Protective role of melatonin on the effect of diazepam on proliferative activity, morphological changes and testosterone levels in the testes of rats

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Abstract

Objective: There are evidences indicating that diazepam administration inhibits cell proliferation both *in vitro* and *in vivo*. The benefits of melatonin against diazepam-induced proliferative activity, morphological and level of testosterone changes were investigated in this study.

Materials & methods: Three equal-sized groups of male rats [control, diazepam (3 mg/kg) and diazepam plus melatonin (10 mg/kg)] were used. Levels of testosterone hormone in the serum were measured. Cell proliferation was detected by autoradiography and the morphological alterations were investigated.

Results: Results documented decreased both ³H-labelling index (proliferative activity) and the levels of testosterone with marked histopathological alterations in the seminiferous tubules of diazepam-administered rats versus those of controls. When melatonin was given to diazepam-administered rats, it stimulates the decrease of labelling index, restored the levels of testosterone and preserves the alterations and number of different germ cell types in all stages of the seminiferous tubules.

Conclusion: These results suggest long-term hazard in use of drugs such as diazepam may be toxic and damage terminates in complex testes dysfunction and infertility. Furthermore, melatonin may be useful in combating testes injury resulting from hazard and/or repeated diazepam administration.

Keywords: Melatonin, diazepam, ³H-labelling index, seminiferous tubule

Introduction

Benzodiazepines (BDZ) are the most frequently prescribed class of psychotropic drugs may be worldwide [1]. Benzodiazepines, such as diazepam, are commonly used for their sedative and anxiolytic effects, i.e., by their action on high affinity receptor sites coupled to gammaaminobutyric acid A (GABA-A) receptor complex, present in the central nervous system (CNS) [2]. In addition to the central receptors described for BDZ, peripheral-type binding sites (PBRs) have been identified for them in liver cells [3], endocrine steroidogenic tissues [4] and immune cells, such as macrophages and lymphocytes [5], and also in tumor cells [6,7].

Peripheral-type benzodiazepine receptor expression has also been associated with both tumor progression and aggressive-

ness, since higher levels of its expression were found in tumor cells that display increased rate of proliferation, such as breast cancer cells [6], hepatic cancer cells [8] and glioma cells [9].

There are many evidences indicating that diazepam inhibits cell proliferation both *in vitro* and *in vivo*. Clark and Ryan [10] reported that diazepam exerted an antiproliferative effect on 3T3 fibroblasts. The antiproliferative action of diazepam on the proliferation of mouse spleen lymphocytes *in vitro* was noticed by Pawlikowski et al. [11,12]. Also, it was found that diazepam inhibited the *in vitro* proliferation of rat C6 glioma and mouse neuroblastoma cells [13] and cerebral cortical neurons [14]. *In vivo* studies of Pawlikowski et al. [15] showed that diazepam decreased the mitotic activity of the parietal cerebral cortex and the anterior pituitary gland in rats. Diazepam administration

was shown to inhibit the mitotic index of the adrenocortical cells [16] and suppress the proliferation of spleen lymphocytes in rats [17]. In contrast, El-Sokkary [18] found increased labeling index and ^3H -thymidine incorporation (DNA synthesis) in the liver of rats administered with diazepam. Diazepam had antifertility effects on male rats through attenuating steroidogenesis and testosterone production by inhibiting the pituitary gonadal axis hormones and the StAR gene expression through its effects on calcium ions [19].

Melatonin, a hormone which is synthesized by the pineal gland, is a potent antioxidant [20,21]. Although melatonin altered the spermatogenesis, it could not change the morphometric parameters such as tubular diameter and heights of the epithelium significantly [22]. Melatonin, by having lipophilic and hydrophilic compounds, can pass freely from the morpho-physiological barrier of different organs [23]. The direct effect of melatonin was observed in the male reproductive system and in the synthesis of testosterone from the Leydig cells in animals [24].

In general, it is accepted that a higher percentage of motile sperm is associated with improved fertilization rates and Ortiz et al. has shown that the addition of melatonin to seminal samples can improve the overall motility and the percentage of progressively motile spermatozoa [25,26]. Melatonin also appears to inhibit apoptosis in spermatozoa, with a reduction in early apoptotic events being demonstrated in human sperm thus prolonging sperm survival [27]. These effects would serve to improve sperm quality, therefore increasing the probability of successful fertilization. Melatonin, through its neutralization of reactive oxygen and nitrogen species, has been shown in both animal and human studies to improve seminal quality in-vitro. Studies in rats have shown that melatonin has a positive effect on sperm that have been subjected to oxidative stress, improving sperm number, viability and motility [28,29]. Similar results have been found in a small human study in which in-vitro melatonin-treated samples showed a higher percentage of sperm motility and a lower proportion of non-viable spermatozoa [30].

Materials and Methods

Animals

Thirty adult male Sprague-Dawley rats, weighing approximately 150 g, were used in this work. The animals were housed conventionally in cages (3-4 rats per cage) and fed with standard food and tap water ad libitum. The rats were maintained in 12:12 light:dark cycle at constant temperature ($25\pm 2^\circ\text{C}$). An automatic timer controlled light and dark exposure with lights off 19.00h to 07.00h. The care and treatment of the animal was approved and performed according to the approval ethics of Assiut University.

Chemicals

Diazepam (Hoffman La Roche, Basel, Switzerland) and melatonin (Sigma Co. St. Louis MO) were suspended in few drops

of ethanol and the final volumes adjusted with saline. Titrated thymidine (^3H TdR) was purchased from PerkinElmer (Boston, MA). Kodak NTB₂ emulsion, Kodak D-76 developer and Kodak fixer were from Eastman Kodak (Rochester, New York). Fertigenix-Testo-Easia kit (Biosource, USA). All other chemicals were of higher quality available.

Experimental Design and procedures

The rats were divided into three equal-sized groups 10 rats each. The first group served as control and given vehicle (0.9% NaCl solution contained 0.5% ethanol) only. The second group was administered with diazepam (3 mg/kg) according to El-Sokkary [18]. The third group was given a similar injection of diazepam which was preceded, 30 min earlier, by melatonin administration (10 mg/kg) according to El-Sokkary et al. [20]. All of these injections were subcutaneously repeated daily for 30 days. At the end of the experiment, the rats were sacrificed and the testes were removed and fixed in 10% neutral buffered formalin.

Autoradiography and quantitation of autoradiographs

Twenty four hours after the last injections, 5 rats from each group received a subcutaneous injection of 1 $\mu\text{Ci/g}$ body weight of ^3H TdR and they were killed 2 hours later. Paraffin sections (5 mm thick) were prepared. The deparaffinized sections were dipped in Kodak NTB₂ emulsion (diluted 1:1 with distilled water) and kept for 25 days in the dark at 4°C . All autoradiographs were stained with Harris's haematoxylin and eosin. A spermatogonial cells were scored as being labelled when 5 or more grains located over their nuclei. All labelled and unlabelled spermatogonia in the 8 stages of the seminiferous tubules were counted. The ^3H -labelling index (LI) was expressed as the percentage of labelled spermatogonia. The LI represent the kinetics of cellular proliferation [31].

Measurement of serum testosterone

Serum samples were collected for testosterone determination using ELISA. Levels were measured by using the above kit according to the manufacturer's instructions.

Histology and quantitation of seminiferous epithelial cycle and Leydig cells

5 rats from each group were sacrificed concomitantly with the previous rats and specimens of testes were taken, fixed, embedded in paraffin, sectioned and stained with haematoxyline and eosin. The identification of the different stages of seminiferous epithelial cycle (50 seminiferous tubules for each animal) was based on the morphological changes of the germ cell nuclei and the local arrangement of the spermatids [32,33].

The number of Leydig cells/ mm^2 (unit area) was counted using the square grid (area/ mm^2) at 1000X magnification and 35 unit areas were counted. The nuclear volume of the Leydig cells was performed using ocular micrometer at 1000X magnification. A total number of 300 nuclei were measured/animal.

The measurement was carried out by concerning rounded nuclei of Leydig cells where the formula of the sphere was applied ($V=4/3\pi R^3$), where R=nuclear diameter/2.

Statistical analysis

Quantitative results were expressed as means $\pm$ S.E.M. Differences between means were tested by one way analysis of variance (ANOVA) followed by the Student-Newman-Keuls t-test. The percent of stimulation (S%) or inhibition (I%) in the mean values was calculated.

Results

The seminiferous tubules

In the diazepam-treated rats, the diameters of seminiferous tubules decreased and their architecture was altered (**Figures 2 and 5**) when compared with those of control animals (**Figures 1 and 4**). A lot of degenerating germ cells that contain vacuoles of varying sizes, congested blood vessels and peritubular edema were detected in large number of seminiferous tubules of diazepam-treated rats as shown in **Figures 2 and 5**. Also, the number of germ cells in all stages of the seminiferous tubule markedly decreased in diazepam-treated rats compared to those of controls (**Figure 5**). Melatonin administration to diazepam-treated rats restored the changes of the germ cells towards those of controls (**Figures 3 and 6**).

The quantitative results of the seminiferous tubules cycles are presented in **Table 1**. Statistically, there was a significant reduction ($P<0.01$) in the mean number of different germ cell types in all stages of the seminiferous tubules in diazepam-treated rats versus those of control animals. When melatonin was given to diazepam-treated animals, the number of different germ cell types in all stages of the seminiferous tubules was significantly increased ($P<0.01$) and appeared near to those of control numbers.

The interstitial cells of Leydig

As seen in **Figure 2** there was a sharp drop in the number of Leydig cells in the testes of diazepam-treated rats when

Figures 1. T.S of testis of control.

Showing the alterations in the seminiferous tubules and Leydig cells.

Figure 2. Diazepam-treated.

Showing the alterations in the seminiferous tubules and Leydig cells. V: vacuoles, ed: edema, cog: congested blood vessels (H&E).

Figure 3. Diazepam +melatonin treated rats.

Showing the alterations in the seminiferous tubules and Leydig cells.

Figure 4. T.S of testis of control.

Showing different stages the seminiferous tubule cycle (H&E).

compared with those of controls (**Figure 1**). The quantitative results of the number and nuclear volume of Leydig cells

Figure 5. Diazepam-treated.

Showing different stages the seminiferous tubule cycle (H&E). The stages of the diazepam-treated rats present several degenerative germ cells and vacuoles (V).

Figure 6. Diazepam +melatonin treated rats.

Showing different stages the seminiferous tubule cycle (H&E).

are presented in **Table 2**. As shown, there was a significant decrease ($P<0.01$) in the mean number of Leydig cells/mm (unit area) in diazepam-treated rats versus controls. Also, the quantitative data revealed that there was a significant reduction ($P<0.01$) in the mean volume of nuclei of Leydig cells in diazepam-treated rats versus controls. Again, melatonin administration to diazepam-treated animals increased both of the mean number of Leydig cells and the mean volume of their nuclei. The stimulation in the two parameters was significant ($P<0.01$).

Testosterone concentration

Serum testosterone concentrations were significantly decreased ($P<0.01$) by 36% in diazepam-treated animals versus controls (**Table 3**). When melatonin was administered to diazepam-treated rats, it stimulated the concentration of testosterone hormone by 18.6%. Statistically this stimulation was significant ($P<0.05$).

Autoradiography and spermatogonial proliferation

The number of labelled spermatogonia in the different stages of seminiferous tubule cycle was lower in diazepam-treated rats than those of controls, as shown in **Figures 7 and 8**. The quantitative results of autoradiographic study are presented in **Table 4**. As shown, the mean ^3H -labelling index (LI) was significant decreased ($P<0.01$) in spermatogonia of each stage in the seminiferous tubule cycle of diazepam-treated rats versus those of controls. In addition, the mean value of LI in the spermatogonia of all stages of the seminiferous tubule cycle as a whole decreased significantly ($P<0.01$) in rats given diazepam versus control ones. The above data revealed that there was a sharp suppression in the proliferative activity of

Table 1: Mean number of cells ($\pm$ S.E) in different stages of the seminiferous epithelial cycle in control and treated rats.

Stages Experiment	Sertoli cells	Spermatogonia			Primary Spermatocytes	Secondary Spermatocytes	Spermatids		Spermatozoa
		Type A	Type B	Total			Rounded	Elongated	
Control group	4.64 ^a $\pm$ 0.171	28.7 ^a $\pm$ 0.981	8.2 ^a $\pm$ 0.181	36.9 ^a $\pm$ 0.932	52.94 ^a $\pm$ 1.194	7.84 ^a $\pm$ 0.182	49.3 ^a $\pm$ 1.568	9.86 ^a $\pm$ 0.293	12.28 ^a $\pm$ 0.316
DZ group	2.52 ^b $\pm$ 0.162	17.8 ^b $\pm$ 0.762	4.7 ^b $\pm$ 0.162	22.5 ^b $\pm$ 0.814	32.58 ^b $\pm$ 1.057	3.24 ^b $\pm$ 0.101	32.58 ^b $\pm$ 1.057	8.3 ^b $\pm$ 0.179	9.1 ^b $\pm$ 0.222
DZ+melatonin	3.68 ^c $\pm$ 0.165	22.3 ^c $\pm$ 0.684	6.9 ^c $\pm$ 0.244	29.1 ^c $\pm$ 0.738	43.6 ^c $\pm$ 1.298	5.42 ^c $\pm$ 0.172	43.6 ^c $\pm$ 1.298	9.28 ^a $\pm$ 0.252	10.98 ^c $\pm$ 0.207

Values in the same column with unlike superscript letters are significantly differing at $P<0.05$.

Table 2: Means of number and nuclear volume of Leydig cells in control and treated rats.

Groups	Measurements	Mean number of Leydig cells $\pm$ SD	Mean nuclear volume (mm ³) of Leydig cells $\pm$ SD
Control group		24.8 $\pm$ 4.3 ^a	89.6 $\pm$ 8.2 ^a
DZ group		12.2 $\pm$ 2.3 ^b	43.5 $\pm$ 5.2 ^b
DZ+melatonin		17.6 $\pm$ 1.9 ^c	60.7 $\pm$ 4.3 ^c

Values in the same column with unlike superscript letters are significantly differing at $P<0.05$

Table 3: Means of testosterone concentrations ($\pm$ SE), inhibition (I%) and/or stimulation (S%) in control and treated rats.

Animal group	Control	DZ group		DZ+melatonin		
			I % versus Cont.		I % versus Cont.	S % versus DZ group
Measurements						
Testosterone (ng/ml)	0.857 $\pm$ 0.02 ^a	0.548 $\pm$ 0.02 ^b	I= 36.0	0.673 $\pm$ 0.02 ^c	I= 21.5	S= 18.5

Values in the same row with unlike superscript letters are significantly differing at $P < 0.05$.

Figure 7. T.S of testis of control. showing the distribution of labelled spermatogonia (arrows) in different stages of seminiferous tubule cycle (H&E).

Figure 8. Diazepam-treated. showing the distribution of labelled spermatogonia (arrows) in different stages of seminiferous tubule cycle (H&E). The 3H-labeled spermatogonia sharply decreased in diazepam-treated rats.

the spermatogonia of diazepam-treated rats. When melatonin administered to the diazepam-treated rats, it was blocking the inhibitory property of diazepam in the proliferative activity. The number of labelled spermatogonia in the different stages of seminiferous tubule cycle was increased than those of diazepam-treated rats (**Figure 9**). As seen in **Table 4**, the LI was significant increased ($P < 0.01$) in spermatogonia of each stage in the seminiferous tubule cycle.

Discussion

The results presented above showed clearly that diazepam administration had suppressed the proliferative activity of the spermatogonia. Various factors may be involved in the mechanisms of the inhibitory effect of diazepam on cell proliferation. It was reported that diazepam can affect the secretion of ACTH. Racagni et al [34] found that diazepam decreased

Figure 9. Diazepam +melatonin treated rats. showing the distribution of labelled spermatogonia (arrows) in different stages of seminiferous tubule cycle (H&E).

Table 4: Mean 3H-labelling indices ($\pm$ SE) in spermatogonia of all stages of seminiferous tubules in control and treated rats.

Stages	I	II	III	IV	V	VI	VII	VIII	Mean
Groups									
Control group	17.1 ^a $\pm$ 0.037	9.9 ^a $\pm$ 0.40	11.8 ^a $\pm$ 0.38	17.7 ^a $\pm$ 0.36	12.2 ^a $\pm$ 0.37	21.4 ^a $\pm$ 0.43	18.2 ^a $\pm$ 0.34	11.2 ^a $\pm$ 0.33	14.9 ^a $\pm$ 1.47
DZ group	8.25 ^b $\pm$ 0.20	6.45 ^b $\pm$ 0.25	7.1 ^b $\pm$ 0.27	8.56 ^b $\pm$ 0.22	6.5 ^b $\pm$ 0.28	9.15 ^b $\pm$ 0.21	8.54 ^b $\pm$ 1.05	6.4 ^b $\pm$ 0.29	7.62 ^b $\pm$ 0.40
DZ+melatonin	12.95 ^c $\pm$ 0.27	8.05 ^c $\pm$ 0.22	8.9 ^c $\pm$ 0.24	11.35 ^c $\pm$ 0.38	8.55 ^c $\pm$ 0.26	15.4 ^c $\pm$ 0.47	13.85 ^c $\pm$ 0.28	10.25 ^c $\pm$ 0.31	11.16 ^{ac} $\pm$ 0.95

Values in the same row with unlike superscript letters are significantly differing at $P < 0.05$.

ACTH secretion; accordingly, the secretion of adrenocortical hormones decreased [35]. The inhibitory effect of diazepam on cell proliferation may due to the induction of chromosomes malsegregation. It was found that diazepam inhibit centrioles shifting and induces the formation of monopolar spindle during the metaphase-anaphase transition [36].

Another possibility, which does not exclude the preceding mechanisms, is the effect of diazepam on calcium ions. It was reported that BZD receptors block the voltage-dependent calcium channels [37]. It is well established that calcium ions are needed in the later G₁ phase of the cell cycle for the initiation of DNA synthesis [38]. Moreover, it was reported that BZD inhibits the calmodulin-dependent protein kinase in the brain. Jones et al. [39] found that the role of calcium in the initiation of DNA synthesis is mediated by the formation of calcium –calmodulin complex.

Our results showed that melatonin administration enhances the proliferative activity of the spermatogonia and the number of labelled cells increased matching those of controls. The fascinating feature of this hormone is that it makes the normal cells proliferate and makes the cancerous cells undergo apoptosis [40].

Increasing evidence demonstrates that melatonin has an anti-apoptotic effect [41-43]. In vivo, melatonin alleviated ischemia-reperfusion induced apoptosis in neural cells through repression of the mitochondrial permeability transition pore [44,45]. Wang et al. [46] found that melatonin attenuated lipopolysaccharide-induced apoptotic liver damage in mice [46]. Li et al. [47] reported that melatonin protected kidney grafts from ischemia/reperfusion injury through the repression of the apoptosis after experimental kidney transplantation. In the study of Ji et al. [48], they found that melatonin alleviated Cd-induced testicular histopathological damage. Importantly, the frequency of tubules with more than six apoptotic germ cells was significantly reduced when mice were administered with melatonin. In addition, melatonin significantly reduced the percentage of tubules with apoptotic germ cells and the number of apoptotic germ cells per tubule. Also, melatonin attenuated 2-bromopropane-induced testicular germ cell apoptosis in rats [49]. These data demonstrate that melatonin could protect against germ cell apoptosis in testes.

In the current study, degeneration in the cells of the seminiferous tubules, vacuolation in their cytoplasm, and reduced seminiferous epithelial layers, edema, and hyalinization of intertubular tissue were detected in diazepam treated animals. The same results was reported previously by other investigators [50,51], who described the appearance of necrosis, degeneration, desquamation, disorganization, reduction in germinal cells, vacuolization in Sertoli cells, multinucleated giant cell formation, interstitial edema, and congestion in the intertubular tissue.

In the present study, the concomitant administration of melatonin with diazepam led to a marked improvement in the histopathological changes.

These changes were in agreement with those observed by some researchers [52-54], who reported that melatonin may alleviate cadmium-induced cellular stress and germ cell apoptosis in testes.

Moreover, Take et al. [55] evaluated the protective effect of melatonin against irradiation-induced damage to rat testes that included amelioration of germ-cell depletion and apoptotic changes. At the ultrastructural level, Hussein et al. [56] observed the disappearance of the characteristics of apoptosis (condensation of the nuclei, vacuolization of the cytoplasm, increased cytoplasmic density, and apoptotic bodies) when the irradiated animals were pretreated with melatonin.

A lot of studies have explained the possible mechanism of melatonin protection as being dependent on its antioxidative action. Lena and Subramanian [57] reported that melatonin has the ability to scavenge reactive species, which makes melatonin a potent antioxidant and a free radical scavenger. They concluded that melatonin could control the oxidative abuse by (i) directly scavenging a variety of radicals and ROS, (ii) inducing antioxidative enzymes which reduce the steady state levels of ROS, (iii) inhibiting nitric oxide synthase, which generates nitric oxide, and (iv) stabilizing cell membranes that aid them in reducing oxidative damage.

Gutierrez-Cuesta et al. [58] reported that melatonin improved prosurvival signals and reduced pro-death signals. Zhang et al. [59] have suggested that melatonin may potentially attenuate testicular damage by improving morphological changes and reducing germ cell apoptosis in mice.

The development and growth of male reproductive ducts and seminiferous tubules is dependent on the increase of testosterone concentration [60]. Testosterone is essential for the survival of the spermatogenic endothelium and the significant decrease in testosterone hormone causes an increase in sperms abnormal morphology and decrease in sperm viability [61].

The results of the present study had shown that treatment with diazepam led to significant decrease in testosterone level in serum of treated animals. Testosterone hormone is the principle male hormone; it is synthesized by Leydig cells from cholesterol [62]. This decrement of testosterone level may be due to the effect of diazepam on serum cholesterol which is a precursor of testosterone synthesis by its action on the Leydig cells [63]. Therefore, the decreased testosterone concentration produced by the drug could be explained either by direct effect at Leydig cell level or an indirect effect by disturbing the hormonal milieu at hypothalamic-pituitary axis [64].

The reduced level of the testosterone has been suggested as being caused by the oxidative damage to the Leydig cell population [65] and might be related to the decreased numbers of Leydig cells [66]. Melatonin is attracting increased attention in recent years due to its known ability to reduce oxidative stress [20], with negligible toxicity even in very high doses [67-69]. Melatonin is not only an effective hydroxyl radical

scavenger [70] but also has the capacity to detoxify other ROS and reactive nitrogen species as well as their metabolites, peroxynitrous acid, and intermediates H₂O₂ [71]. Moreover, MLT enhances the antioxidant potential of the cell by the upregulation of several antioxidant enzymes [72].

Melatonin treatment to diazepam-administered rats ameliorated the reduction in the testosterone level. The increase of testosterone level after melatonin administration to diazepam-treated rats lends credence to melatonin's protection against the primary causes of damage of the testis. Because testosterone plays a key role in the process of spermatocyte meiosis [73] and the relationship between melatonin and testosterone has been well documented [74], it is suggested that modulation of the testosterone level by this hormone potentially protected the meiosis of spermatocytes and production of normal sperm quality, and this effect might be attributed to its capacity to control cellular redox state.

Finally, in this study, it was found that melatonin could provide good protection against diazepam-induced testicular damage. Several clinical trials should be conducted in the future to combine melatonin treatment with other chemotherapeutic and toxic drugs to reduce their cellular damage and toxicity.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	GHS	HHR	HAY
Research concept and design	✓	✓	✓
Collection and/or assembly of data	✓	✓	✓
Data analysis and interpretation	✓	✓	✓
Writing the article	✓	✓	✓
Critical revision of the article	✓	✓	✓
Final approval of article	✓	--	--
Statistical analysis	✓	--	--

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References

- Stiefel F, Berney A and Mazzocato C. **Psychopharmacology in supportive care in cancer: a review for the clinician. I. Benzodiazepines.** *Support Care Cancer*. 1999; **7**:379-85. | [PubMed](#)
- Costa E, Guidotti A, Mao CC and Suria A. **New concepts on the mechanism of action of benzodiazepines.** *Life Sci*. 1975; **17**:167-85. | [PubMed](#)
- Woods MJ, Zisterer DM and Williams DC. **Two cellular and subcellular locations for the peripheral-type benzodiazepine receptor in rat liver.** *Biochem Pharmacol*. 1996; **51**:1283-92. | [Article](#) | [PubMed](#)
- Zilz A, Li H, Castello R, Papadopoulos V and Widmaier EP. **Developmental expression of the peripheral-type benzodiazepine receptor and the advent of steroidogenesis in rat adrenal glands.** *Endocrinology*. 1999; **140**:859-64. | [Article](#) | [PubMed](#)
- Zavala F. **Benzodiazepines, anxiety and immunity.** *Pharmacol Ther*. 1997; **75**:199-216. | [Article](#) | [PubMed](#)
- Hardwick M, Fertikh D, Culty M, Li H, Vidic B and Papadopoulos V. **Peripheral-type benzodiazepine receptor (PBR) in human breast cancer: correlation of breast cancer cell aggressive phenotype with PBR expression, nuclear localization, and PBR-mediated cell proliferation and nuclear transport of cholesterol.** *Cancer Res*. 1999; **59**:831-42. | [Article](#) | [PubMed](#)
- Han Z, Slack RS, Li W and Papadopoulos V. **Expression of peripheral benzodiazepine receptor (PBR) in human tumors: relationship to breast, colorectal, and prostate tumor progression.** *J Recept Signal Transduct Res*. 2003; **23**:225-38. | [Article](#) | [PubMed](#)
- Corsi L, Geminiani E, Avallone R and Baraldi M. **Nuclear location-dependent role of peripheral benzodiazepine receptor (PBR) in hepatic tumoral cell lines proliferation.** *Life Sci*. 2005; **76**:2523-33. | [Article](#) | [PubMed](#)
- Brown RC, Degenhardt B, Kotoula M and Papadopoulos V. **Location-dependent role of the human glioma cell peripheral-type benzodiazepine receptor in proliferation and steroid biosynthesis.** *Cancer Lett*. 2000; **156**:125-32. | [Article](#) | [PubMed](#)
- Clarke GD and Ryan PJ. **Tranquillizers can block mitogenesis in 3T3 cells and induce differentiation in Friend cells.** *Nature*. 1980; **287**:160-1. | [PubMed](#)
- Pawlikowski M, Stepień H and Kunert-Radek J. **Diazepam inhibits proliferation of the mouse spleen lymphocytes in vitro.** *Pol J Pharmacol Pharm*. 1986; **38**:167-70. | [PubMed](#)
- Pawlikowski M, Lyson K, Kunert-Radek J and Stepień H. **Effect of benzodiazepines on the proliferation of mouse spleen lymphocytes in vitro.** *J Neural Transm*. 1988; **73**:161-6. | [PubMed](#)
- Regan CM, Gorman AM, Larsson OM, Maguire C, Martin ML, Schousboe A and Williams DC. **In vitro screening for anticonvulsant-induced teratogenesis in neural primary cultures and cell lines.** *Int J Dev Neurosci*. 1990; **8**:143-50. | [PubMed](#)
- Regan CM, Gorman AM, Larsson OM, Maguire C, Martin ML, Schousboe A and Williams DC. **In vitro screening for anticonvulsant-induced teratogenesis in neural primary cultures and cell lines.** *Int J Dev Neurosci*. 1990; **8**:143-50. | [PubMed](#)
- Pawlikowski M, Stepień H, Mroz-Wasilewska Z and Pawlikowska A. **Effects of diazepam on cell proliferation in cerebral cortex, anterior pituitary and thymus of developing rats.** *Life Sci*. 1987; **40**:1131-5. | [PubMed](#)
- Zieleniewski J, Nowakowska-Jankiewicz B and Stepień H. **Influence of diazepam on regenerating adrenal cortex in Wistar rats.** *Cytobios*. 1990; **61**:85-8. | [PubMed](#)
- Morsi GA. **Autoradiographic studies on the effect of diazepam on the proliferation of rat spleen lymphocytes.** *J. Egypt. Ger. Soc. Zool*. 1991; **6**:229-244.
- El-Sokkary GH. **Melatonin and vitamin C administration ameliorate diazepam-induced oxidative stress and cell proliferation in the liver of rats.** *Cell Prolif*. 2008; **41**:168-76. | [Article](#) | [PubMed](#)
- Taher MA, and Anber, Z.N. **Effect of diazepam on the reproductive system in male rats.** *World J. Pharm. Pharm. Sci*. 2015; **4**:60-78.
- El-Sokkary GH, Nafady AA and Shabash EH. **Melatonin administration ameliorates cadmium-induced oxidative stress and morphological changes in the liver of rat.** *Ecotoxicol Environ Saf*. 2010; **73**:456-63. | [Article](#) | [PubMed](#)
- El-Sokkary GH, Ismail, I.A. and Saber H.S. **Bright face of melatonin against breast cancer progression and metastasis.** *Eur. J. Biomed. Pharm. Sci.(EJBPS)*. 2016; **3**:105-113.
- Mirhoseini M, Talebpour Amiri F, Karimpour Malekshah AA, Rezanejad Gatabi Z and Ghaffari E. **Protective effects of melatonin on testis histology following acute torsion-detorsion in rats.** *Int J Reprod Biomed (Yazd)*. 2017; **15**:141-146. | [PubMed Abstract](#) | [PubMed FullText](#)
- Tahan G, Akin H, Aydoğan F, Ramadan SS, Yapıcıer O, Tarcin O, Uzun H, Tahan V and Zengin K. **Melatonin ameliorates liver fibrosis induced by bile-duct ligation in rats.** *Can J Surg*. 2010; **53**:313-8. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
- Reiter RJ. **Functional diversity of the pineal hormone melatonin: its role**

- as an antioxidant. *Exp Clin Endocrinol Diabetes*. 1996; **104**:10-6. | [Article](#) | [PubMed](#)
25. Ortiz A, Espino J, Bejarano I, Lozano GM, Monllor F, Garcia JF, Pariente JA and Rodriguez AB. **High endogenous melatonin concentrations enhance sperm quality and short-term in vitro exposure to melatonin improves aspects of sperm motility.** *J Pineal Res*. 2011; **50**:132-9. | [Article](#) | [PubMed](#)
26. Espino J, Ortiz A, Bejarano I, Lozano GM, Monllor F, Garcia JF, Rodriguez AB and Pariente JA. **Melatonin protects human spermatozoa from apoptosis via melatonin receptor- and extracellular signal-regulated kinase-mediated pathways.** *Fertil Steril*. 2011; **95**:2290-6. | [Article](#) | [PubMed](#)
27. Espino J, Bejarano I, Ortiz A, Lozano GM, Garcia JF, Pariente JA and Rodriguez AB. **Melatonin as a potential tool against oxidative damage and apoptosis in ejaculated human spermatozoa.** *Fertil Steril*. 2010; **94**:1915-7. | [Article](#) | [PubMed](#)
28. Nasiraei-Moghadam S, Parivar K, Ahmadiani A, Movahhedini M and VaezMahdavi M. **Protective effect of melatonin against inequality-induced damages on testicular tissue and sperm parameters.** *Int J FertilSteril* 2014; **7**:313-322.
29. Liu C, Gao P, Xu SC, Wang Y, Chen CH, He MD, Yu ZP, Zhang L and Zhou Z. **Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived cell line: a protective role of melatonin.** *Int J Radiat Biol*. 2013; **89**:993-1001. | [Article](#) | [PubMed](#)
30. du Plessis SS, Hagenaar K and Lampiao F. **The in vitro effects of melatonin on human sperm function and its scavenging activities on NO and ROS.** *Andrologia*. 2010; **42**:112-6. | [Article](#) | [PubMed](#)
31. Hamatani K, Kawahara A and Amano M. **Quantitative study of deoxycytidine incorporation in large and small lymphocytes of the mouse.** *Cell Tissue Kinet*. 1983; **16**:557-70. | [PubMed](#)
32. Clermont Y. **Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal.** *Physiol Rev*. 1972; **52**:198-236. | [Article](#) | [PubMed](#)
33. Franca, L R, Ye, S-J, Ying, L, Sandberg, M and Russell L.D. **Morphology of rat germ cells during spermatogenesis.** *Anat. Rec*. 1995; **241**:181-204.
34. Racagni G, Apud JA, Cocchi D, Locatelli V and Muller EE. **GABAergic control of anterior pituitary hormone secretion.** *Life Sci*. 1982; **31**:823-38. | [PubMed](#)
35. Guyton C.A. **The adrenocortical hormones.** In: *Textbook of Medical Physiology*. Dreibelbis, D. (ed), W.B. Saunders Company Philadelphia. 1986; 909-922.
36. Callaini G, Dallai R and Riparbelli MG. **Diazepam induces abnormal mitosis in the early Drosophila embryo.** *Biol Cell*. 1989; **67**:313-20. | [Article](#) | [PubMed](#)
37. Cantor EH, Kenessey A, Semenuk G and Spector S. **Interaction of calcium channel blockers with non-neuronal benzodiazepine binding sites.** *Proc Natl Acad Sci U S A*. 1984; **81**:1549-52. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
38. Hesketh TR, Moore JP, Morris JD, Taylor MV, Rogers J, Smith GA and Metcalfe JC. **A common sequence of calcium and pH signals in the mitogenic stimulation of eukaryotic cells.** *Nature*. 1985; **313**:481-4. | [PubMed](#)
39. Jones A, Boynton AL, MacManus JP and Whitfield JF. **Ca-calmodulin mediates the DNA-synthetic response of calcium-deprived liver cells to the tumor promoter TPA.** *Exp Cell Res*. 1982; **138**:87-93. | [Article](#) | [PubMed](#)
40. Gholami M, Saki G, Hemadi M, Khodadadi A and Mohammad-di-Asl J. **Effect of Melatonin on the Expression of Apoptotic Genes in Vitrified-thawed Spermatogonia Stem Cells Type A of 6-Day-Old Mice.** *Iran J Basic Med Sci*. 2013; **16**:906-9. | [PubMed Abstract](#) | [PubMed FullText](#)
41. Feng Z and Zhang JT. **Melatonin reduces amyloid beta-induced apoptosis in pheochromocytoma (PC12) cells.** *J Pineal Res*. 2004; **37**:257-66. | [Article](#) | [PubMed](#)
42. Luchetti F, Canonico B, Curci R, Battistelli M, Mannello F, Papa S, Tarzia G and Falcieri E. **Melatonin prevents apoptosis induced by UV-B treatment in U937 cell line.** *J Pineal Res*. 2006; **40**:158-67. | [Article](#) | [PubMed](#)
43. Choi SI, Joo SS and Yoo YM. **Melatonin prevents nitric oxide-induced apoptosis by increasing the interaction between 14-3-3beta and p-Bad in SK-N-MC cells.** *J Pineal Res*. 2008; **44**:95-100. | [Article](#) | [PubMed](#)
44. Andrabai SA, Sayeed I, Siemen D, Wolf G and Horn TF. **Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism responsible for anti-apoptotic effects of melatonin.** *FASEB J*. 2004; **18**:869-71. | [Article](#) | [PubMed](#)
45. Jou MJ, Peng TI, Hsu LF, Jou SB, Reiter RJ, Yang CM, Chiao CC, Lin YF and Chen CC. **Visualization of melatonin's multiple mitochondrial levels of protection against mitochondrial Ca(2+)-mediated permeability transition and beyond in rat brain astrocytes.** *J Pineal Res*. 2010; **48**:20-38. | [Article](#) | [PubMed](#)
46. Wang H, Xu DX, Lv JW, Ning H and Wei W. **Melatonin attenuates lipopolysaccharide (LPS)-induced apoptotic liver damage in D-galactosamine-sensitized mice.** *Toxicology*. 2007; **237**:49-57. | [Article](#) | [PubMed](#)
47. Li Z, Nickkholgh A, Yi X, Bruns H, Gross ML, Hoffmann K, Mohr E, Zorn M, Buchler MW and Schemmer P. **Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-kB and apoptosis after experimental kidney transplantation.** *J Pineal Res*. 2009; **46**:365-72. | [Article](#) | [PubMed](#)
48. Ji YL, Wang H, Meng C, Zhao XF, Zhang C, Zhang Y, Zhao M, Chen YH, Meng XH and Xu DX. **Melatonin alleviates cadmium-induced cellular stress and germ cell apoptosis in testes.** *J Pineal Res*. 2012; **52**:71-9. | [Article](#) | [PubMed](#)
49. Huang F, Ning H, Xin QQ, Huang Y, Wang H, Zhang ZH, Xu DX, Ichihara G and Ye DQ. **Melatonin pretreatment attenuates 2-bromopropane-induced testicular toxicity in rats.** *Toxicology*. 2009; **256**:75-82. | [Article](#) | [PubMed](#)
50. Jalali AS, Hasanzadeh S and Malekinejad H. **Crataegus monogyna aqueous extract ameliorates cyclophosphamide-induced toxicity in rat testis: stereological evidences.** *Acta Med Iran*. 2012; **50**:1-8. | [Article](#) | [PubMed](#)
51. Tripathi DN and Jena GB. **Astaxanthin inhibits cytotoxic and genotoxic effects of cyclophosphamide in mice germ cells.** *Toxicology*. 2008; **248**:96-103. | [Article](#) | [PubMed](#)
52. Ji YL, Wang H, Meng C, Zhao XF, Zhang C, Zhang Y, Zhao M, Chen YH, Meng XH and Xu DX. **Melatonin alleviates cadmium-induced cellular stress and germ cell apoptosis in testes.** *J Pineal Res*. 2012; **52**:71-9. | [Article](#) | [PubMed](#)
53. Jang SS, Kim WD and Park WY. **Melatonin exerts differential actions on X-ray radiation-induced apoptosis in normal mice splenocytes and Jurkat leukemia cells.** *J Pineal Res*. 2009; **47**:147-55. | [Article](#) | [PubMed](#)
54. Tunon MJ, San Miguel B, Crespo I, Jorquera F, Santamaria E, Alvarez M, Prieto J and Gonzalez-Gallego J. **Melatonin attenuates apoptotic liver damage in fulminant hepatic failure induced by the rabbit hemorrhagic disease virus.** *J Pineal Res*. 2011; **50**:38-45. | [Article](#) | [PubMed](#)
55. Take G, Erdogan D, Helvacioğlu F, Goktas G, Ozbey G, Uluoglu C, Yucel B, Guney Y, Hicsonmez A and Ozkan S. **Effect of melatonin and time of administration on irradiation-induced damage to rat testes.** *Braz J Med Biol Res*. 2009; **42**:621-8. | [Article](#) | [PubMed](#)
56. Hussein MR, Abu-Dief EE, Abou El-Ghait AT, Adly MA and Abdelraheem MH. **Morphological evaluation of the radioprotective effects of melatonin against X-ray-induced early and acute testis damage in Albino rats: an animal model.** *Int J Exp Pathol*. 2006; **87**:237-50. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
57. Lena PJ and Subramanian P. **Evaluation of the antiperoxidative effects of melatonin in ammonium acetate-treated Wistar rats.** *Pol J Pharmacol*. 2003; **55**:1031-6. | [Pdf](#) | [PubMed](#)
58. Gutierrez-Cuesta J, Tajas M, Jimenez A, Coto-Montes A, Camins A and Pallas M. **Evaluation of potential pro-survival pathways regulated by melatonin in a murine senescence model.** *J Pineal Res*. 2008; **45**:497-505. | [Article](#) | [PubMed](#)
59. Zhang K, Lv Z, Jia X and Huang D. **Melatonin prevents testicular damage in hyperlipidaemic mice.** *Andrologia*. 2012; **44**:230-6. | [Article](#) | [PubMed](#)
60. Guillete L.J., Pickford D.B., Crain D.A. and Percival H.F. **Reduction in penis size and plasma testosterone concentration in juvenile alligators living**

- in a contaminated environment. *Gen. and Comp. Endocrine*. 1996; **101**: 32-42.
61. Einstein R., Jones R.S., Knifton A. and Srammer G.A. **Principles of veterinary therapeutics**. Singapore, Longman Scientific and technical, 1994: 288-89.
 62. Chaudhary R., Ashish R.S. and Mali P.C. **Reversible contraceptive efficacy and safety evaluation of ethanolic extract of Maytenusemargineta in male albino rats**. *J. pharm. Res*. 2011; **4**:213-16.
 63. Sandeep G, Dheeraj A, Sharma NK, Jhade D and Bharti A. **Effect of plumbagin free alcohol extract of Plumbago zeylanica Linn. root on reproductive system of female Wistar rats**. *Asian Pac J Trop Med*. 2011; **4**:978-84. | [Article](#) | [PubMed](#)
 64. Pineda M. H. and Dooley M.P. McDonald s. **Veterinary Endocrinology and reproduction**. 5th ed. , Iowa state press, A Blackwell publishing company. 2003; **17**:239-56.
 65. Aguilar-Mahecha A, Hales BF and Robaire B. **Chronic cyclophosphamide treatment alters the expression of stress response genes in rat male germ cells**. *Biol Reprod*. 2002; **66**:1024-32. | [PubMed](#)
 66. Nakamura D, Yanagiba Y, Duan Z, Ito Y, Okamura A, Asaeda N, Tagawa Y, Li C, Taya K, Zhang SY, Naito H, Ramdhan DH, Kamijima M and Nakajima T. **Bisphenol A may cause testosterone reduction by adversely affecting both testis and pituitary systems similar to estradiol**. *Toxicol Lett*. 2010; **194**:16-25. | [Article](#) | [PubMed](#)
 67. El-Sokkary GH, Abdel-Rahman GH and Kamel ES. **Melatonin protects against lead-induced hepatic and renal toxicity in male rats**. *Toxicology*. 2005; **213**:25-33. | [Article](#) | [PubMed](#)
 68. Gurpinar T, Ekerbicer N, Uysal N, Barut T, Tarakci F and Tuglu MI. **The effects of the melatonin treatment on the oxidative stress and apoptosis in diabetic eye and brain**. *ScientificWorldJournal*. 2012; **2012**:498489. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
 69. Reiter RJ, Rosales-Corral SA, Manchester LC and Tan DX. **Peripheral reproductive organ health and melatonin: ready for prime time**. *Int J Mol Sci*. 2013; **14**:7231-72. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
 70. Reiter RJ, Tan DX, Gitto E, Sainz RM, Mayo JC, Leon J, Manchester LC, Vijayalaxmi, Kilic E and Kilic U. **Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage**. *Pol J Pharmacol*. 2004; **56**:159-70. | [Pdf](#) | [PubMed](#)
 71. Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, Mayo JC, Kohen R, Allegra M and Hardeland R. **Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger**. *Curr Top Med Chem*. 2002; **2**:181-97. | [Article](#) | [PubMed](#)
 72. Fischer TW, Kleszczynski K, Hardkop LH, Kruse N and Zillikens D. **Melatonin enhances antioxidative enzyme gene expression (CAT, GPx, SOD), prevents their UVR-induced depletion, and protects against the formation of DNA damage (8-hydroxy-20-deoxyguanosine) in ex vivo human skin**. *J. Pineal Res*. 2013; **54**:303-312.
 73. Jin P, Wang X, Chang F, Bai Y, Li Y, Zhou R and Chen L. **Low dose bisphenol A impairs spermatogenesis by suppressing reproductive hormone production and promoting germ cell apoptosis in adult rats**. *J Biomed Res*. 2013; **27**:135-44. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)
 74. Casao A, Cebrian I, Asumpcao ME, Perez-Pe R, Abecia JA, Forcada F, Cebrian-Perez JA and Muino-Blanco T. **Seasonal variations of melatonin in ram seminal plasma are correlated to those of testosterone and antioxidant enzymes**. *Reprod Biol Endocrinol*. 2010; **8**:59. | [Article](#) | [PubMed Abstract](#) | [PubMed FullText](#)

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