ORIGINAL ARTICLE

Melatonin's Potential Protection against Testicular Damage Caused by Formaldehyde

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Abstract

Background: Hospitals and industrial settings both use the chemical molecule formaldehyde. The pineal gland releases the endogenous hormone melatonin. There are antioxidant properties of melatonin. **Aim of Study:** This study sought to identify the aberrant histological alterations in adult albino rats' testicles brought on by formaldehyde as well as any potential preventive benefits of taking a melatonin medication in addition to formaldehyde. **Material and Methods:** Three sets of 30 mature rats weighing between 190 and 210 g were created; Group I (control): For a month, rats in this group received intraperitoneal injections of 5% ethanol once every 2 days. Group II (Group treated with formaldehyde): For a month, rats received intraperitoneal injections of formaldehyde at a dose of 10 mg-kg BW 1 time every other day. The last group, which was treated with both melatonin and formaldehyde, involved injecting rats intraperitoneally with 10 mg-kg BW of formaldehyde and 25 mg/kg BW of melatonin an hour later. For a month, both medications were administered once every other day. The testes were removed from the rats, and prepared for light microscopy using hematoxylin and eosin. **Results:** Rats given formaldehyde showed tubular degeneration, thicker tunica albuginea, loss of normal tubule architecture, degenerated nuclei with thickened and indented nuclear envelope, and a karyolitic nucleus. Rats given both formaldehyde and melatonin treatment showed a clear improvement in the prior changes. **Conclusion:** It is necessary to provide melatonin to employees in the anatomy department and food industries because it was found that formaldehyde caused histological changes in the testis. These changes were improved by the use of melatonin. However, more research is needed to determine the appropriate dosage and administration method for humans.

Key words: Formaldehyde, histology, melatonin, rats, testis

INTRODUCTION

Formaldehyde is a chemical compound produced by burning wood stoves, power plants, and refineries. In addition, formaldehyde is included in building supplies such as paint, chipboard, and varnish. Hospitals and industrial settings also employ it. Some fruits naturally contain it, whereas humans and other mammals produce it endogenously as a byproduct of oxidative metabolism.[1] In pathology and anatomy laboratories, it is used to preserve tissues and embalm cadavers. In addition, the chemical is employed as a sterilizer and preservative in the manufacturing of furniture, cosmetics, and food preservation. Through the skin, respiration, and digestion, formaldehyde enters the body. It binds firmly to proteins, DNA, and RNA to cause mutagenicity and carcinogenicity.[2] It has been shown to cause spermatogenic cell exfoliation, basement membrane thickening, seminiferous tubule degeneration, and increased collagen fiber deposition in the tissues that lie between the seminiferous tubules.[2] The pineal gland releases the endogenous hormone melatonin. It readily passes across all biological membranes, including the blood–brain barrier, and possesses lipophilic and hydrophilic properties.[3] Numerous physiological systems, including the stimulation of the

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immune system and the control of endocrine rhythms, are known to be impacted by it. The substance is an antioxidant. Thus, it protects testicular tissue from oxidative damage and apoptosis brought on by formaldehyde. Melatonin uses its binding sites on reproductive organ cell membranes to produce its protective antioxidant activity.[4] The current study set out to identify any aberrant histological alterations brought on by formaldehyde in the testes of rats. Clarifying the potential protective function of combining the melatonin medication with formaldehyde was another goal.

MATERIALS AND METHODS

Formaldehyde

Available as a 40% commercial solution, each rat received an intraperitoneal injection of 5 ml of the diluted solution with (5 ml) of water 1 time every other day at a dose of 10 (mg-kg-BW). Each 3 mg tablet of melatonin was dissolved in 5% ethanol before being administered. A dose of 25 mg-kg-BW of melatonin was administered intraperitoneally once every other day, 60 min after formaldehyde injection. In this study, 30 mature rats weighing (190–210 g) on average were produced locally at the animal house. The rats were kept at a constant temperature, were subjected to light-dark cycles that occur naturally every day, and were given unlimited access to food and water. The Ethics Committee and PSA University's requirements were followed and authorized for all animal handling and treatments. Every animal experiment was conducted in compliance with the Health's National Institute of Standards for the use and care of laboratory animals. Three groups of 10 rats each were created from the rats: Group I (control): For a month, rats in this group received intraperitoneal injections of (5%) ethanol 1 time every other day. In addition; Group II (group treated with formaldehyde): For a month, rats in this group received intraperitoneal injections of formaldehyde at a dose of (10 mg-kg BW) 1 time every other day.

Rats in Group III (group treated with formaldehyde and melatonin) received intraperitoneal injections of formaldehyde (10 mg-kg BW) once every other day for 1 month, followed by intraperitoneal injections of melatonin (25 mg-kg BW) once every other day for an hour. The rats were given an intraperitoneal injection of ketamine at a dose of (200 mg-kg BW) to induce anesthesia, and they were then slaughtered at the conclusion of each experiment. A vertical midline cut was made that extended from the pubic symphysis to the xiphoid. The muscles and skin covering the abdomen were reflected horizontally. Pushing forward into the body hole separated the retractable testes.

Every rat's testes were cut transversely from the midline and immersed in Bouin's fixative for a full day. To get rid of the yellow tint from picric acid, the testis parts were cleaned for 72 h using 50% and 70% ethanol changes after 24 h. After that, tissues were processed as usual to dry them using increasing alcohol grades and then cleared with pure xylene. Paraffin section preparation involved impregnating tissues with melted paraffin wax using an automated processor. Paraffin blocks were created. The rotary microtome was used to prepare these blocks for sectioning at 4–5 microns. To display the overall structure of the tissue, sample sections were gathered and stained with hematoxylin and eosin.

RESULTS

The first control group specimens examined with hematoxylin and eosin under a light microscope revealed the normal architecture of the testis, including seminiferous tubules coated with germinal epithelium, and spermatogonia sitting on a thin basement membrane. The Sertoli cells and primary spermatocytes were seen. In between seminiferous tubules, Leydig cells, and tiny blood veins with thin walls are visible [Figure 1].

The second group (formaldehyde-treated group): specimens examined with hematoxylin and eosin under a light microscope revealed tissue exudates between the tubules, a significantly thicker fibrous capsule, and the separation of the germinal epithelium from the basement membrane. Vacuolated cytoplasm, a perforated basement membrane, and exfoliation of the germinal epithelium into the tubule core are all visible in seminiferous tubules.

Seminiferous tubule architecture is completely disrupted, spermatozoa are gone, germinal epithelium exfoliates into the core region of the tubules, mononuclear cells infiltrate, and blood extravasates between the tubules. There is evidence of a disturbed basement membrane [Figure 2].

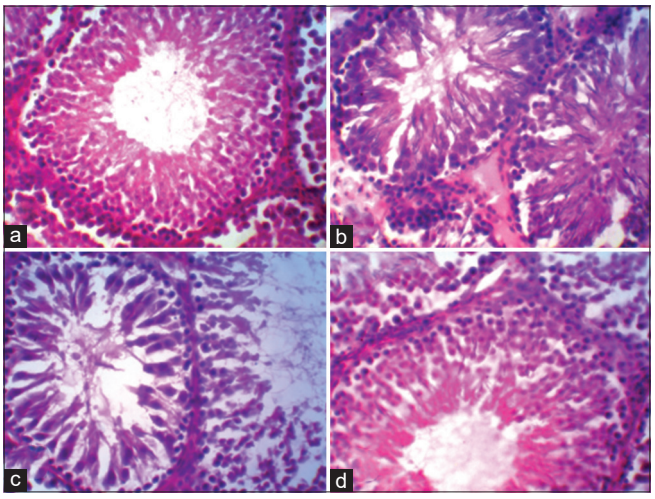

Figure 1: Different images of the testes of the control group demonstrating the typical architecture of the testicular seminiferous tubules coated with germinal epithelium (a-d: H&E. ×400)

The last group (formaldehyde and melatonin treated group): specimens of the testis stained with hematoxylin and eosin under a light microscope revealed seemingly normal testicular architecture, including seminiferous tubules lined with germinal epithelium and spermatogonia resting on a thin basement membrane among primary spermatocytes, spermatozoa, and Sertoli cells. Between seminiferous tubules, Leydig cells, and tiny blood veins with thin walls were visible. Spermatogonia type B, rounded spermatids, primary spermatocytes, and spermatozoa with flattened myoid cell nuclei encased in a thin basement membrane are among the various types of germinal epithelial cells found in a seminiferous tubule. Seminiferous tubules with plenty of spermatozoa have a normal design. A small number of tubules have a lot of spermatozoa in the center of the seminiferous tubule with exfoliated germinal epithelium [Figure 3].

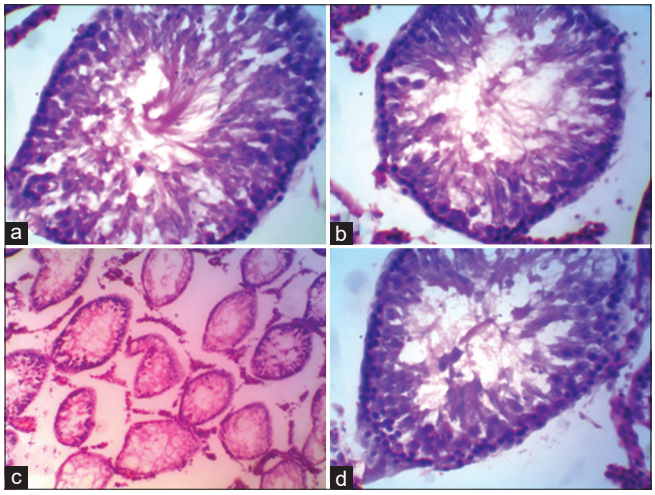

Figure 2: Various pictures of the group that had formaldehyde treatment show that the germinal epithelium has separated from the basement membrane and deteriorated, displaying a completely disrupted architecture (a and b) H&E (×400) (c) (×200) (d) (×400)

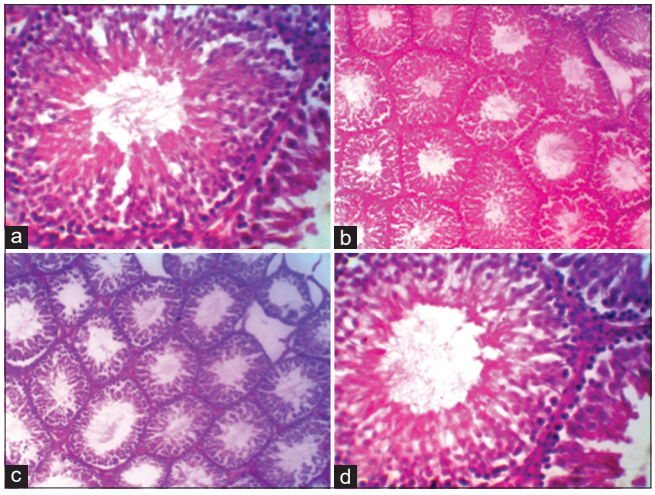

Figure 3: Images of the group treated with formaldehyde and melatonin show that the testicular architecture has returned to normal (a) H&E (×400) (b and c) (×200) (d) (×400)

DISCUSSION

In the current study, the testis of the second group that received formaldehyde treatment showed degeneration of seminiferous tubules and thickening of the basement membrane. A previous study that subjected the experimental mice to formaldehyde vapor found similar outcomes.[5] Exfoliation of spermatogenic cells was discovered in Group II in the current study. This result was consistent with that of Ulucam and Bakar who ascribed the breaking of the intercellular bridge to the lumen of seminiferous tubules. It showed signs of cellular debris and germinal epithelium sloughing.[6] Group II in the current investigation displayed germinal epithelium vacuolization. This was consistent with the findings of a previous study,[7] which reported that prolonged exposure to formaldehyde caused the germinal epithelium to degenerate and exhibit significant cytoplasmic vacuolation.[7] Leydig cells in the rat testes of Group II appeared to be normal. This contrasted with Take *et al*.'s investigation, which found that Leydig cells were degenerating.[8] Examining rat testis specimens in Group III, however, showed less thought of the tubules' basement membrane. This was consistent with another study's findings,[9] which they ascribed to melatonin's potent anti-inflammatory and antioxidant properties.

Melatonin's beneficial effects as a potent antioxidant were demonstrated by light microscopic analysis of the rat testes of Group III (the group that received both formaldehyde and melatonin administration); it improved all histological changes brought on by the injection of formaldehyde, including tissue fibrosis, cytoplasmic vacuolation, degenerated seminiferous tubules, and Sertoli and Leydig cells degeneration. According to a study by similar researches,[5,10] melatonin may shield the testis from the damaging oxidative damage and degeneration of cells caused by injecting rats with formaldehyde. They ascribed its antioxidant action to elevated levels of antioxidant enzymes, such as superoxide dismutase, and inhibition of lipid peroxidation. Melatonin has been shown to stop apoptosis in a variety of tissues, such as the rat hippocampal degeneration brought on by iron and cyanic acid.[11]

CONCLUSION

It is necessary to provide melatonin to employees in the anatomy department and food industries because it was found that formaldehyde caused histological changes in the testis of rats that could be improved using melatonin. However, more research is needed to determine the appropriate dosage and administration method for humans.

ACKNOWLEDGMENTS

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AVAILABILITY OF DATA AND MATERIALS

The data are available upon request from the authors.

ETHICS APPROVAL

All series of steps that were implemented in this study that included animal models were in compliance with the Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board. (SCBR-093-2024).

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