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Melatonin pretreatment attenuates diazinon-induced testicular damage in mice

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Abstract

Background: Diazinon is one of widely used organophosphrous pesticides, can affect both animals and man even after a single exposure. It has a dual toxicity due to acetylcholinestrase inhibition and formation of free oxygen radicals .So, the current work aimed to evaluate the effects of diazinon on the mice testes and the possible protective effect of melatonin.

Material and Methods: Male CD-1 adult mice were divided into 6 groups, (1) control group,(2) melatonin group 10mg/kg,(3) diazinon group (30mg/kg), (4) diazinon group (60mg/kg),(5) diazinon 30mg + melatonin and (6) diazinon 60mg/kg + melatonin. Diazinon was orally administrated 1 and 28 days of treatment, whereas, melatonin was administrated intraperitoneally at a single dose. Testicular damage was examined by using hematoxyline and eosin staining.

Results: Diazinon treated groups diminished the plasma acetylcholinestrase activity on day 1 of treatment. Morphometrical analysis showed a decrease in seminiferous thickness (day 1 and 28), with increased testicular superoxide dismutase (SOD) activity (day28). Melatonin pre-treatment prevented alterations induced by diazinon, except diminution of acetylcholinestrase activity.

Conclusion: These results suggest that testicular damage observed post-treatment might be due to elevated concentration of free oxygen radicals (ROS) with diazinon while, pretreatment with a single dose of melatonin is a potentially beneficial agent to reduce testicular damage in adult mice probably by decreasing oxidative stress.

Key words: Diazinon, Melatonin, Superoxide dismutase, Germinal epithelium Acetylcholinestrase, Testosterone

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Introduction

Organophosphorus (OP) compounds are among the pesticides most commonly used and utilized as insecticides, helminthecides, ascaricides, nematicides, fungicides and bactericides for 5 decades (**Reece and Handson, 1982 and Yehia *et al.*, 2007**). Pesticides are occasionally used indiscriminately in large amounts causing environmental pollution. Residual amounts of OP pesticides have been detected in the soil, water bodies, vegetables, grains and other food products (**John *et al.*,2001 and Poet *et al.*,2004**). Toxicities of OP pesticides cause adverse effects on hematological and biochemical parameters (**De-Blaquiere *et al.*, 2000; Yehia *et al.*,2007 and Al-Shinnawy,2008**). Their mechanism of action is based on the inhibition of acetyl cholinesterase (AChE) activity through covalent binding to its serine residues, thus producing a detention of the nerve impulses that leads to death (**Kalender *et al.*,2006**). Amongst the most frequently used OP insecticides, O-O diethyl O-2- isopropyl-6-methylpyrimoidine-4-yl-phosphothioate (diazinon), a synthetic nonsystemic subst-

ance, which is used to control pest insects in soil on ornamental plants, and on fruits and vegetable field crops (**Grafft *et al.*, 2002**). It has also veterinary uses against fleas and ticks, to eliminate crop and cattle plagues, as well as in household pest control (**ATSDR, 1997**). In the environment, diazinon is quickly broken down into a variety of other chemicals (**ATSDR, 2006**). Diazinon exerts its toxicity by binding its oxygen analog to the neuronal acethlcholinesterase (AChE) enzyme, resulting in the accumulation of endogenous acetylcholine in nerve tissues and organs (**Mayer *et al.*, 1991**). Diazinon can be absorbed through the digestive system, the skin, or via the respiratory tract when inhaled. Although it is mainly eliminated by the kidney, microsomal enzymes in the liver oxidize diazinon producing more potent acetylcholinesterase inhibitors, such as dizoxon, hydroxydizoxon and hydroxydiazinon (**WHO, 1998**).

Infertility in humans has been correlated with exposure to pesticides (**Strohmer *et al.*, 1993**). It has been observed that the spermatogenesis and spermiogenesis are the spermatogenic stages most susceptible to environmental toxicants, therefore being able, directly or indirectly, to permanently damage the testis and affect male reproduction (**Bjorge *et al.*, 1996**). Pesticides also produce endocrine alterations, leading to changes in diverse reproductive parameters. It is known that quinalphos affects the union of androgens and/or estrogens to their receptors (**Sarkar *et al.*, 2000**). On the other hand, degenerative changes in seminiferous tubules were observed by OP diazinon in mice (**Sarabia *et al.*,2009**), malathion in rats (**Uzun *et al.*,2009**) and lindane induced a decrease in plasmatic testosterone levels as a consequence of alterations in steroidogenesis probably through adverse effects on the Leydig cells **(Ronco *et al.*, 2001**). These cells are the main producers of testosterone, a hormone that contributes to normal spermatogenesis, normal Sertoli cells function and to development of the secondary glands of male reproductive system (**Dadoune and Demoulin, 1993**). Moreover, the OP chlorpyriphos caused a significant reduction in the activity of superoxide dismutase (SOD), an enzymatic defensive system which permits the dismutation of the superoxide ion into hydrogen peroxide, the accumulation of which is avoided by the Catalase/glutathione peroxidase (CAT/GPX) system by transforming it into water and molecular oxygen or oxidized glutathione, respectively (**Pierrefiche and Laborit, 1995**). They added that these systems fail or get surpassed, there is an over production of superoxide ions and hydrogen peroxide which, when not completely detoxified, give rise to the highly toxic hydroxyl radical.

5-Methoxy-N-acetyltriptamine (melatonin) is an indol synthesized from tryptophan in various organs such as the pineal gland, retina, intestine, bone marrow cells and skin (**Tan *et al.*, 2003**). It is very lipophilic and crosses all lipidic membranes, including the cytoplasmic membrane. As a result, melatonin reaches the cytosol of all cells; thereby its function is not restricted solely to a certain group (**Karbowinik and Reiter, 2000**). At the molecular level, its participation in steroidogenesis has been demonstrated (**Vera *et al.*, 1997**). The finding of mel 1a receptors in Leydig cells in vitro has been associated with melatonin's capacity to block human chorionic gonadotropin (hCG) stimulation and production of necessary cAMP for the synthesis of enzymes which participate in the transformation of cholesterol into

testosterone, like the 17- and the 3-hydroxysteroid dehydrogenase. Like-wise, melatonin diminishes the expression of the steroidogenic acute regulation (StAR) protein, which is essential for steroidogenesis (**Frungieri *et al.*, 2005**). Melatonin can reduce oxidative stress by stimulating important enzymes such as SOD and glutathion peroxidase (GSH-Px) (**Onur *et al.*, 2004**). Taking into consideration that diazinon-induced oxidative stress and reactive oxygen species (ROS) generation that has been demonstrated after acute toxicity in vivo murine model (**Teimouri *et al.*, 2006; Giordano *et al.*, 2007 and Sutcu *et al.*, 2007**), and although the protective mechanism of melatonin in most cases has not been completely clarified (Reiter *et al*., 1995; Reiter, 2002).

It is interesting to elucidate if the antioxidant molecule melatonin could prevent the toxic effects provoked by an organophosphate pesticide like diazinon under conditions of acute toxicity on the testicular tissue. Additionally, it is important to establish if testosterone production by Leydig cells persists without alteration at late times after OP exposure.

Material & Methods

In this study 60 sexually mature male mice of CD-1 strain were used at 10-14 weeks of age and at least 30 g in weight. They were obtained from high institute of health. Mice were acclimated to ambient conditions (room temperature 23±2°C, relative humidity 50±10%, 12h light-dark cycle) for at least week prior the experiment. They were fed a commercial pellet and tap water ad libitum. Mice were segregated in 6 cages of 10 individual each and divided into the equal groups:

Group 1. served as control. Male mice from that group were fed orally with a saline.

Group 2. Males were injected with a single dose of melatonin (10mg/kg) at day 1.

Group 3. The experimental mice were fed with low exposure diazinon (diz) 30 mg/kg.

Group 4. Mice administrated diazinon (diz) 60 mg/kg.

Group 5. Mice treated with diazinon (30mg/kg) and injected with melatonin.

Group 6. Mice treated with diazinon (60 mg/kg) with melatonin.

All groups were evaluated on day 1 and 28. The groups treated with both melatonin and diazinon received melatonin 30 minutes prior to diazinon. The chosen dose of melatonin was 10 mg/kg of body weight (Gultenkin *et al*., 2001).The insecticide used in this study was: diazinon (diz) (30% EC; Egychem chemical company, Egypt).

Biochemical analysis

Determination of plasma acetylcho-linestarse activity (AChE)

Plasma was obtained by cardiac puncture at the moment of anesthesia, using a heparinized tuberculin syringe. Enzymatic activity was determined using the Ellman's method for cholinesterases, involving the substrate acetylthiocholine. Its hydrolysis product, thiocholine, reacts with dithiobis-2-nitrobenzoate (DTNB producing 5-thionitrobenzoate, an ion that absorbs strongly at 412nm (Ellman *et al.*, 1961).

Determination of superoxide dismutase activity (SOD)

After surgical extraction of the right testis, it was dissected out, cleaned with phosphate buffer solution (PBS) pH 7.3 at 41C, and weighed; then, it was placed in a volume of PBS 45 times bigger than the testicular tissue weight. Each testis was homogenized separately in a T-line homogenizer laboratory stirrer (model 106) with 25 strokes and centrifuged at 33,000g at 41C during 45 min in a Sorvall Dupont RC-5B ultracentrifuge, and followed by supernatant freezing at201C until the moment of the determinations. Total SOD activity was determined on the basis of the epinephrine–adrenochrome autoxidation (Misra and Fridovich,1977). The method is based on the SOD capacity to inhibit epinephrine autoxidation and subsequent adrenochrome formation. Epinephrine conversion has a maximum absorption peak at 480nm, and this was read in a Hewlett

Packard 8452 spectrophotometer Results were expressed in units U (mg/mL proteins) (Misra and Fridovich,1977).

Determination of plasma testosterone

This assay was performed in heparinized plasma through direct radio- immunoassay using a commercial kit (Coat-A-Count Total Testosterone, Diagnostic Products Co., Los Angeles, CA), with a minimum sensibility of 0.14nM and an interassay coefficient with variation of 11%. The Coat-A-Count kit consists in a solid-phase radioimmunoassay based on a specific antibody for testosterone that is immobilized on the wall of a polypropylene tube. The I 125-tagged testosterone competes with testosterone from the plasma sample for the binding spaces of the antibody. After leaving the tube to decant, the antibody-attached testosterone is measured using a gamma counter.

Testicular sperm extraction and count

One testis of each mouse was placed in 1 ml of phosphate buffer saline immediately after dissection. The tunica albuginea was cut by surgical blades and removed, and the remaining seminiferous tubules were mechanically minced by using surgical blades in 1ml of phosphate buffer saline. The testicular cell suspension was pipetted several times to form a homogenous cell suspension. One drop of the suspension was placed on a ‘‘Makler Counting Chamber” and the testicular sperm concentration was determined under a phase contrast microscope at 200 magnifications and expressed as million sperm cells per ml of suspension. Sperm counting must include complete spermatozoa only (head and tail). Loose heads must be counted separately (Bustos-Obregon *et al*., 2003).

Morphometrical analysis

It includes seminiferous tubular diameter (STD), seminiferous tubular epithelium (STE), seminiferous tubular lumen (STL) and interstitial tissue (IST). Round-shaped seminiferous tubules were measured using 10X objective and fields were calibrated using a microscopic eyepiece. For each (control and experimental samples) 200 tubules were analyzed, using digitalized microscopic images (10X) (Wing and Christensen, 1982). To perform the morphometric analysis selecting one every ten successive 5µm-thick sections. The 10 most circular seminiferous tubules were randomly identified in each section of the testis, and their diameters were measured with an ocular micrometer using the 10X lens. The mean seminiferous tubule diameter in micrometers was determined for each testis.

Histopathology of testicular tissue

After treatment, the animals were sacrificed and anesthetized by over dose of ether; the testes were extracted, fixed in Bouin's solution and subjected to routine histological techniques, obtaining 5-µm-thick cross-sections. Testes were paraffin embedded and stained with hematoxylin-Eosin and trichrome staining. Epithelial discontinuity and vacuolization, cell degeneration and tubular tissue alterations were evaluated.

Statistical analysis

The obtained data were statistically analyzed between control and experimental groups by using SPSS 11.0 windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons (Montgomery, 1997). Values of $P \leq 0.05$ were regarded as statistically significant.

Results

The present work evaluates and compares the morphometrical and histological analysis of testicular tissue sample obtained from adult male CD-1 mice that were exposed to 30mg/kg and 60mg/kg diz since day 1 until the 28th day with those from a similar control and melatonin-treated groups during the same time.

Treatment with diz (one day) provoked a significant decrease in plasma AChE when compared to control, even regardless of the previous treatment with melatonin ($p \leq$ 0.05,Table 1). On day 28 the detected levels of AChE in all groups were approached to the control values (Table 1).

The treatments with melt, diz or the combination of both produced no significant variations in SOD activity levels when compared to control at day 1. Nevertheless, at day 28 a significant

increase in SOD activity was observed in all groups treated with diz, independently of the presence or absence of previous melatonin treatment ($p \leq 0.05$, Table 1).

On day 1, treatments with melt, diz or the combination of both did not produce any significant variation in plasma testosterone levels compared to control. Significant difference occurred with the different treated groups on day 28 when compared to control ($p \leq 0.05$, Table 1).

The melatonin-treated group did not differ significantly from the control group at the end of the 4th week in terms of sperm counts, but the diazinon and melatonin plus diazinon-treated mice on

day 28 of treatment had significantly lower sperm counts than the control group ($p \leq 0.05$; Table 1). However, the melatonin plus diazinon-treated mice had significantly higher sperm counts than the diazinon-treated group ($p \leq 0.05$, Table 1).

The obtained morphometrical results of each group are shown in table 1. Morphometric analysis showed that in all diazinon-treated groups of 30mg and 60mg on day 28 the seminiferous tubules (the active site of spermatogenesis) diameter (STD) values were significantly lesser than in the control group ($p \leq 0.05$). However, these values were significantly increased in the melatonin plus diazinon treated animals compared with the diazinon treated groups. Overall, no statistically significant differences were determined between the melatonin-treated group and the control group ($p \leq 0.05$, Table 1). On day 1, diz 60mg did not produce a significant decrease in STD compared to control (Table 1). Also, the group treated with diz 30mg did not show significant changes. Similarly, the group treated with melt+diz 30mg and melt+diz 60mg did not show changes in STD with respect to control (Table1). Nevertheless, the STD of the melt+diz 60mg group on day 1 was nonsignificantly higher than that observed in the groups treated with only diz 60mg and 30mg and melt +diz 30mg (Fig. 1, Table 1).

On day 28, the groups treated with diz 30mg and 60mg showed a significant reduction in seminiferous tubule epithelium (STE) in comparison with control. Unlike observed results in the groups treated with melt+diz 30mg and melt+ diz 60mg, no changes were evident in the STE with respect to control ($p \leq 0.05$ Table 1). STE in these groups of animals were significantly higher than those observed in the groups treated with diz 30mg and diz 60mg (Fig. 2, Table 1).

Regarding STL, the results showed an increase in the luminal area in the groups treated with diazinon compared to the control (Fig 2, Table 1). Analysis of the STL detected significant increase in groups of diz 30mg and diz 60mg at 28 day, whereas in groups of diz plus melt this increase was statistically significant. In all diazinon experimental groups tendency of higher luminisation of seminiferous tubules was observed (Fig 2, Table 1).On the other hand, no significant wideness in the seminiferous lumen was observed on day 1 in all experimental groups comparable to control groups.

On day 1, no significant differences were detected in the interstitial tissue (IST), neither in groups treated with diz 30mg and diz 60mg nor in groups previously treated with melt+diz 30mg and melt+diz 60mg with respect to control. On day 28, an adverse results were obtained to that of day 1; significant differences took place in testis interstitial tissue, either in groups treated with diz 30mg and diz 60mg) or in groups treated before with melt+diz 30mg and melt+diz 60mg with respect to control (Fig 2,Table 1).

Qualitative histopathological evaluation of testes in control group revealed normal structure of germinal epithelium representing different developmental stages of spermatogenesis. The shape of the tubules was oval or rounded, without any alterations. It was observed that the testis tissue in the control group was covered with an albugineous layer and the seminiferous tubule cells were present (Figs.1 & 2). The histological structure in the melatonin-treated group resembled that of the control group (Figs.1&2).

In contrast, after 4 weeks of diazinon exposure, dilatation of blood capillaries in interstitium, ruptured basal membrane and occurrence of empty spaces in the seminiferous epithelium with detached

spermatogonia from basal membrane were noticed (Fig. 2). Most seminiferous tubules were highly disturbed, corregated and reduced in size. In addition, there was reduction in number of spermatogenic cells, and necrosis in some seminiferous tubules.Along with the reduction in size of seminiferous tubules and germinal epithelium the seminiferous tubules appeared free from spermatozoa. Edema or empty spaces with detached spermatogonia was observed in seminiferous epithelium and interstitial tissue (intertubular spaces) (Fig. 2). An apoptosis occurred in the spermatogenic cells after diazinon administration. The pronounced changes were degeneration of some tubular epithelial cells and widened lumen compared to those of control.

The number of spermatogenic cells per a seminiferous tubules was decreased in the experimental diazinon plus melatonin groups after 28 day, with the occurrence of empty spaces and detached germinal epithelium as well as the interstitial spaces representing mild edema (Fig 2).

The number of spermatogenic cells in the treated groups with melatonin and diazinon (30mg & 60mg) at the first day was increased compared to the diazinon treated groups (30mg and 60mg). Also, there was an improvement in the seminiferous tubule structure in these animals (Fig.1). The interstitial connective tissue between the seminiferous tubules appeared nearly normal, and the tubules appeared to be uniform in size and shape.

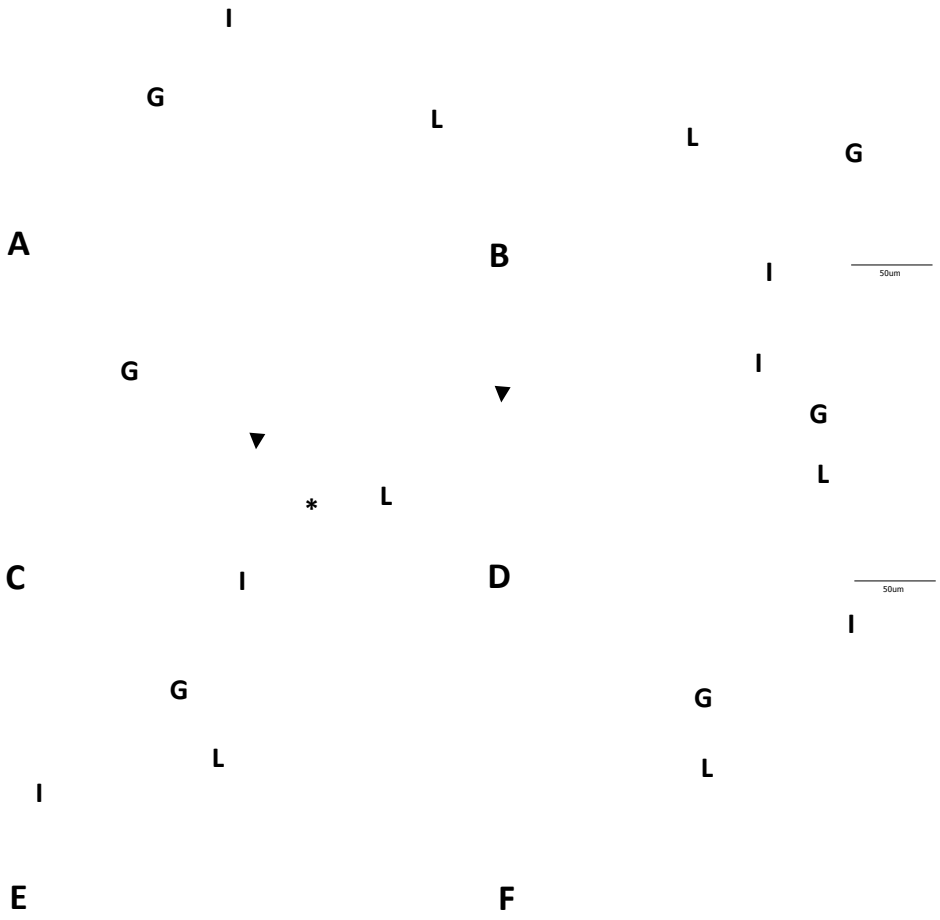

Fig.(1): Photomicrographs of testicular sections from CD-1 mice at 1 day of treatment of control(A); melatonin treated(B); diazinon 30mg(C); diazinon 60mg(D); melatonin+diazinon 30mg(E); and melatonin+diazinon 60mg(F). Note testicular structure is normally formed by germinal epithelium (G), lumen (L), interstitial tissue (I). After an experimental diazinon administration the structure of germinal epithelium was altered with predominant occurrence of hypocellularity (*), widened lumen (L), detached germinal cells (arrowhead) suggesting abnormal course of spermatogenesis. H&E. 400X.

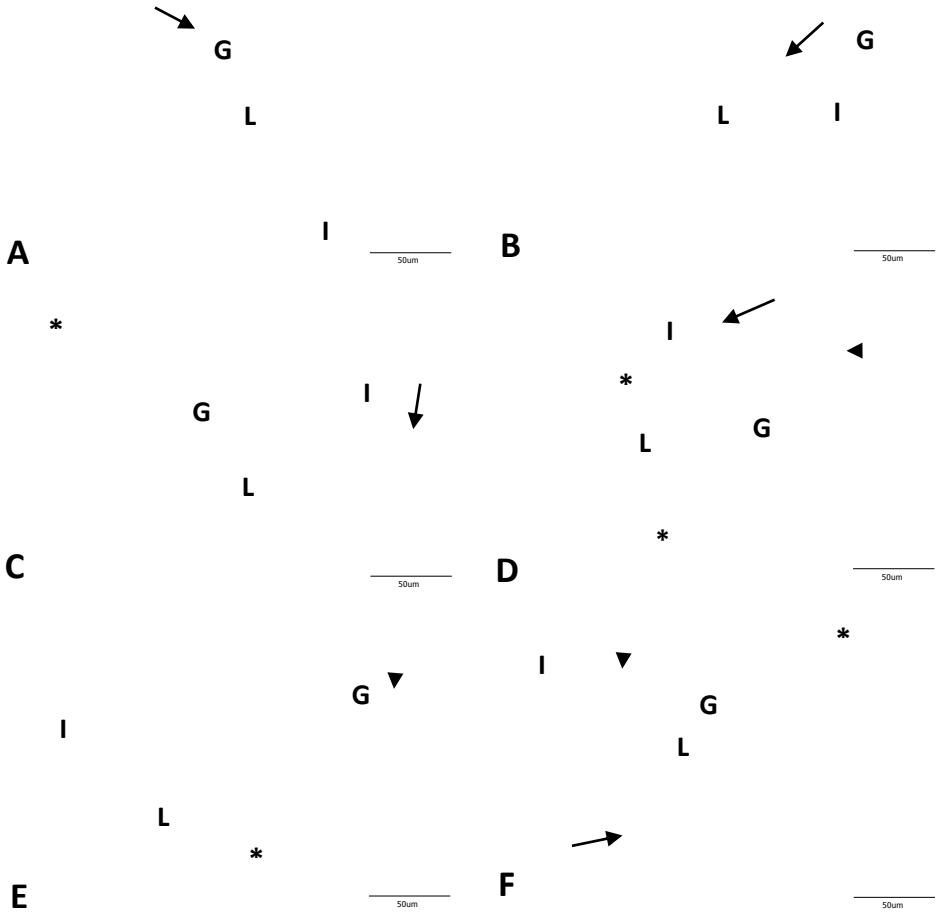

Fig.(2): Representative photomicrographs of the testes of CD-1 mice showing effects of melatonin on diazinon-induced testicular histopathological changes at 28 day post-treatment of control(A); melatonin treated(B); diazinon 30mg(C); diazinon 60mg(D); melatonin+diazinon 30mg(E); and melatonin+diazinon 60mg(F). Atrophy seminiferous tubules with detached germ layers (*), disturbed and reduced Leydig cells (arrow), corrugated seminiferous tubules (arrowhead), hypocellularity of germ cells (G), wide interlobular space (I), seminiferous lumen (L) free from spermatozoa. H&E staining. 400X.

Table (1): Melatonin and diazinon effects on testicular parameters of CD-1 mouse at day 1 and 28 (mean ± standard deviation).

	Cont.		**Melt.**		**Diz. 30mg**		**Diz.60 mg**		**Diz.30mg + melt**		**Diz.**
	1 day	**28 day**	**1 day**	**28 day**	**1 day**	**28 day**	**1 day**	**28 day**	**1 day**	**28 day**	**1 day**
	0.95±0.42	1.32±0.73	0.96±0.37**	1.34±0.69**	0.17±0.11*	1.30±0.62**	0.29±0.22*	1.31±0.33**	0.20±0.03*	1.37±0.25**	0.17±0.0
	15.1±2.8	13.2±3.2	15.2±5.2**	13.5±4.1**	15.3±5.7**	19.5±2.8*	14.2±7.5**	24.4±2.7*	14.4±3.4**	21.2±2.9*	15.1±2.7
	110±23	112±14	111±32**	110±19**	109±42**	93±24*	108±21**	82±04*	110±29**	109±51**	109±12
	28.34±0.96	28.21±0.19	29.25±0.62**	27.55±0.10**	26.54±0.15**	19.53±0.60*	25.32±0.43**	18.40±0.58*	28.12±0.26**	23.52±0.19*	27.31±0.5
	277±5.7	277.5±3.1	276.2±2.3**	275.1±3.4**	275.7±4.2**	253.3±2.5*	275.9±9.9**	240.2±8.1*	275.2±9.1**	265.4±1.3*	276.4±4.
	73±4	75±3	74±2**	72±5**	74±3**	65±8*	71±5**	60±4*	73±4**	72±4**	71±2**
	62.5±1.4	62.4±4.3	62.2±3.2**	62.0±1.6**	60.9±4.1**	82.3±2.6*	60.4±2.8**	90.5±2.4*	62.1±3.4**	74.5±8.6*	61.7±2.9
	9±1	9±1	9±1**	9±1**	11±2**	15±2*	10±1**	16±1*	11±2**	13±2*	8±1**

Cont: Control, Diz: Diazinon, Melt: Melatonin, AChE: Acetylcholinestrase, $p \leq 0.05$ comarison to control

* Significance

**nonsignificance

Discussion

The present study showed that diazinon in male CD-1 mice caused testicular dysfunction, and melatonin treatment improved these functional impairment by providing protection of seminiferous tubules and the loss of spermatogenic cell series. In this study, the first parameter determined was the degree of animal poisoning by measuring acetylcholinest-erase activity using the Ellman's method; the present results showed that all groups treated with diazinon had a significant reduction in plasma acetylcholinesterase activity levels. However, these decreases appeared only on day 1, and pretreatment with melatonin did not prevent it on any evaluated group or post-treatment time. As previously mentioned, the pesticide-induced inhibitory mechanism of acetyl-cholinesterase activity may be due to the synergestic effect of both the formation of free radicals or through direct enzyme inhibition. On day 28, the lack of differences in acetylcholinesterase activity observed between the treated and control groups might be due to the presence of hepatic esterases with detoxification functions, as proposed by Sams and Mason (1999).

Regarding SOD, the present findings showed that diazinon produced an increase in the testicular levels of this enzyme at day 28. In a previous study (**Onur *et al.,* 2004**), melatonin was injected in rats every day during 30 days until sacrifice; this chronic treatment explains their findings of augmented SOD levels. In this experiment, melatonin did not increase SOD levels, neither 1 nor 28 day, since it was injected on a single dose only. However, although melatonin did not significantly increase the levels of SOD on the studied days, it cannot discard that melatonin might increase the levels of SOD if administered during the whole experimental period, as found by Onur *et al*. (2004). Other studies demonstrated that pesticides such as pyrethroids derivatives (**Kale *et al.,* 1999**) or the organophosphate malathion (**Munoz-Hoyos *et al.*,1998**) or diazinon (**Sarabia *et al* 2009**) produced an increase in SOD activity in the liver. This suggests an activation of compensatory mechanisms as a response to the pesticide, a hypothesis which coincides with findings reported by Akhgari *et al.* (2003), and that might explain the increase of SOD activity 28 days after diazinon treatment.

The decrease in the thickness of the seminiferous epithelium without changes in tubule diameter in the group treated with diazinon (on both days 1 and 28) suggests that the pesticide specifically affects the proliferation of spermatogonia, the spermatogenic cell type in active mitotic process. This would explain the fact that the decrease in the seminiferous epithelium thickness observed on day 28 of treatment might be possibly due to the reduction in the number of all cell types. No changes were observed in the interstitial area of the testes, the seminiferous tubule diameter measurements or in plasma testosterone on day 1. Also, on day1, a decrease in the height of the epithelium only in the group treated with30 and 60mg of diazinon was noted, a phenomenon possibly more likely associated to an augment in apoptotic events than to a reduction in proliferative processes. This suggests that an acute exposure to diazinon induces apoptosis in the male germ cells, which leads to a lower height in the seminiferous epithelium as observed on day 28 of treatment, even with a concentration of 30mg of diazinon On day 28, the decreased seminiferous epithelium may be due to a combination of reduced proliferation and augmented apoptotic events. Between germ cells, spermatogonia are most sensitive to pro-oxidant agents when compared to spermatocytes and spermatids (**Bjorge *et al.*,1996 and sarabia *et al* 2009**). The reason for this is that they reside and develop below the junctional complex between adjacent Sertoli cells (**Billig *et al.,* 1996**).

It has been shown that pesticides can cause various histopathological and cytopathological changes in the reproductive system of male mammals (**Mahgoub and El-Medany, 2001 and Uzunhisarcikli *et al.*, 2007**). These changes include decreased spermatogenesis and sperm counts. For example, acephate, an OP insecticide, decreased the number of spermatogenic cells in the testes (**Farag *et al.*, 2000**), while (**Khan *et al.*, 2001**) reported that phosphorothionate, an OP insecticide, inhibited spermatogenesis. In addition, Xu *et al*. (2004) found that male rats exposed to phoxim, an OP insecticide, along with fenvalerate, a pyrethroid insecticide, showed decreased daily sperm production. In addition, OP insecticides not only decreased sperm counts, but also reduced sperm motility (**Farag *et al.*, 2000; Khan *et al.* 2001 and Uzunhisarcikli *et al.*, 2007**). Moreover, pesticide-exposed experimental animals produced significantly higher numbers of dead or abnormal sperm (**Contreras and Bustos-Obregon,1999; Burruel *et al.*,2000 and Uzunhisarcikli *et al.*, 2007**). These changes were concentration-dependent and intensify the longer the animals were exposed. Malathion treatment also reduced sperm counts has been shown previously by Contreras and Bustos-Obregon (1999), who found that mice treated intraperitoneally for 18days with malathion exhibited decreased sperm counts. (**Uzunhisarcikli *et al.* 2007**) also noticed that rats exposed for 4 and 7 weeks to a close relative of malathion, methyl parathion, had decreased sperm counts. It is likely that these effects of malathion and other OPs relate, may be due to their ability to cross the blood–testis barrier (**Uzunhisarcikli *et al.*, 2007**), after which they induce oxidative stress and lipid peroxidation that damage the biological membranes in the testes. This in turn may cause the degeneration of the spermatogenic and Leydig cells, which disrupts spermatogenesis and reduces sperm counts. Supporting this the present results showed that sub acute exposure to diazinon-induced histopathological changes in the seminiferous tubules, namely, necrosis and edema in the seminiferous tubules and interstitial tissue. The sperm themselves may also be damaged by the oxidative effects of OPs, which affect the activities of mitochondrial enzymes and the structure of the microtubules in the sperm. This in turn reduces their motility. The reactive oxygen species may contribute to infertility caused by defective sperm function has been reported previously by (**Latchoumycandane *et al.*, 2002, Sarabia *et al*.,2009 and Uzun *et al*.,2009**).

For a proper spermatogenesis, Leydig cells located in the testicular interstitium and Sertoli cells in testicular parenchyma must participate. The Leydig cell is the main testosterone producer (**Dadoune and Demoulin, 1993**). When the testosterone levels diminish, the Sertoli cell activity become reduced. Therefore, spermatids and spermatocytes may be affected in their metabolism and differentiation, thus causing alterations in the spermatogenic process. Finally, OPs may also affect male reproductive function by decreasing testosterone level. Significant alterations in testosterone level have been reported after exposure to certain pesticides (**Elbetieha and Da'as, 2003 and Maitra and Mitra, 2008**). For example, exposure to methyl parathion has been reported to significantly reduced testosterone level (**Maitra and Mitra, 2008**). It has been shown that exposure to environmental contaminants adversely affects testicular function by reducing Leydig cell steroidogenesis (**Akingbemi *et al*.,2004 and Murugesan *et al.*, 2007**). In the present study, the testosteron level in the diazinon treated mice was significantly lower than the levels in the control mice at the end of the 4th week (28 day). Thus, sub acute diazinon exposure suppressed testosteron secretion. Notably, however, cotreatment with melatonin did not have a protective effect on testosteron levels. These results may be explained by the putative androgen receptor antagonistic property of diazinon: it is known that androgen receptor antagonist substances can alter the glycosylation of gonadotrophins, which results in the suppression of testosteron levels (**Naz, 1999**).

Together with gonadotrophins, testosterone is a key hormone that regulates spermat-

ogenesis. The secretion of testosterone by the Leydig cells is dependent upon the secretion of LH by the pituitary gland. Various OPs have been studied for their effect on testosterone levels. Quinalphos treatment was shown to significantly reduce the plasma concentration of testosterone and the testicular testosterone levels (**Ray *et al.*, 1992**). However, (**Okamura *et al.* 2005**) did not observe that OPs significantly change plasma testosterone levels. In this study, diazinon exposure for 4 weeks was associated with a decrease in plasma testosterone levels. This may be because diazinon induces pathological changes in the Leydig cells in the interstitial tissues. Almost all circulating testosterone in male blood plasma is secreted by Leydig cells located in the testicular interstice. Therefore, a reduction in plasma testosterone levels indicates an alteration in these cells (**Bustos-Obregon and Gonzales-Hormazabal, 2003**), which are of great importance for the progression of the spermatogenic process (**Bustos-Obregon and Croxatto, 2003**).

(**Frungieri *et al*. 2005**) demonstrated that melatonin binds to mel 1A receptors in Leydig cell in vitro, and that this binding blocks both the stimulus of hCG and the production of cAMP necessary for the synthesis of enzymes which participate in the transformation of cholesterol into testosterone (e.g.17- and 3-b-hydroxysteroid dehydrogenase) and also reduce the expression of the StAR protein. Nevertheless, in this study we found differences in the levels of plasmatic testosterone between the groups treated with diazinon, melatonin or the combination of both in any of the exposure periods studied. This rules out a direct, local effect of the assayed compounds on the local synthesis or metabolism of testosterone. The administration of melatonin, via any route, results in a rapid rise in blood melatonin concentrations. Since melatonin has both highly lipophilic and hydrophilic properties, it passes rapidly through all biological membranes and enters the cells and their subcellular compartments. The widespread subcellular distribution of melatonin may allow it to reduce oxidative damage in both the lipid and aqueous environments of the cell. This is an advantage for melatonin over some other antioxidants, which penetrate cells more slowly. (**Hussein *et al.* 2005**) showed that a single and high dose of melatonin (100 mg/kg, i.p.) provided protection in X-ray-induced skin damage in rats. Several lines of evidence indicated that melatonin had an influence on cell proliferation and cell differentiation (**Hill and Blask, 1988 and Garcia-Santos *et al*., 2006)**. Melatonin induced a reduction in the total number of neuroblastoma cells in vitro. The inhibition was dose and time dependent and reached its maximum in cells treated for 6 days (**Sainz *et al*., 2003 and Garcia-Santos *et al*., 2006**). Consequently, the present results showed that melatonin administered at the given dose (10 mg/kg) and for the one given dose reduced the increase in basement membrane thickness of seminiferous tubules histologically and morphometrically in diazinon treated groups. Results of the present study detected that in concurrent treatment with diazinon, melatonin (a molecule with powerful antioxidant properties) preventd this damage, suggesting that the toxic effect of diazinon on the testicle is mediated mainly by ROS generation. ROS are thought to be neutralized with melatonin, since damages are not observed in diazinon-treated individuals that previously received melatonin. Furthermore, as it was reported in a previous study with a rat varicocele model, the increase in the height of the seminiferous epithelium on day 28 of treatment with melatonin could be only due to the fact that melatonin increases antioxidant enzymes such as GSH-Px and catalase, which avoid ROS increase and hence the activation of the pro-apoptotic protein bax, thus reducing apoptosis of spermatogonia, spermatocytes and spermatid cells (Onur *et al*., 2004). This is of great importance for the seminiferous epithelium, as it has been shown that this tissue has an elevated apoptosis rate (Kirsi *et al.*, 2004).

Thus, in summary, in this study, we found that acute exposure of diazinon causes testicular toxicity, but that the antioxidant melatonin is protective in terms of morphometry, sperm counts, and

histopathological changes. However, it does not protect diazinon-exposed mice from the effects of diazinon on biochemical parameters. Diazinon can indirectly stimulate a local increase in SOD activity in the testicular tissue as a result of a cell self-protection mechanism.

References

1. **Akhgari M, Abdollahi M, Kebryaeezadeh A, Hosseini R and Sabzevari O (2003):** Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. Hum. Exp. Toxicol., 22: 205–211.
2. **Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR and Hardy MP (2004)**: Inhibition of testicular steroidogenesis by xenoestrogens bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzymes gene expression in rat Leydig cells. Endocrinology, 145: 592–603.
3. **Al-Shinnawy MS (2008):** effect of orally fed diazinon on some biochemical parameters of male albino rats. Egyp.J.Hosp.Med., 33: 559-568.
4. **(ATSDR) Agency for Toxic Substances and Disease Registry (1997):** Available in: http://www.atsdr.cdc.gov/es/es_index.html.
5. **(ATSDR) Agency for Toxic Substances and Disease Registry (2006):** Diazinon, CAS#333-41-5,Division of Toxicology and environmental medicine Tox.FAQs ™ < http://www.atsdr.ede.gov./tfacts86.html>
6. **Billig H, Chun S, Eisenhauer K and Hsueh A (1996):** Gonadal cell apoptosis: hormone-regulated cell demise. Hum. Reprod., 2: 103–117.
7. **Bjorge C, Brunborg G, Wiger R Holme J, Scholz T, Dybing F and Soderlund E (1996):** A comparative study of chemically induced DNA damage in isolated human and rat testicular cells. Reprod.Toxicol., 10: 509-519.
8. **Burruel VR, Raabe OG, Overstreet JW, Wilson BM and Wiley LM (2000):** Paternal effects from methamidophos administration in mice. Toxicol. Appl. Pharm., 165: 148–157.
9. **Bustos-Obregon E and Croxatto H (2003):** Gametogenesis. In: Alfredo Perez Sanchez,A. (Ed.), Ginecologia. Editorial Mediterraneo, Chile, pp. 60–75.
10. **Bustos-Obregon E and Gonzales-Hormazabal P (2003):** Effect of a single dose of malathion on spermatogenesis in mice. Asian J. Androl., 5: 105–107.
11. **Contreras HR and Bustos-Obregon E (1999):** Morphological alterations in mouse testis by a single dose of malathion. J. Exp. Zool., 284: 355–359.
12. **Dadoune J and Demoulin A (1993):** Structure and Function of the Testis.In: Thibault,C.,Levasseur,M.(Eds.). Reproduction in mammals and man.RHF Hunter, France, pp.197-225.
13. **De-Blaquiere GE, Waters L, Blain PG and Williams FM (2000):** Electrophysiological and biochemical effects of single and multiple doses of the organophosphate diazinon in the mouse. Toxicol. Appl. Pharmacol., 166: 81-91.
14. **Elbetieha A and Da'as SI (2003):** Assessment of antifertility activities of abamectin pesticide in male rats. Ecotoxicol. Environ. Saf., 55: 307–313.
15. **Ellman G, Courtney K, Andres V and Feather-Stone R (1961):** A new rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88–95.
16. **Farag AT, Ewediah MH and El-Okazy AM (2000):** Reproductive toxicology of acephate in male mice. Reprod. Toxicol., 14: 457–462.
17. **Frungieri M, Mayerhofer A, Zitta K, Pigataro O, Calandara R and Gonzalez-Calvar S (2005):** Direct effect of melatonin on Syrian hamster testes:melatonin subtype 1a receptors, inhibition of androgen production, and interaction with the local corticotrophin-releasing hormone system. Endocrinology, 140: 1541-1551.
18. **Garcia-Santos G, Antolin I, Herrera F, Martin V, Rodriguez-Blanco J, del Pilar Carrera M and Rodriguez C (2006):** Melatonin induces apoptosis in human neuroblastoma cancer cells. J. Pineal Res., 41: 130–135.
19. **Giordano G, Afsharinejad Z, Guizzett M, Vitalote A, Kavanagh T, and Costa L (2007):** Organophosphorous insecticides chorpyrifos and dizinon and oxidative stress in neuronal cells in a genetic model of glutathione deficiency. Toxicol. Appl. Pharmacol., 219: 181-189.
20. **Grafft SJ, Jones K, Mason Hj and Cocker J (2002):** Exposure to the organophosphate diazinon data from a human volunteer study with oral and dermal doses.Toxicol. Lett., 134: 105-113.
21. **Gultenkin F, Delibas N, Yasar S and Kilinc I (2001):** In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch. Toxicol., 75: 88–96.
22. **Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S and Uysal N (2008):** Effect of melatonin on testicular damage in steptozotocin-induced diabetes rats. Eur. Surg. Res., 40: 354-360.
23. **Hill SM and Blask DE (1988):** Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. Cancer Res., 48: 6121–6126.
24. **Hussein MR, Abu-Dief EE, Abd El-Reheem MH and Abd-Elrahman A (2005):** Ultrastructural evaluation of the radioprotective effects of melatonin against X-ray-induced skin

damage in Albino rats. Int J. Exp Pathol., 86: 45–55.

25. **John S, Kale M, Rathore N and Bhantgar D (2001):** Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J. Nutr. Biochem.,12: 500-504.
26. **Kale M, Rathore N, John S, and Bhatnagar D (1999):** Lipid peroxidative damage on pyrethroid exposure and alteration in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicol. Lett., 105: 197–205.
27. **Kalender Y, Uzunhisarcikl M, Ogutu A, Acikgoz F, Kalender S (2006):** Effects of diazinon on pseudocholinesterase activity and haematological indices in rats: the protective role of vitamin E. Environ.Toxicol. Pharmacol., 22: 46-51.
28. **Karbowinik M and Reiter R (2000):** Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc. Soc. Exp. Biol. Med., 225: 9-22.
29. **Khan IA, Reddy BV, Mahboob M, Rahman MF and Jamil K (2001):** Effects of phosphorothionate on the reproductive system of male rats. J. Environ. Sci. Health B., 36: 445–456.
30. **Kirsi J, Chrysis D, Hou M, Parvinen M, Eksborg S and Soder O (2004):** Increased apoptosis occurring during the first wave of spermatogenesis is stage-specific and primarily affects midpachytene spermatocytes in the rat testis. Biol. Reprod., 70: 290–296.
31. **Latchoumycandane C, Chitra KC, and Mathur PP (2002):** The effect of methoxychlor on the epididymal antioxidant system of adult rats. Reprod. Toxicol., 16: 161–172.
32. **Mahgoub AA and El-Medany AH (2001):** Evaluation of chronic exposure of the male rat reproductive system to the insecticide methomyl. Pharm. Res., 44: 73–80.
33. **Maitra SK and Mitra A (2008):** Testicular functions and serum titers of LH and testosterone in methyl parathion-fed resoringed parakeets. Ecotoxicol. Environ. Saf., 71: 236–244.
34. **Mayer dF, Lurden CA and Williams RF (1991):** Tralomethrin insecticide and domestical pollinator. Am. Rec.J., 132: 461.
35. **Misra H and Fridovich I (1977):** Superoxide dismutase : a photochemical augmentation assay. Arch. Biochem. Biophys.,181: 308–314.
36. **Montgomery DC (1997): Design and Analysis of Experiments, fourth ed. John Wiley and Sons, Inc., USA.Munoz-Hoyos A, Sanchez-Forte M, Molina-Carballo A, Escames G, Martin-Medina E, Reiter R et al. (1998):** Melatonin's role as an anticonvulsant and neuronal protector: experimental and clinical evidence. J. Child Neurol., 13: 501–509.
37. **Murugesan P, Muthusamy T, Balasubr-amanian K and Arunakaran J (2007):** Effects of vitamins C and E on steroidogenic enzymes mRNA expression in polychlorinated biphenyl (Aroclor 1254) exposed adult rat Leydig cells. Toxicology, 232: 170–182.
38. **Naz R (1999):** Endocrine Disruptors, Effects on Male and Female Reproductive Systems. CRC Press, Florida.USA.
39. **Okamura A, Kamijima M, Shibata E, Ohtani K, Takagi K, Ueyama J, Watanabe Y, Omura M, Wang H, Ichihara G, Kondo T and Nakajima T (2005):** A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. Toxicology, 213: 129–137.
40. **Onur R, Semercioz A, Orhan I and Yekeler H (2004):** The effects of melatonin and the antioxidant defense system on apoptosis regulator proteins(Bax and Bcl-2) in experimentally induced varicocele. Urol. Res., 32: 204-208.
41. **Ozdemir D, Tugyan K, Uysal N, Sonmez U Sonmez A Acikgoz et al. (2005):** Protective effect of melatonin against head trauma-induced hippocampal damage and spatial memory deficits in immature rats. Neurosci. Lett.,385: 234-239.
42. **Pierrefiche G and Laborit H (1995):** Oxygen free radicals, melatonin, and aging. Exp. Gerontol.,30: 213-227.
43. **Poet TS, Kousba AA, Dennison SI and Timechalk C (2004):** Physiologicallybased pharmacokinetic/phamrmacodynamic model for the orgnophosphorous pesticide dizinon. Neurotoxicology, 25: 1013-1030.
44. **Ray A, Chatterjee S, Ghosh S, Bhattacharya K, Pakrashi A and Deb C (1992):** Quinalphos-induced suppression of spermatogenesis, plasma gonadotrophins,testicular testosterone production, and secretion in adult rats. Environ. Res., 57: 181–189.
45. **Reece RL and Handson P (1982):** Observation on the accidental poisoning of birds by organophosphate insecticides and other toxic substance.Vet. Res., 111: 453-455.
46. **Rieter R (2002):** Melatonin lowering the high price of free radicals. News Physiol.Sci.15, 246-250.
47. **Reiter R, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, etal. (1995):** A review of the evidence supporting melatonin's role as an antioxidant. J. Pineal Res.,18: 1-11.
48. **Reiter RJ, Tan Dx, Osuna C and Gitto E (2000):** Actions of melatonin in the reduction of oxidative stress: a review.J. Biomed. Sci.,7: 444-458.
49. **Ronco A, Valdes K, Marcus DL and lanos M (2001):** The mechanism for lindane-induced inhibition of steroidogenesis in cultured rat Leydig cells. Toxicology, 159: 99-106.
50. **Sainz RM, Mayo JC, Tan DX, Lopez-Burillo S, Natarajan M and Reiter RJ (2003):** Antioxidant activity of melatonin in Chinese hamster ovarian cells: changes in cellular proliferation and differentiation.

Biochem. Biophys. Res. Commun., 302: 625–634.

51. **Sams C and Mason H (1999):** Detoxification of organophosphates by A-esterases in human serum. Hum. Exp. Toxicol., 18: 653–658.
52. **Sarabia I, Maurer E and Bustos-Obregon (2009):**Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on themouse testis. Ecotoxicol. Environ. Safety, 72:938-942.
53. **Sarkar R, Mohanakumar K and Chwdhury M (2000):** Effects of an organophosphate pesticide,quinalphos, on the hypothalamo-pituitary-gonadal axis in adult male rats. J. Reprod. Fertil., 118: 29-38.
54. **Strohmer H, Boldizsar A, Plockinger B, Feldner-Busztin M and Feichtinger W (1993):** Agricultural work and male infertility. Am. J. Ind. Med.,24: 587-592.
55. **Sutcu R, Altuntas I, Buyukvanli B, Akturka O, Ozturka O, Koylu H and Delibas N (2007):** The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat erythrocytes: role of vitamins E and C. Toxicol. Ind. Health, 23: 13-17.
56. **Tan D, Manchester L, Hard R, Lopez-Burillo S, Mayo J, Sainz R, etal. (2003):** Melatonin: a hormone, a tissue factor,an autocoid,a paracoid, and an antioxidant vitamin .J. Pineal Res.,34: 75-78.
57. **Teimouri F, Amirkbirian N, Esmaily H, Mohammadirad A, Aliahmadi A and Abdollah M (2006):** Alteration of hepatic cells glucose metabolism as a noncholinergic detoxcation mechanism in counteracting diazinon-induced oxidative stress. Hum.Exp.Toxicol., 25: 697-703.
58. **Tugyan k, Uysal N, Ozdemir D, Sonmez U, Pekeetin C, Erbil G and Sonmez A (2006):** Protective effect of melatonin against maternal deprivation-induced acute hippocampal damage in infant rats. Neurosci.lett., 398: 145-150.
59. Uzun F K, Kalender S, Durak D,Demir F and Kalender Y(2009): Malathion-induced testicular toxicity in male rats and the protective effect of vitaminsC and E. Food Chem.Toxicol.,47:1903-1908.
60. **Uzunhisarcikli M, Kalender Y, Dirican K, Kalender S, Ogutcu A and Buyukkomurcu F (2007):** Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pestic. Biochem. Phys., 87: 115–122.
61. **Vera H, Tijmes M and Valladares L (1997):** Melatonin and testicular function: characterization of binding sites for 2-[1251]-iodomalatonin in immature rat testes. Steroids, 62: 226-229.
62. **Wing T and Christensen K (1982):** Morphometric studies on rat seminiferous tubules Am. J. Anat.,165: 13-25.
63. **(WHO) World Health Organization (1998):** PNUMA. International program on chemical safety, diazinon. Geneva Editions.
64. **Xu L, Zhan N, Liu R, Song L and Wang X (2004):** Joint action of phoxim and fenvalerate on reproduction in male rats. Asian J. Androl., 6: 337–341.
65. **Yehia MA, Elbanna SG and Okab AB (2007):** Diazinon toxicity affects histophysiological and biochemical parameters in rabbits. Exp. Toxicol. Pathol., 59: 515-225.

المعالجة بالميلاتونين تخفف من الأضرار التي يسببها الدايازينون للخصيتين في الفئران

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ديازينون واحد من مبيدات الأفات و هو ينتمى الى مجموعة الفوسفات العضوية يستخدم على نطاق واسع ، وهو يؤثر على كل من الحيوان والإنسان، حتى بعد التعرض لجرعة واحدة. ويرجع ذلك الى السمية المزدوجة على انزيم الأسيتل كولين استيريز والآثار السامة المتعلقة بتكوين الشوارد الحرة، ولذلك يهدف البحث الحالي الى تقييم الآثار الناتجة عن تعاطى جرعة واحدة من المبيد و التأثير الوقائى المحتمل للميلاتونين فى خصية الفئران البالغة.

ذكور الفئران البالغين من سلألة CD-1 قسمت إلى 6 مجموعات ، (1) المجموعة الضابطة ، (2) مجموعة الميلاتونين (3) مجموعة ديازينون 30 مج/كجم/وزن الجسم، (4) مجموعة ديازينون 60مج/كجم/وزن الجسم (5) مجموعة ديازينون30 مج/كجم + الميلاتونين و (6) مجموعة ديازينون 60 مج/كجم + الميلاتونين وعوملت الذكور بجرعة يومية من الديازينون عن طريق الفم لمدة يوم وبعد اليوم 28 من المعاملة ، في حين كان تعاطى الميلاتونين عن طريق الغشاء البريتونى في جرعة واحدة من 10 مج/كجم فى اليوم الاول.

وكانت النتائج كالتالى:

تقلص نشاط انزيم الأسيتيل كولين استيريز في اليوم الأول بعد تلقي العلاج. كما أظهرت التحاليل المظهرية انخفاضا في سمك جدار الأنيبيبات المنوية (أيام 1 و 28) ، في حين كانت زيادة ملحوظة فى نشاط انزيم السوبرأوكسيد ثنائى الميوتيز (SOD) الفائق (28 يوم). ووجد أن المعاملة بالميلاتونين قبل العلاج تمنع كل الأضرارالتي يحدثها ذلك المبيد، إلا تناقص نشاط انزيم الأسيتيل كولين استيريز .

هذه النتائج تشير إلى أن الضرر الذى حدث بعد المعاملة بالديازينون فى الخصيتين قد يكون نتيجة لارتفاع تركيز الأكسجين الحر الراديكالي (الشوارد الحرة)، غير أن المعالجة بجرعة واحدة بالميلاتونين قد أدت الى تقليل الآثار في الخصيتين وربما من خلال تقليل عنصر مؤكسد الإجهاد.