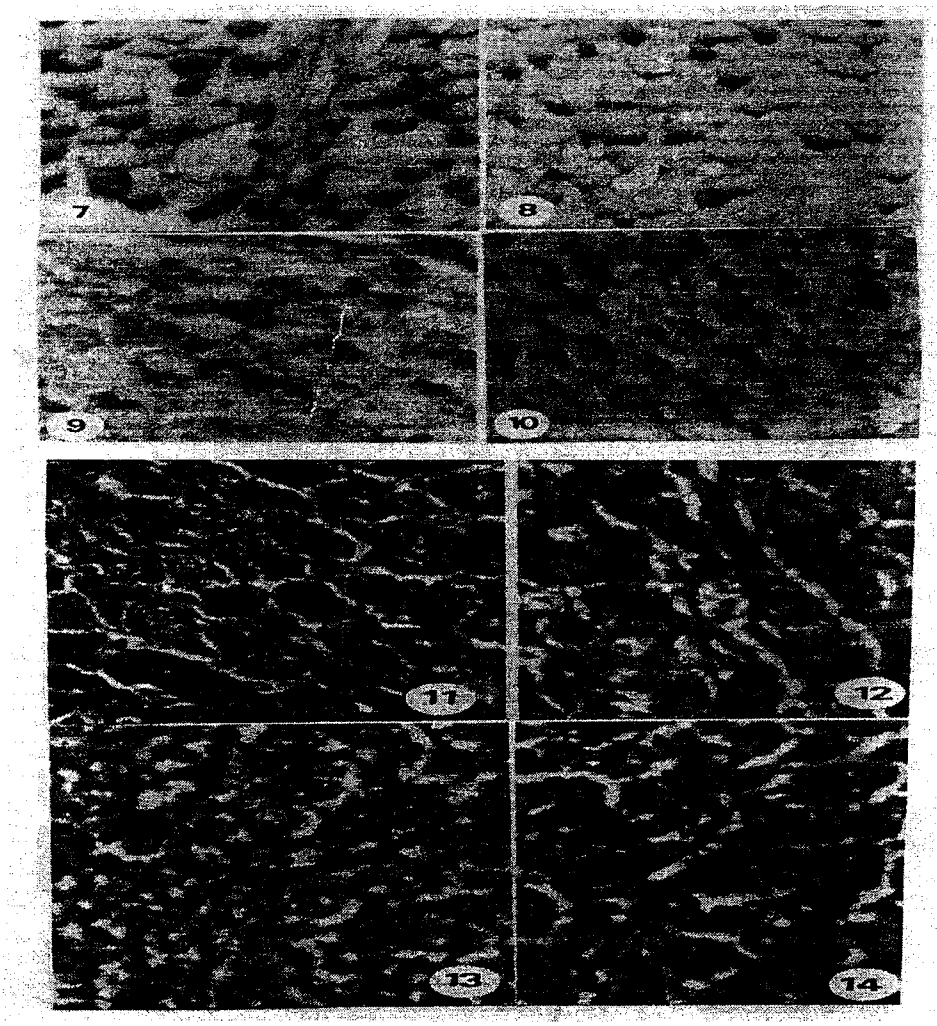

Fig.7. Liver section of a control rat showing distribution of glycogen in the cytoplasm of the hepatocytes, X 400.
Fig.8. Reduction of glycogen in hepatocytes of a rat treated with fenvalerate and examined after 2 weeks, X 400.
Fig.9. Marked loss of glycogen in hepatocytes of a rat treated with fenvalerate and examined after 4 weeks, X 400.
Fig.10. An increase of glycogen in hepatocytes after 4 weeks of treatment with fenvalerate and melatonin for X 400.
Fig.11. Normal protein content in the liver of a control rat, X 400.
Fig.12 Diminution of total proteins of hepatocytes of an animal treated with fenvalerate and examined 2 weeks, X 400
Fig.13. Marked reduction of proteins in hepatocytes of a rat treated with fenvalerate for 4 weeks, the cells showed cytoplasmic vacuolization X 400.
Fig.14. Restoration of total proteins in the hepatocytes of an animal examined 4 weeks after treatment with fenvalerate and melatonin,X 400.

The present results also showed that rats treated with fenvalerate showed a marked decrease in liver proteins. Similarly, Sakr et al., (2002) found that tetramethrin reduced total proteins in the hepatic cells of albino rats. Abdeen et al., (1994) indicated that fenvalerate caused a decrease in protein content of mice hepatocytes The reduction in protein content observed in this work may be attributed partially to the decreased level of hepatic protein synthesis in the cells suffering from pathological changes due to the hyperactivity of hydrolytic enzymes (Sivaprasada et al., 1983).

The present results showed that melatonin has an inhibitory effect on histological and histochemical alterations induced by the pyrethroid ,fenvalerate. Melatonin is a very potent and efficient endogenous free radical scavenger. It reacts with the highly toxic radical and provides one side protection against oxidative damage. Melatonin acts as a primary nonenzymatic antioxidative defense against the devastating action of the extremely reactive free radicals (Abou-El-naga et al., 2002). Abdel wahab (1997) reported that melatonin has beneficial effect on histopathological changes produced by carbon tetrachlorid and caused marked decrease in parameters of hepatic damage . Melatonin was found effective in protecting membrane lipids, nuclear DNA and protein from oxidative damage both in vivo and in vitro (Reiter et al., 1998 a,b; Lusardi et al.,2000; Atkinson et al., 2003) . In addition , melatonin influence the growth of spontaneous and induced tumor in animals and inhibits the proliferation of cultured epithelial breast cancer cell and malignant melanoma cell in dose dependent manner (Molis et al., 1994) , and reduces hepatic DNA damage of rats exposed to the carcinogen safrol (Tan et al., 1994).

The inhibitory effect of melatonin may be due to: (1) Its ability to scavenger the free radical induced by toxic materials , (2) It also functions as an indirect antioxidant by stimulating mRNA levels and the activities of superoxide dismutase , glutathione peroxidase and glutathione reductase (Pablose et al., 1998). These enzymes function to reduce OH generation by metabolizing as precursors to non-toxic products. Thus one or more mechanisms of melatonin is responsible for the inhibitory effect of melatonin observed in the present work.

REFERENCES

Abdeen, A. M., T. A. M. Amer, E. M., El-Habibi and E. M. Kamel, 1994. Histological and histochemical studies on the effect of fenvalerate insecticide on some organs of Albino mice. J. Union Arab Biol., 21: 129-166.

Abdel Wahab, M. H. 1997. Protective effect of melatonin against carbon tetrachloride-induced hepatic damage. J. Pharmacol. Sci. 19: 179-189.

Abou-EL-Naga, A.M.; Abdel-Hamid, A. and EL-Habibi, E.M. 2002. The effect of melatonin on the uterus in ovariectomized albino rats intoxicated with nicotine. J. Union Arab Biol. Cairo. 17A: Zoology 397-413.

Atkinson, G.; Drust, B.; Relliy, T. and Waterhouse, J. 2003. The relevance of melatonin to sports medicine and scince. Sports Med. 33(11):809-831.

Casida, J.E.(1973): Pyrethrum, the natural insecticide. New York, Academic Press.

Drijfhout,W.J.; Van-Der-Linde, A.G.; De-Vries, J.B.; Grol, C.J. and Westerink, B.H.1996. Microdialysis reveals dynamics of coupling between noradrenaline release and melatonin secretion in conscious rats. Neurosci-Lett. 202(3): 185-193.

EI-Banhawy, M. A., A. Mahmoud, S. A. Gaber, A. M. Mostaffa, 2000. Histopathological studies on the liver of adult and embryonic stages of mice under the effect of Ezalo insecticide. J. Egypt. Ger. Soc. Zool., 32: 89-105.

Hotchkiss, R. D., 1948. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem., 16-131.

Laurido, C.; Pelissie, T.; Sotomoyano, R.; Valladares, L.; Flores, F. and Hernandez, A. 2002. Effect of melatonin on rat spinal cord nociceptive transmission. Neuroreport. 13(1):89-91.

Lusardi, P.; Piazza, E. and Fogari, R. 2000. Cardiovascular effects of melatonin in hypertension controlled by infedipine: a 24 hour study. Br. J. Clin. Pharmacol. 49:423-430.

Maestroni, G.; Conti, A. and Reiter, R. 2001. Therapeutic potential of melatonin. Frontiers of hormone research. Karger publishers copyright 2001.

Mazia, M., P. A. Brewer and M. Affert, 1953. The cytochemical staining and measurements of protein with mercuric bromophenol blue. Biol. Bull., 104 : 57-67.

Molis, T.M.; Spriggs, L.L. and Hill, S.M. 1994. Modulation of estrogen receptor mRNA expression by melatonin in McF-7 human breast cancer cells. Mol.Endocrinol. 8 : 1681-1690.

Ohkawa H, Kaneko H, Tsuji H, Miyamoto J (1979): Metabolism of fenvalerate (Sumicidin) in rats. *J Pestic Sci* 1979, 4, 143-155

Okuno, Y., Ito, S.,Seki, T., Hiromori, T., Murakami, M.,Kadota, T.,Miyamoto,J. (1989): Fenvalerate induced granulomatous changes in rats and mice. J.Toxicol Sci.11(1):53-66.

Pablos, M.I.; Reiter, R.J.; Ortiz, G.G.; Guerreto, J.M. and Agapito, M.T. 1998. Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. Neurochem. Int. 5: 61-66.

Pieri, C.; Moroni, F.; Marra, M.; Recchioni, R. and Marcheselli, F. 1994. Melatonin: A peroxyl radical scavenger more effective than vitamin E. Life Sci. 55: 271-276.

Pilo, B. and S. P. Mehan, 1987. Effect of cholinesterase inhibitors on acetylcholine and insulin induced glucose uptake and certain hepatic enzymes in pigeon liver: an in vitro study. Indian J. Physiol. Pharmacol., 31: 159-169.

Poeggeler, B; Reiter, R.J.; Hardeland, R.; Sewerynek, E; Melchiorri, D. and Barlow- Reiter, R.J. 1998. Oxidative damage in the central nervous system: protection by melatonin. Prog. Neurobiol. 56: 339-384.

Reiter, R.J.; Tan, D.X. and Qi, W. 1998a. A brief survy of observations and mechanisms related to suppression of oxygen toxicity by melatonin. Acta. Pharmacol Sci. 19: 575-581.

Reiter, R.J.; Tan, D.X.; Kim, S.J. and Qi, W. 1998b. Melatonin as apharmacological agant against oxidative damage to lipids and DNA. Proc. West Pharmacol Soc. 44: 229-236.

Sakr, S. A., 1999. Pyrethroid inhalation-induced hepatotoxicity in Albino Rats. Oxford Res. Forum J., 1:27-31.

Sakr, S.A., El-Mesady, F.A.,El-Desouky, N.I.(2002): Pyrethroid inhalation induced histochemical changes in the liver of albino rats. The Sciences2(1):24-28.

Shakoori, A. R., S. S. Ali and M. A. Saleem, 1988. Effect of six months feeding of cypermethrin on the blood and liver of albino rats. J. Biochem. Toxicol., 3: 59-72,

Sivaprasada, K., K. R. Sombasiva and K. V. Ramana, 1983. Effect of parathion on tissue ionic changes in fish *Channa punctatus* Geobios. JODHPUR, 10; 60-62

Tan, D.X.; Chen, L.D. .; Poeggeler, B.; and Reiter, R.J. 1993. Melatonin a potent endogenous hydroxyl radical scavenger. J. Endocrine.1: 53-60.

Tan, D.X.; Reiter, R.J.; Chen, L.D.; Poeggeler, B.; Manchester, L.C. and Barlow-Walden, L.R. 1994. Both physiological and pharmalogical levels of melatonin reduse DNA adduct formation induced by the carcinogen safrole. Carcinogenesis 15: 215- 218.