Light and Electron Microscopic Study on the Possible Protective Effect of Melatonin on Formaldehyde Induced Testicular Damage in Adult Albino Rats

Original Article

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ABSTRACT

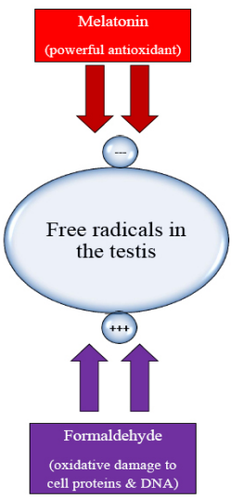
Background: Formaldehyde is a chemical compound used in industrial field and hospitals. Melatonin is an endogenous hormone released by the pineal gland. Melatonin has an antioxidant effects. This work aimed to detect the abnormal histological changes in the testis of adult albino rats induced by formaldehyde and the possible protective role of using a melatonin drug in combination with formaldehyde.

Material and Methods: Thirty adult albino rats weighing 180-220 gm were divided into three groups; Group I (control): Rats of this group were intraperitoneally injected with 5% ethanol once every other day for one month. Group II (Formaldehyde treated group): Rats were intraperitoneally injected with formaldehyde at a dose of 10 mg/kg body weight once every other day for one month. Group III (Formaldehyde and melatonin treated group): Rats were intraperitoneally injected with formaldehyde at a dose of 10mg/kg body weight and after one hour they were intraperitoneally injected with melatonin at a dose of 25 mg/kg body weight. Both drugs were injected once every other day for one month. The rats were sacrified, the testes were dissected, processed for light microscopy using) H&E ,Masson's trichrome, PAS(, electron microscope, morphometric and statistical measurements were also done.

Results: Examination of formaldehyde treated rats revealed thickening of tunica albuginea, tubular degeneration, loss of normal architecture of tubules, marked increase in the collagen fibers deposition, degenerated nuclei with indented and thickened nuclear envelope, ballooned mitochondria, karyolitic nucleus of a head of a sperm. Rats treated with melatonin together with formaldehyde showed obvious improvement in the previous alterations.

Conclusion: It could be concluded that formaldehyde induced histological alterations in the testis of albino rats that could be improved by using melatonin, so, it is essential to supply workers in anatomy department and food industries with melatonin but the human application needs further studies to adjust the dose and the route of administration.

GRAPHICAL ABSTRACT



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Key Words: Formaldehyde, melatonin, testis.

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INTRODUCTION

Formaldehyde is a chemical compound released as a result of the burning process from refineries, power plants and wood stoves. Building materials such as paint, chipboard and varnish also contain formaldehyde. It is used also in industrial fields and hospitals. It naturally occurs in some fruits and it forms endogenously in mammals, including humans, as an end product of the oxidative metabolism. It is used for preserving tissues and embalming cadavers in pathology and anatomy laboratories. Moreover, the compound is used as a preservative, sterilizer in the production of cosmetics and furniture and in preserving food. Formaldehyde enters the body through digestion, respiration, and via the skin. It induces carcinogenicity and mutagenicity by strongly binding to proteins, DNA and RNA. It has been identified to induce degeneration of seminiferous tubules, exfoliation of spermatogenic cells, thickening of basement membrane and increased deposition of collagen fibers in the interstitial tissues between the seminiferous tubules[1,2,3].

Melatonin is an endogenous hormone released by the pineal gland. It has lipophilic and hydrophilic characters, and easily penetrates all biological membranes including the blood-brain barrier. It is known to be involved in a variety of physiological processes such as the regulation of endocrine rhythms and stimulation of the immune function. It is an antioxidant material. So, it has a protective effect against apoptosis and oxidative damage in testicular tissue induced by formaldehyde. Melatonin exerts its protective antioxidant effect via its binding sites located on cell membranes in the reproductive organs[4,5,6,7]. The aim of the present work was to detect the abnormal histological changes in the testes of adult albino rats induced by formaldehyde. Also, another aim was to elucidate the possible protective role of using melatonin drug in combination with formaldehyde.

MATERIAL AND METHODS

Chemicals

1-Formaldehyde: It was available in the form of 40% commercial solution, each rat was injected with 10mg/kg body weight of formaldehyde solution, so each rat was intraperitoneally injected with 5 ml of the solution mixed with 5 ml of drinking water once every other day. 2-Melatonin: It was supplied in the form of 3 mg containing tablets (Natures, Bounty, USA), each tablet was dissolved in 5% ethanol. Melatonin was intraperitoneally injected once every other day, one hour following formaldehyde administration, at a dose of 25 mg/kg body weight.

Animals

Thirty adult male albino rats, locally bred at the animal house of Kasr El-Aini, Faculty of Medicine, Cairo University", Egypt, with an average weight of 180-200 g were used in the present study. The animals were housed at an ambient temperature of 25 ± 1oC, exposed to natural daily light–dark cycles, and had free access to food and water ad libitum. All animal handling and procedures were followed and approved by the ethical committee and the guidelines of Kasr El-Aini animal house. All animal experimental procedures were carried out in accordance with the guidelines of National Institutes of Health for the care and use of Laboratory animals. The rats were divided into three groups 10 rats each:

Group I (Control): Rats of this group were intraperitonially injected with 5% ethanol once every other day for one month.

Group II (Formaldehyde treated group): Rats of this group were intraperitonially injected with formaldehyde at a dose of 10 mg/kg body weight once every other day for one month[8].

Group III (Formaldehyde and melatonin treated group): Rats of this group were intraperitonially injected with formaldehyde at a dose of 10mg/kg body weight once every other day and after one hour they was intraperitonially injected with melatonin at a dose of 25 mg/kg body weight once every other day for one month[8].

A- Histological study

At the end of each experiment, the rats were anesthetized by ketamine 200 mg/kg body weight, intraperitoneally injected[9] and sacrificed. A midline cut was made in the vertical manner reaching out from xiphoid to the pubic symphysis. Skin covering the abdomen alongside the muscles was horizontally reflected. The retractable testes were detached by pushing frontward into the body hole. Testes of all rats were transversely sectioned from midline and submerged in Bouin's fixative for 24 hours. After 24 hours, the pieces of testis were washed for 72 hours with changes of 50% and 70% ethanol to remove picric acid's yellow color. Then the processing of tissues was done in a usual way to dehydrate in ascending grades of alcohol (50, 70, 80, 90 and 100%), clearing by pure xylene.

1-Light microscopic examination

Preparation of paraffin sections: Impregnation of tissues in molten paraffin wax in an automatic processor was done. Blocks of paraffin were formulated. These Blocks were prepared for sectioning at 4-5 microns by the rotary microtome. Sections of samples were collected on glass slides, deparaffinized, and stained by Hematoxylin and Eosin to show the general structure of the tissue, periodic acid Schiff (PAS) to show the glycogen content and Masson's trichrome to visualize the deposition of collagen fibers distribution[10]. Slides were examined by the light electric microscope (LEICA ICC50 W), Faculty of Agriculture, Cairo University.

2- Electron microscopic examination[11,12,13]

Immediately after sacrificing the animals, 10–12 small pieces were fixed in 5% gluteraldehyde for 24 h followed by washing in phosphate buffer (pH 7.2-7.4) three to four times for 20 min. The specimens were then post fixed in 1% osmium tetroxide for 2 h. Then, after that washed in a similar buffer four times for 20 min each. Dehydration was carried out using ascending grades of alcohol

(30, 50, 70, 90, and absolute alcohol) each for 2 h. They were cleared in propylene oxide and then embedded in Epon 812 using a gelatin capsule. These samples were kept in an incubator at 35°C for one day, then at 45°C for another day, and finally at 60°C for three days. Semithin section (about 0.5-1 µm) are cut with the ultramicotome and attained with toluidine blue to be examined by light microscope. Ultrathin sections (50–100 nm) from selected areas of trimmed blocks were prepared and collected on copper grids. The ultrathin sections were then contrasted in uranyle acetate for 10 min and lead citrate for 5min. The grids were examined and photographed using a transmission electron microscope (JEOL JEM-1200 EX II, Japan) operated at 60-70 kV, at (Electron Microscope Unit), pathology department, National Cancer Institute (NCI).

B- Morphometric study using the image analyzer computer system with Leica Qwin 500 software (Cambridge, England) to determine area% of the stained collagen fibers around seminiferous tubules per high-power field. Ten different non overlapping randomly selected fields at a magnification of 400 were examined for each slide.

C- Statistical analysis: The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 22 (SPSS Inc, USA). The mean and standard deviation were calculated. ANOVA (Analysis of variance) was used to test the difference about mean values of measured parameters among groups) to compare different groups with the control group. The results were expressed as mean±SD. The differences were considered statistically significant if probability value *P-value* was less than 0.05 and highly significant if *P value* less than 0.001.

RESULTS

Histological results

Light microscopic results

Group I (control): Light microscopic examination of rat testis specimens stained with haematoxylin and eosin showed normal testicular architecture of semineferous tubules that are lined with germinal epithelium; spermatogonia resting on thin basement membrane together with Sertoli cells, primary spermatocytes. Leydig cells and thin walled small blood vessels can be visualized between semineferous tubules (Figure 1). A semineferous tubule with higher magnification demonstrating different types of germinal epithelial cells spermatogonia, Sertoli cells , spermatocytes and spermatozoa surrounded by thin basement membrane (Figure 2). Masson's trichrome stained sections visualized a normal pattern of collagen deposition; a thin layer of collagen fibers around the blood vessels and between the semineferous tubules (Figure 3). PAS stained sections of rat testis of the same group featured germinal epithelium of semineferous tubules resting on thin regular basement membrane (Figure 4). Toluidine blue stained sections displayed a semineferous tubule with higher magnification demonstrating different types of germinal epithelial cells; spermatogonia type B, spermatogonia type A dark cells, spermatogonia type A light cells, Sertoli cells, primary spermatocytes, rounded spermatids, heads of sperms and spermatozoa surrounded by thin basement membrane with flattened nuclei of myoid cells (Figure 5).

Electron microscopic examination of rat testis specimens of the same group revealed nuclei of spermatogonia type A light cells and spermatogonia type B cells with prominent nucleoli resting on thin basal lamina. Both types exhibit mitochondria with intact cristae (Figure 6). Nuclei of spermatogonia type A dark cells and Sertoli cells with prominent nucleoli resting on thin basal lamina . Both types exhibit mitochondria with intact cristae (Figure 7). Nuclei of primary spermatocytes, rounded spermatids. Both types exhibit mitochondria with intact cristae and smooth endoplasmic reticulum (Figure 8). Nuclei of rounded spermatids with vesicular cap, nuclei, of sperms surrounded by sheath. Tails of sperms can be observed (Figure 9)

Group II (Formaldehyde treated group): Light microscopic examination of rat testis specimens stained with haematoxylin and eosin showed markedly thickened fibrous capsule, separation of germinal epithelium from the basement membrane and tissue exudates inbetween the tubules (Figure 10). The semineferous tubules exhibit: exfoliation of germinal epithelium into the central part of semineferous tubules, vacuolated cytoplasm and disrupted basement membrane (Figure 11). Completely disturbed architecture of semineferous tubules and absent spermatozoa, exfoliation of germinal epithelium into the central part of semineferous tubules with mononuclear cell infiltration, extravasated blood inbetween the tubules. Disrupted basement membrane can be observed (Figure 12). Separation of germinal epithelium from the basement membrane and degenerated germinal epithelium, completely disturbed architecture of semineferous tubles and a markedly thickened congested blood vessel (Figure 13). Masson's trichrome stained sections visualized marked increase in the collagen fibers between semineferous tubules and around the blood vessels (Figures 14,15). PAS stained sections of rat testis of the same group featured germinal epithelium of semineferous tubules resting on markedly thickened basement membrane (Figure 16). Toluidine blue stained sections displayed extremely degenerated germinal epithelium of a semineferous tubule with moderately thickened basement membrane (Figure 17). Electron microscopic examination of rat testis specimens of the same group revealed extremely degenerated germinal epithelium of a semineferous tubule exhibit: degenerated nuclei with indented nuclear envelope, ballooned mitochondria with lost cristae and a markedly thickened basal lamina (Figure 18). Extremely degenerated germinal epithelium of a semineferous tubule exhibit: degenerated nuclei with indented and thickened nuclear envelope, ballooned mitochondria with lost cristae , increased electron dense granules and karyolitic nucleus of a head of a sperm (Figure 19).

Group III (Formaldehyde and melatonin treated group): Light microscopic examination of rat testis specimens stained with haematoxylin and eosin showed apparently normal testicular architecture- semineferous tubules lined with germinal epithelium; spermatogonia resting on thin basement membrane together with Sertoli cells , primary spermatocytes and spermatozoa. Leydig cells and thin walled small blood vessels could be visualized between semineferous tubules (Figure 20). A semineferous tubule presents different types of germinal epithelial cells; spermatogonia type B, spermatogonia type A dark cells, spermatogonia type A light cells, Sertoli cells, primary spermatocytes, rounded spermatids and spermatozoa surrounded by thin basement membrane with flattened nuclei of myoid cells (Figure 21). Normal architecture of semineferous tubules with abundant spermatozoa. Few tubules present abundant spermatozoa with exfoliated germinal epithelium into the central part of the semineferous tubule (Figure 22). Thin fibrous capsule, tissue exudates in between the tubules that appear either with almost normal architecture or with separated germinal epithelium.Most of tubules display abundant spermatozoa (Figure 23). Masson's trichrome stained sections visualized marked decrease in collagen tissue deposition between semineferous tubules and around blood vessels (Figure 24). PAS stained sections of rat testis of the same group featured germinal epithelium of semineferous tubules resting on a thin regular basement membrane (Figure 25). Toluidine blue stained sections displayed a semineferous tubule that presents different types of germinal epithelial cells; spermatogonia type B, spermatogonia spermatogonia type A light cells, Sertoli cells, primary spermatocytes, rounded spermatids and heads of sperms surrounded by thin basement membrane with flattened nuclei of myoid cells (Figure 26). Electron microscopic examination of rat testis specimens of the same group revealed apparently normal nuclei of spermatogonia type A light cells, spermatogonia type A dark cells, primary spermatocytes, and rounded spermatids resting on mildly thickened basal lamina. Both types exhibit mitochondria with intact cristae (Figure 27). Apparently normal nuclei of rounded spermatids, heads of sperms surrounded by sheath (Figure 28). Apparently normal nuclei of: a primary spermatocyte , Sertoli cells with prominent nucleolus and electron dense granules resting on mildly thickened basal lamina (Figure 29).

Statistical analysis of data

For interpretation of results of tests of significance, significance was adopted at $P \leq 0.05$. Collagen area % was statistically significantly higher in group II (formaldehyde) (13.08 ± 2.2) compared to control (4.26 ± 0.99) and group III (formaldehyde & melatonin)(5.56 ± 1.52), $p < 0.0001$. There was no statistically significant difference between the control and group III (formaldehyde & melatonin) (p = 0.449) (Table1, Histogram1).

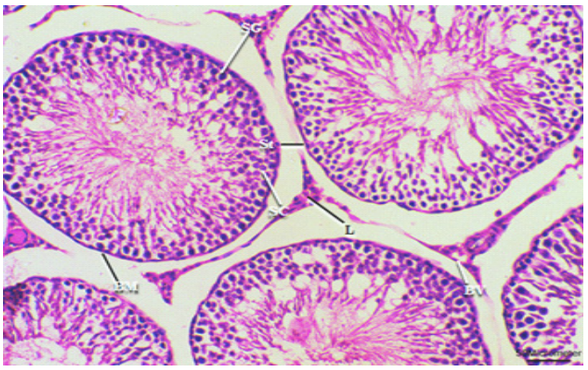


Fig. 1: A photomicrograph of a testicular section of a control rat showing normal testicular architecture of semineferous tubules that are lined with germinal epithelium; spermatogonia (SG) resting on thin basement membrane (BM) together with Sertoli cells (St), primary spermatocytes (SC) and spermatozoa (SZ). Leydig cells (L) and thin walled small blood vessels (BV) can be visualized between semineferous tubules (H & E. X200).

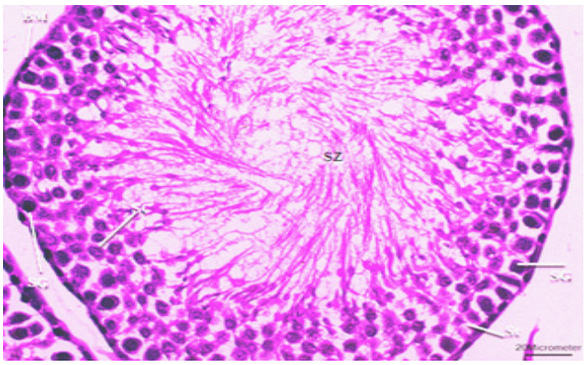


Fig. 2: A photomicrograph of a testicular section of a control rat showing a semineferous tubule with higher magnification demonstrating different types of germinal epithelial cells; spermatogonia type B (B), spermatogonia type A dark cells (AD), spermatogonia type A light cells (AL), Sertoli cells (St), primary spermatocytes (SC), rounded spermatids (RS) and spermatozoa (SZ) surrounded by thin basement membrane (BM) with flattened nuclei of myoid cells (My) (H & E. X400).

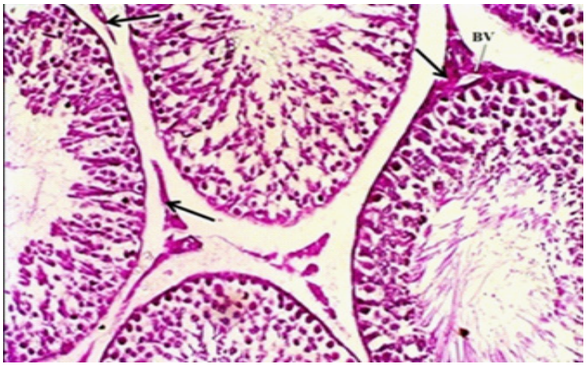


Fig. 3: A photomicrograph of a testicular section of a control rat showing a normal pattern of collagen deposition;a thin layer of collagen fibers (arrows) around the blood vessels (BV) and between the semineferous tubules (Masson trichrome stain x200).

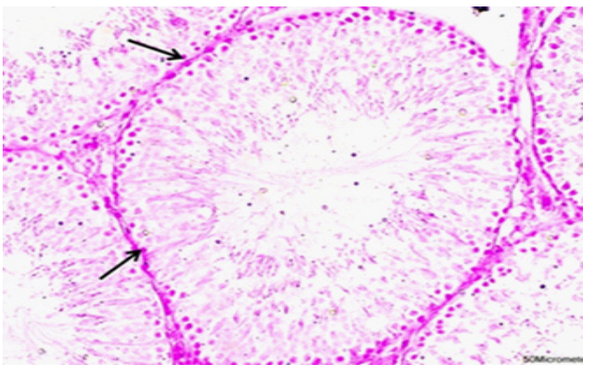


Fig. 4: A photomicrograph of a testicular section of a control rat showing germinal epithelium of semineferous tubules resting on thin regular basement membrane (arrow) (PAS X200).

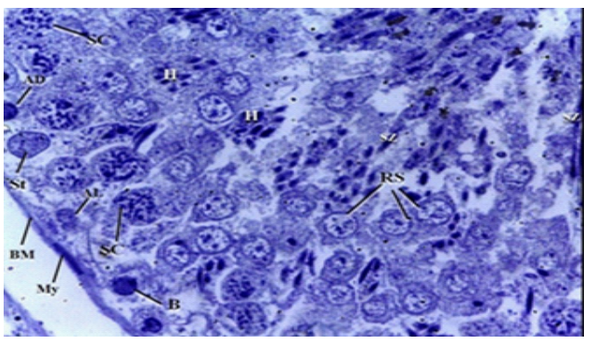


Fig. 5: A photomicrograph of a testicular section of a control rat showing a semineferous tubule with higher magnification demonstrating different types of germinal epithelial cells; spermatogonia type B (B), spermatogonia type A dark cells (AD), spermatogonia type A light cells (AL), Sertoli cells (St), primary spermatocytes (SC), rounded spermatids (RS), heads of sperms (H) and spermatozoa (SZ) surrounded by thin basement membrane (BM) with flattened nuclei of myoid cells (My) (Toluidine blue X1000)

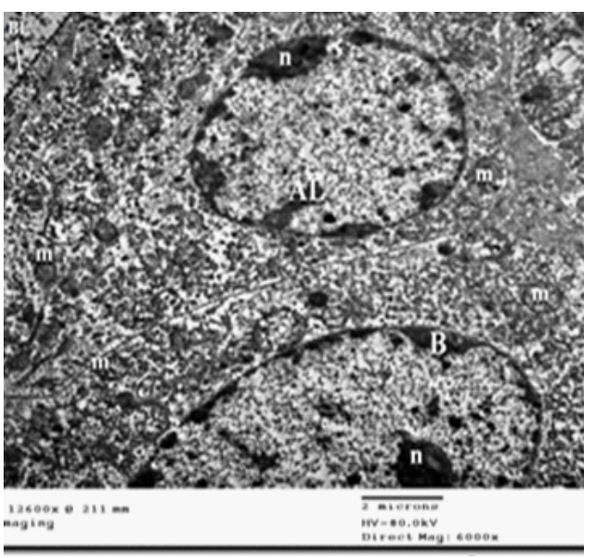


Fig. 6: An electron photomicrograph of a testicular section of a control rat displaying nuclei of spermatogonia type A light cells (AL) and spermatogonia type B cells (B) with prominent nucleoli (n) resting on thin basal lamina (BL). Both types exhibit mitochondria with intact cristae (m) (EM X 6000).

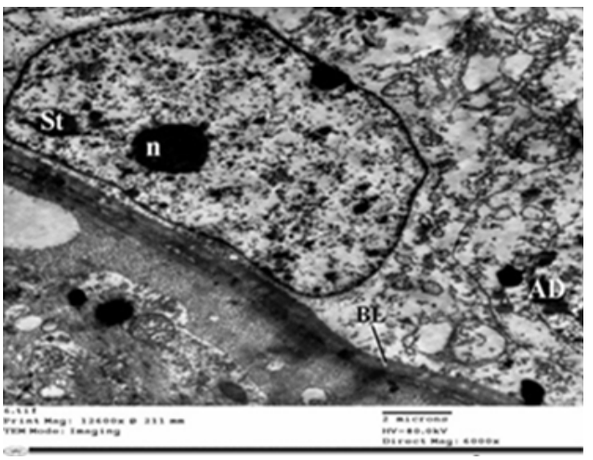


Fig. 7: An electron photomicrograph of a testicular section of a control rat displaying nuclei of spermatogonia type A dark cells (AD) and Sertoli cells (St) with prominent nucleoli (n) resting on thin basal lamina (BL). Both types exhibit mitochondria with intact cristae (m) (EMX 6000).

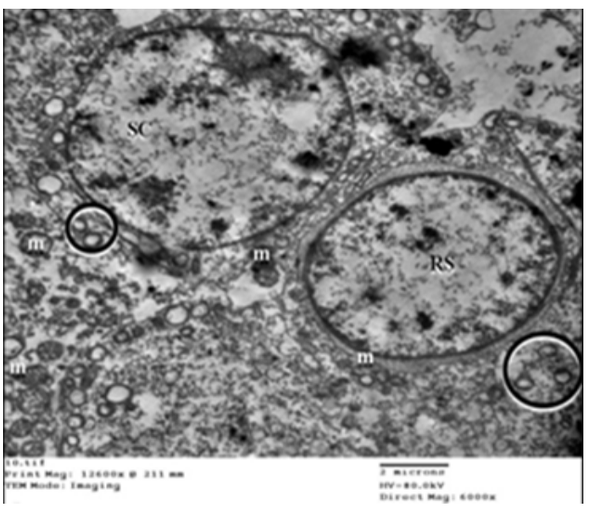


Fig. 8: An electron photomicrograph of a testicular section of a control rat displaying nuclei of primary spermatocytes (SC), rounded spermatids (RS). Both types exhibit mitochondria with intact cristae (m) and smooth endoplasmic reticulum (circle) (EMX 6000).

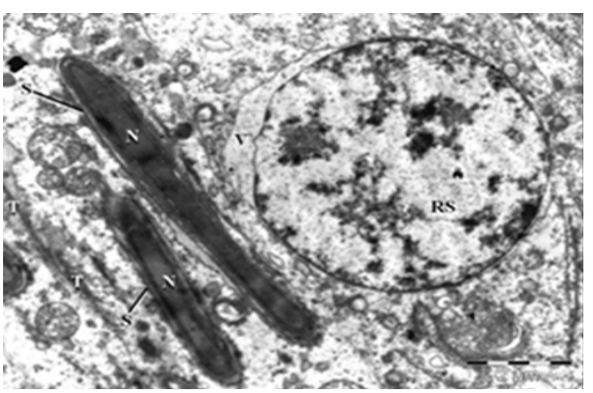


Fig. 9: An electron photomicrograph of a testicular section of a control rat displaying nuclei of rounded spermatids (RS) with vesicular cap (V), nuclei (N) of sperms surrounded by mitochondrial sheath (S). Tails (T) of sperms can be observed (EM X 5600).

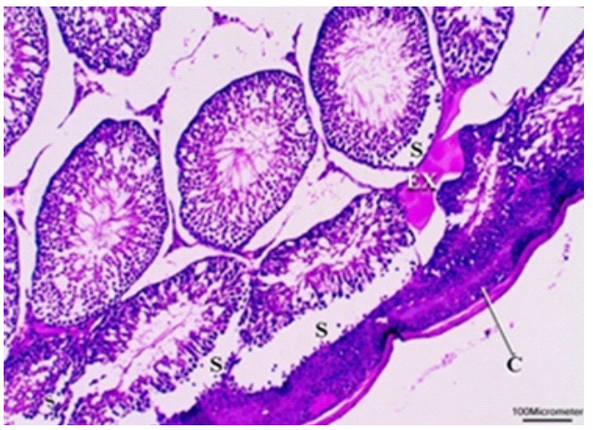


Fig. 10: A photomicrograph of a testicular section from group II (Formaldehyde treated group) showing markedly thickened fibrous capsule (C) , separation of germinal epithelium from the basement membrane (S) and tissue exudates (EX) inbetween the tubules (H & E X100).

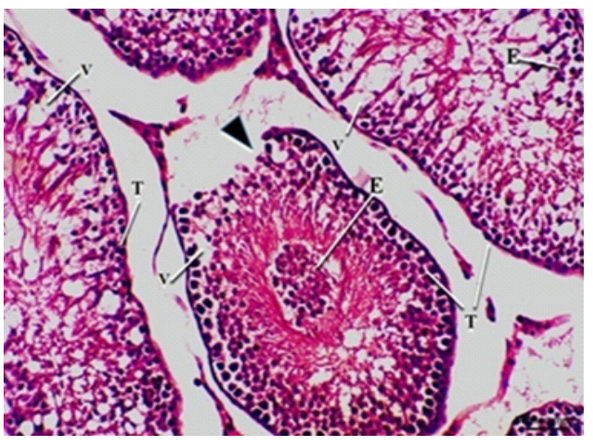


Fig. 11: A photomicrograph of a testicular section from group II showing semineferous tubules (T) that exhibit: exfoliation (E) of germinal epithelium into the central part of semineferous tubules, vacuolated cytoplasm (V) and disrupted basement membrane (arrowhead). (H & E X200).

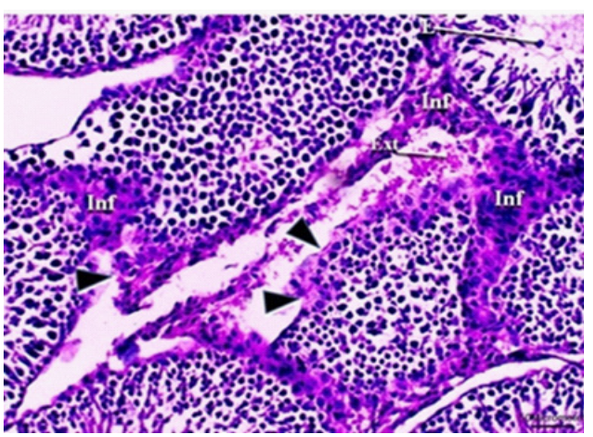


Fig. 12: A photomicrograph of a testicular section from group II showing completely disturbed architecture of semineferous tubules and absent spermatozoa, exfoliation (E) of germinal epithelium into the central part of semineferous tubules with mononuclear cell infiltration (Inf), extravasated blood (Ext) in between the tubules. Disrupted basement membrane (arrowhead) can be observed (H & E X200).

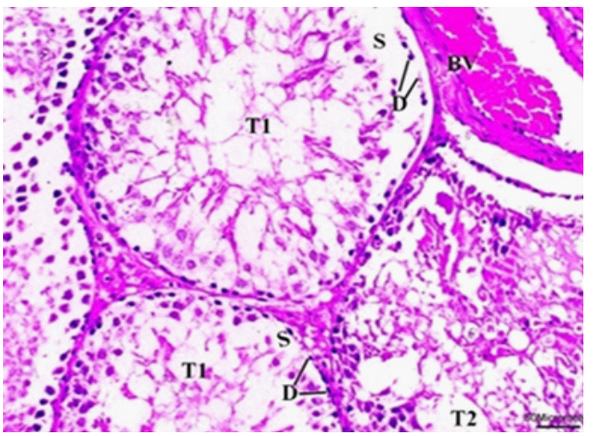


Fig. 13: A photomicrograph of a testicular section from group II showing (T1): that present separation of germinal epithelium from the basement membrane (S) and degenerated germinal epithelium (D), (T2): that displays completely disturbed architecture of semineferous tubles and a markedly thickened congested blood vessel (BV) (H & E X200).

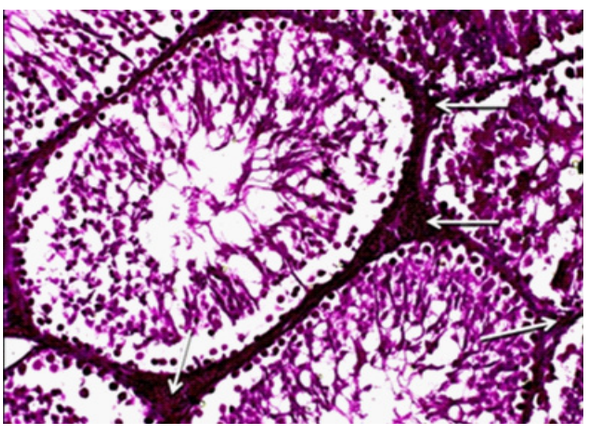


Fig.14: A photomicrograph of a testicular section from group II displaying marked increase in the collagen fibers (arrows) between semineferous tubules (Masson trichrome stain x200)

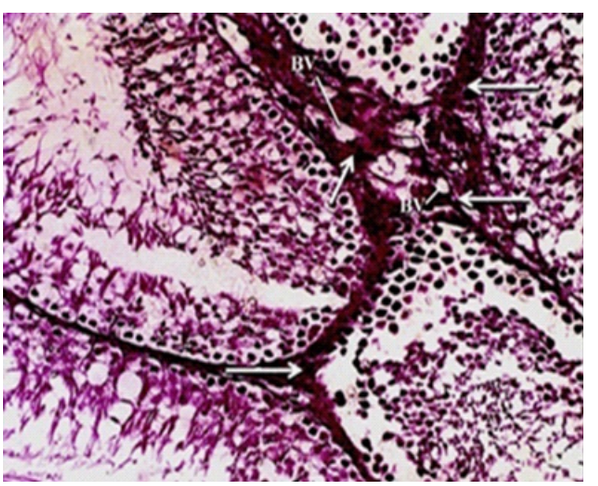


Fig. 15: A photomicrograph of a testicular section from group II displaying marked increase in the collagen fibers (arrows) between semineferous tubules and around the blood vessels (BV)(Masson trichrome stain X200)

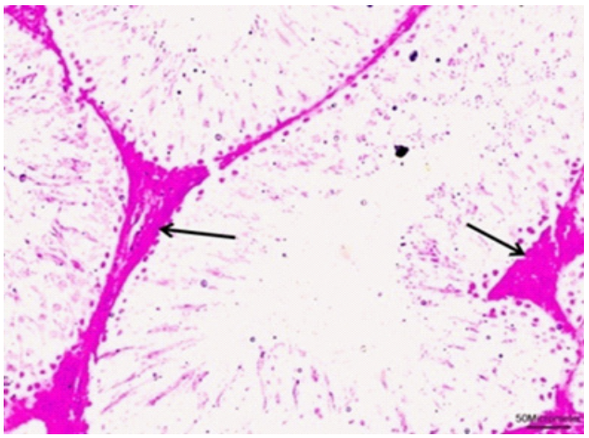


Fig. 16: A photomicrograph of a testicular section from group II showing germinal epithelium of semineferous tubules resting on markedly thickened basement membrane (arrow) (PAS X200).

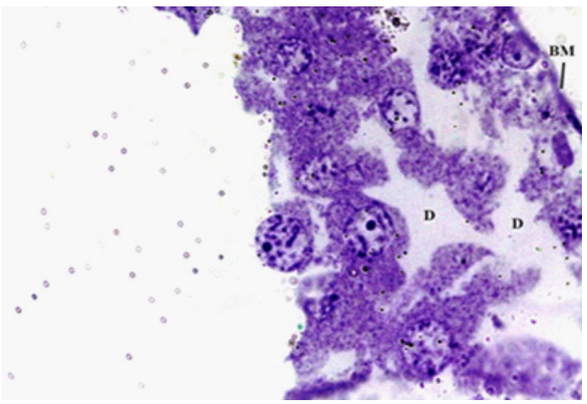


Fig. 17: A photomicrograph of a testicular section from group II showing extremely degenerated germinal epithelium of a semineferous tubule (D) with moderately thickened basement membrane (BM) (Toluidine blue X1000)

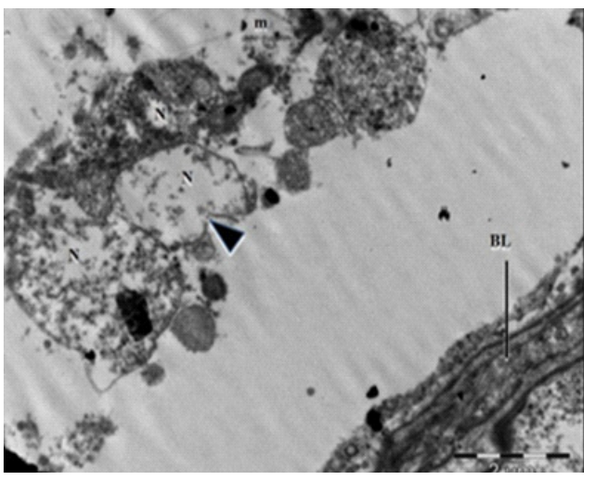


Fig. 18: An electron photomicrograph of a testicular section from group II displaying extremely degenerated germinal epithelium of a semineferous tubule that exhibit: degenerated nuclei (N) with indented nuclear envelope (arrowhead), ballooned mitochondria with lost cristae (m) and a markedly thickened basal lamina (BL) (EM X 5600).

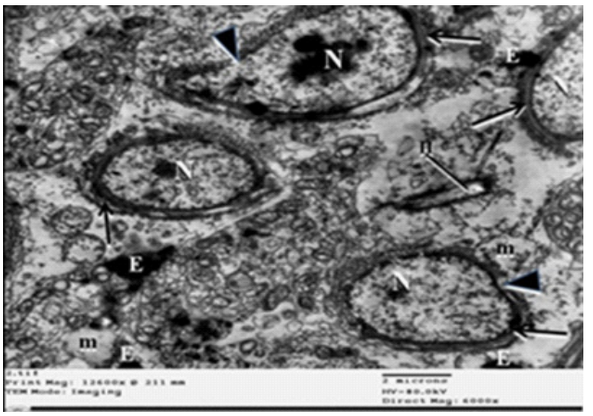


Fig. 19: An electron photomicrograph of a testicular section from group II displaying extremely degenerated germinal epithelium of a semineferous tubule that exhibit: degenerated nuclei (N) with indented (arrowhead) and thickened (arrow) nuclear envelope, ballooned mitochondria with lost cristae (m), increased electron dense granules (E) and karyolitic nucleus of a head of a sperm (H) (EM X 6000).

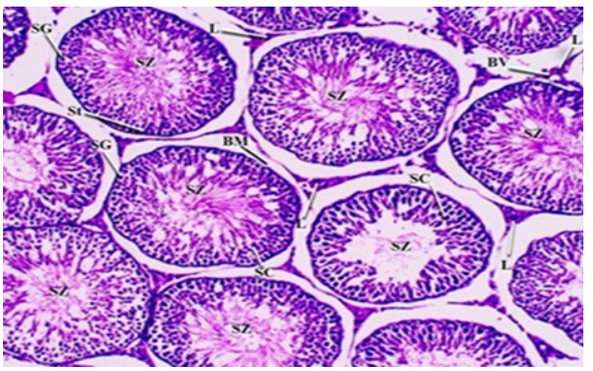


Fig. 20: A photomicrograph of a testicular section from group III (Formaldehyde and melatonin treated group) showing apparently normal testicular architecture- semineferous tubules lined with germinal epithelium; spermatogonia (SG) resting on thin basement membrane (BM) together with Sertoli cells (St), primary spermatocytes (SC) and spermatozoa (SZ). Leydig cells (L) and thin walled small blood vessels (BV) could be visualized between semineferous tubules (H & E. X200).

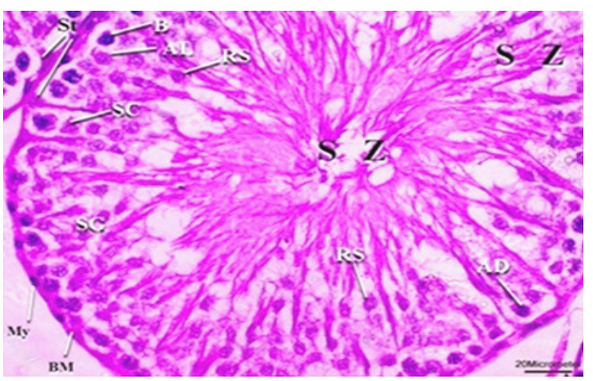


Fig. 21: A photomicrograph of a testicular section from group III showing a semineferous tubule that presents different types of germinal epithelial cells; spermatogonia type B (B), spermatogonia type A dark cells (AD), spermatogonia type A light cells (AL), Sertoli cells (St), primary spermatocytes (SC), rounded spermatids (RS) and spermatozoa (SZ) surrounded by thin basement membrane (BM) with flattened nuclei of myoid cells (My) (H & E. X400).

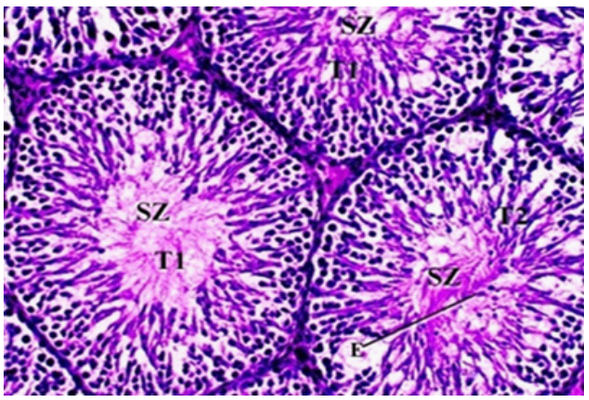


Fig. 22: A photomicrograph of a testicular section from group III showing apparently normal architecture of semineferous tubules (T1) with abundant spermatozoa (SZ). Few tubules (T2) present abundant spermatozoa (SZ) with exfoliated germinal epithelium (E) into the central part of the semineferous tubule (H & E X200).

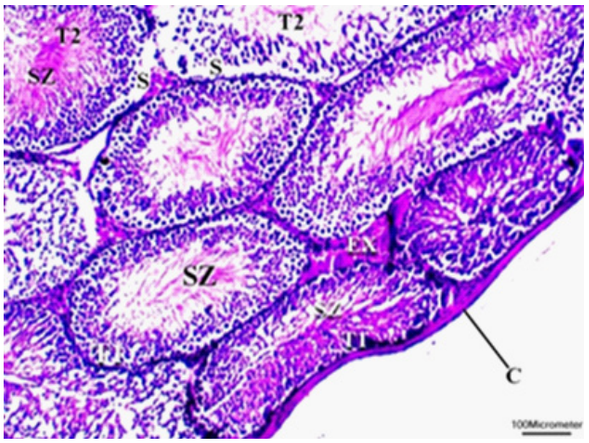


Fig. 23: A photomicrograph of a testicular section from group III showing thin fibrous capsule (C), tissue exudates (EX) inbetween the tubules that appears either with almost normal architecture (T1) or with separated germinal epithelium (S). Note most of tubules display abundant spermatozoa (SZ) (H & EX100).

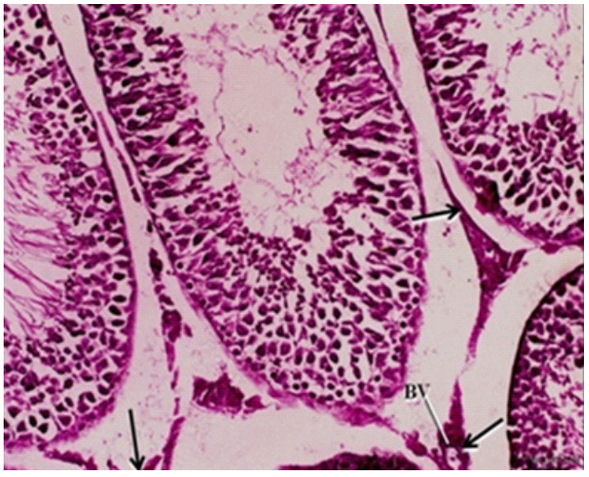


Fig. 24: A photomicrograph of a testicular section from group III showing marked decrease in collagen tissue deposition between semineferous tubules (arrows) and around blood vessels (BV) (Masson trichrome stain x200).

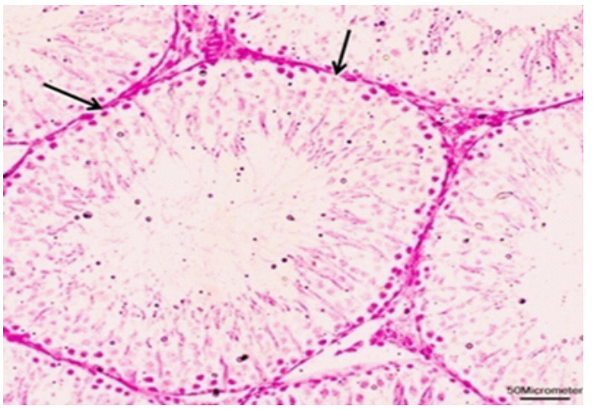


Fig. 25: A photomicrograph of a testicular section from group III showing germinal epithelium of semineferous tubules resting on a thin basement membrane (arrow) (PAS X200).

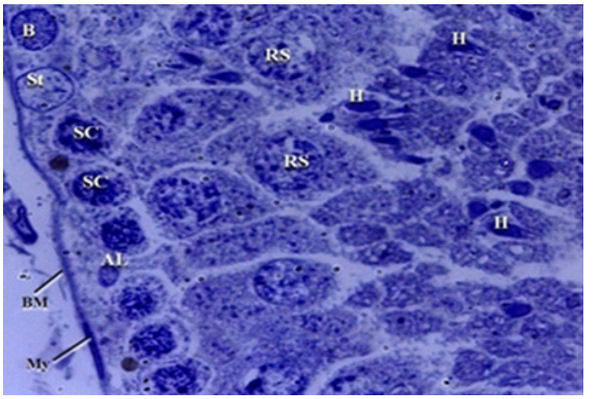


Fig. 26: A photomicrograph of a testicular section from group III showing a semineferous tubule that presents different types of germinal epithelial cells; spermatogonia type B (B), spermatogonia spermatogonia type A light cells (AL), Sertoli cells (St), primary spermatocytes (SC), rounded spermatids (RS) and heads of sperms (H) surrounded by thin basement membrane (BM) with flattened nuclei of myoid cells (My) (Toluidine blueX1000)

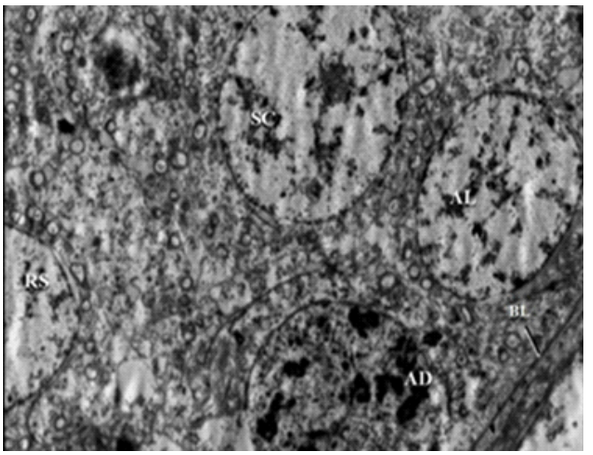


Fig. 27: An electron photomicrograph of a testicular section from group III displaying apparently normal nuclei of spermatogonia type A light cells (AL), spermatogonia type A dark cells (AD), primary spermatocytes (SC), and rounded spermatids (RS) resting on mildly thickened basal lamina (BL). Both types exhibit mitochondria with intact cristae (m) (EMX 3500).

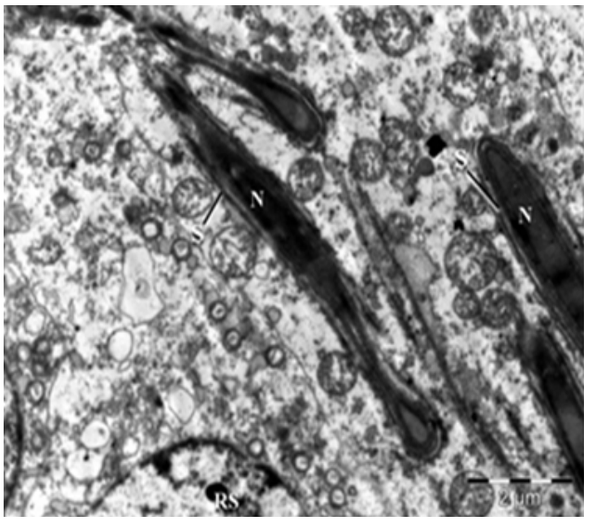


Fig. 28: An electron photomicrograph of a testicular section from group III displaying apparently normal nuclei of rounded spermatids (RS), heads of sperms (N) surrounded by mitochondrial sheath (S) (EM X 5600).

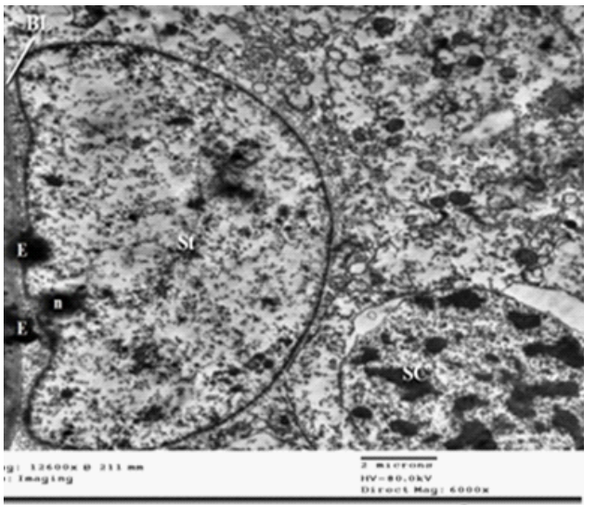
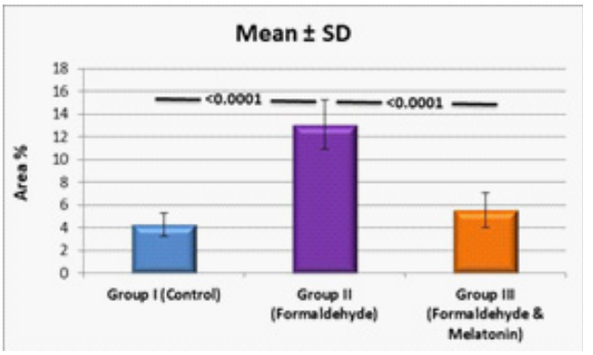


Fig. 29: An electron photomicrograph of a testicular section from group III displaying apparently normal nuclei of: a primary spermatocyte (SC), Sertoli cells (St) with prominent nucleolus (n) and electron dense granules resting on mildly thickened basal lamina (BL). (EM X 6000).



Histogram 1: Mean distribution of the connective tissue (area %) between the seminiferous tubules of the testes obtained from the different groups of the examined animals.

Table 1: A table of area percent of collagen fibers in rat testis obtained from different groups of the examined animals.

	Group I (Control)		Group II (Formaldehyde)		Group III (Formaldehyde & Melatonin)	
	Mean	SD	Mean	SD	Mean	SD
Area %	4.26	0.99	13.08	2.2	5.56	1.52
P-values						
G I vs. G II	<0.0001 (S)					
G II vs. G III			<0.0001 (S)			
G I vs. G III					0.449 (NS)	

DISCUSSION

In the present work, the testis of the group II treated with formaldehyde revealed that there was a thickening of basement membrane and degeneration of seminiferous tubules. Similar results were observed by Jang *et al*.,[14] who exposed the experimental animals to formaldehyde vapor (10 mg/m3 for two weeks). In the current work exfoliation of of spermatogenic cells was found in rat testes of group II. This finding was in accordance with Ulucam and Bakar[3] who attributed the sloughing of germinal epithelium and presence of cellular debris in the lumen of seminiferous tubules to the interruption of intercellular bridge. Another mechanism was described by Sapmaz *et al*.,[2] who explained presence of cells in this site was due to karyokinesis. Mirhoseini *et al*.,[15] attributed decreased spermatogenesis to apoptosis. In the current study, group II showed vacuolization of germinal epithelium. This was in agreement with Sapmaz *et al*.,[16] who stated that long term exposure to formaldehyde resulted in degeneration and extensive cytoplasmic vacuolation of germinal epithelium. Examination of rat testes of group II revealed apparently normal Leydig cells. This was in contrast to Take *et al*.,[17] who observed degeneration of Leydig cells in their study. In the current study, PAS stained sections revealed thickening of basement membranes of semineferous tubules in rats treated with formaldehyde (group II). However, in group III examination of rat testis specimens revealed decreased thinking of the basement membrane of the tubules. This was in accordance with Salehinezhad *et al*.,[18] who attributed that to the powerful antioxidant and anti-inflammatory effects of melatonin. Masson's trichrome-stained sections in the formaldehyde-treated animals showed increased deposition of collagen fibers in the interstitial tissues between the seminiferous tubules and around blood vessels. This was in agreement with Rocha *et al*.,[19] who stated that formation of reactive oxygen species resulted in lipid peroxidation with consequent damage to cell proteins and increase in collagen tissue deposition, Golipour *et al*.,[20] also explained that presence of fibrosis was due the process of aging or might be related to infertility.

Light and ultrastructural microscopic examination of rat testes of group III (Formaldehyde and melatonin administration group) revealed the beneficial effect of melatonin as a powerful antioxidant; it ameliorated all the histological alterations produced by formaldehyde injection; degenerated seminefrous tubules, cytoplasmic vacuolation,

degeneration of Sertoli and Leydig cells and tissue fibrosis. This was in agreement with a study performed by Srinivasan *et al*.,[21] and Vosoughi *et al*.,[22] who reported that melatonin could protect the testis against the harmful oxidative damage and cell degeneration produced by injection of formaldehyde to adult male albino rats. They attributed its antioxidant effect to prevention of lipid peroxidation and increased levels of antioxidant enzymes e.g. superoxide dismutase. This was supported also by Ji *et al*.,[23] who observed action of melatonin in inhibition of degeneration of hepatocytes due to malaria. In addition, the investigators[24,25] stated that melatonin had a beneficial role in amelioration of germinal epithelial apoptosis in rat testes treated with cadmium. Mirhoseini *et al*.,[7] demonstrated the effect of melatonin in repairing injury to seminiferous tubules caused by torsion detorsion of the spermatic cord in adult male albino rats. Golipour *et al*.,[20] observed also in their study the beneficial role of melatonin in management of testicular trauma. Melatonin has been reported to prevent apoptosis in different tissues e.g. cerebral cortex degeneration due to iron and cyanic acid damage of rat hippocampus[26,27].

CONCLUSION AND RECOMMENDATIONS

It could be concluded that formaldehyde induced histological alterations in the testis of albino rats that could be improved by using melatonin, so, it is essential to supply workers in anatomy department and food industries with melatonin but the human application needs further studies to adjust the dose and the route of administration.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

دراسة مجهرية ضوئية وإلكترونية حول التأثير الوقائي المحتمل للميلاتونين على تلف الخصية المستحث بالفورمالدهايد في الجرذان البيضاءالبالغه

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المقدمة: الفورمالدهايد هو ماده كيميائيه تستخدم فى المجالات الصناعية والمستشفيات. الميلاتونين هو هورمون يفرز من الغدة الصنوبرية كما ان للميلاتونين تأثير مضاد للأكسدة. يهدف هذا العمل الي توضيح التغيرات النسيجيه لخصية الفأر الأبيض البالغ الناتجه عن الفورمالدهيد و التاثير الوقائى المحتمل للميلاتونين عند اعطاؤه مع الفورمالدهيد.

مواد وطرق الدراسة: تم استخدام ثلاثون من الجرذان البيضاء البالغة تزن 220-180 جم, قسمت الى ثلاث مجموعات. المجموعه الضابطة: تم اعطاؤها 5% ايثانول بالحقن البريتوني يوم بعد يوم لمده شهر، مجموعات ثانية(المعالجه بالفورمالدهيد) تم اعطاؤها الفورمالدهايد بالحقن البريتوني 10مج/كجم يوم بعد يوم لمده شهر ، مجموعات ثالثة (المعالجه بالفورمالدهيد والميلاتونين) تم اعطاؤها كل من الفورمالدهايد بالحقن البريتوني 10مج/كجم ووبعد ساعه تم اعطاؤها الميلاتونين بالحقن البريتونى 25مج/كجم ,كلاهما يوم بعد يوم لمده شهر.تم التضحية بالفئران,استئصال الخصي وجهزت للفحص بالميكرسكوب الضوئي باستخدام الهيماتوكسيلين والايوسين والماسون ثلاثى اللون وحمض شيف الدوري وللفحص بالمجهر الالكتروني وتم ايضا اجراء دراسه مورفوميتريه واحصائيه.

النتائج: عند فحص الفئران المعالجه بالفورمالدهيد وجد زيادة سمك الطبقة الليفية التى تغطى الخصية و تليف الخصية من الداخل و تدمير الخلايا المبطنة لقنوات الخصية و تدمير النواة و الميتوكوندريا و الشبكة الانوبلازمية و كذلك ظهور اشكال غريبة للحيوانات المنوية الانه قد وجد تحسن ملحوظ في التغيرات المسبقه في الفئران المعالجه بالميلاتونين والفورمالدهيد.

الخلاصه: اوضحت هذه الدراسة ان الفورمالدهايد ينتج تغيرات نسيجيه في خصيه الفار الابيض والتي من الممكن ان تتحسن باستخدام الميلاتونين لذ ا من الضروري اعطاء العمال في اقسام التشريح ومصانع الاغذ يه الميلاتونين بينما التطبيق البشري يحتاج للمزيد من الدراسات لضبط الجرعه وطريقه الاستخدام.