Tikrit Journal of Veterinary Sciences (2025) 4(1): 28-35
DOI : 10.25130/tjvs.4.1.5

IRAQI Academic Scientific Journals

Tikrit Journal of Veterinary Science

Evaluation of the role of melatonin for protect testicular tissues from harmful effects of diclofenac in rat male

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ARTICLE INFO.

Article history:

-Received:25/1/2025

- Received In Revised Form: 18/3/2025

-Accepted:20/3/2025

-Available online: 30/6/2025

Keywords:

Melatonin, Diclofenac, Testis tissues, Rat's male.

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ABSTRACT

The aim of the current study is to evaluate the effect of melatonin as an protect testicular tissues after the harmful effects of diclofenac. Thirteen rats were used in this study, which lasted for month. Divided into 6 groups, each group contain 5 rats. The control group G1 (vehicle treated) received normal saline (0.1 ml/day). The Second group G2 (5 rats) treated with Diclofenac. (0.7 mg/Kg/B.W), The Third group G3 (5 rats) was treated with Melatonin. (0.07 mg/Kg/B.W), The Fourth group G4 (5rats) was treated with Diclofenac.(1.4 mg/Kg/B.W),The fifth group G5(5 rats) was treated with Melatonin + Diclofenac.(0.7 mg/Kg/B.W +0.07 mg/Kg/B.W),and The sixth group G6 (5 rats) was treated with Melatonin + Diclofenac. (1.4 mg/Kg/B.W +0.07 mg/Kg/B.W).

The results indicate that diclofenac sodium induces significant reproductive dysfunction in male rats, characterized by degeneration of spermatogenic cells and Leydig cells. Melatonin administration appears to mitigate some of these adverse effects, particularly at the lower dose of diclofenac (0.7 mg/Kg/B.W), suggesting its potential as a protective agent against diclofenac-induced reproductive toxicity. Further studies are warranted to explore the mechanisms underlying these protective effects and to optimize treatment protocols.

In conclusion, the reproductive toxicity effects of DF these effects on testicular tissues could be attenuated by treatment with melatonin.

1. Introduction

Diclofenac, a conventional non-steroidal anti-inflammatory medication, is frequently utilized for the management of chronic pain and inflammation. Diclofenac (2-[2,6-dichloranilino] phenyl acetic acid) is a nonsteroidal anti-inflammatory drug (NSAID) extensively utilized for the management of chronic inflammatory and degenerative joint disorders, including osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis, and extra-articular rheumatism (2). As with all NSAIDs, diclofenac functions by reducing prostaglandin synthesis through the inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) with comparable potency. Extensive research indicates that the pharmacologic activity of diclofenac extends beyond COX inhibition, encompassing multimodal and, in certain cases, new mechanisms of action (MOA)(3). The administration of the medicine has been linked to antipyretic hepatotoxicity, nephrotoxicity, and reproductive harm(4,5). Sperm are particularly susceptible to reactive oxygen species (ROS) induced damages because they don't have DNA repair mechanisms. Additionally, they exhibit diminished amounts of cytoplasmic antioxidants and elevated levels of polyunsaturated fatty acids(6). Low quantities of reactive oxygen species play an essential role in proper sperm physiology, including fertilization capacity and sperm motility. DIC demonstrated an increase in ROS production, a decrease in mitochondrial membrane potential, and cardiotoxicity leading to cell death [16]. Increased production of ROS induce activation of NF-kB is a transcription factor, regulate expression the production of as pro-inflammatory cytokines, as tumour necrosis factor-a (TNF-a), interleukin-1b (IL-1b), (7).

Research demonstrates that inflammatory cytokines increased free fatty acid (FFA)concentrations, leading to dyslipidaemia, a condition related infertility in male (8). Any pharmacological substance exhibiting anti-dyslipidemic, anti-oxidant, and anti-inflammatory properties may provide therapeutic potential in enhancing fertility in individuals with impaired physiological conditions. Melatonin (N-acetyl-5-methoxytryptamine) possesses immune-enhancing and anti-inflammatory activities, and it plays homeostatic roles inside the mitochondrion. Melatonin may confer a protective effect against testicular damage induced by chemotherapy, partially via reducing apoptosis and modifying the proliferative activity of germ cells. Melatonin receptors are abundantly expressed in hypothalamic neurons situated within the pituitary gonadotrophs. (9) (10).

Material and methods

Experimental design:

Thirteen male albino rats with an average weight of about (165.3 ± 3.269 g) were used. The rats were housed in group cages and given free access to food and water. Then they were left for one week before starting the experiment for acclimatization. Prior to the beginning and throughout the experiment, the rats were housed at 24˚C room temperature and 12 hours' light: 12 hours' dark cycle. The rats were divided randomly into 6 main groups; untreated control & 5-treated groups each containing 5 animals. The experimental protocol was approved by the Local Ethical Committee and by the Institutional Review Board of the Faculty of Veterinary Medicine, Tikrit University- salah aldin-iraq (Tu. Vet.52).

sixty adult male rats were grouped into four major groups as showed in figure 3.1.

- The first group G1 (5 rats) control (normal saline)
- The Second group G2 (5 rats) treated with Diclofenac. (0.7 mg/Kg/B.W)
- The Third group G3 (5 rats) was treated with Melatonin. (0.07 mg/Kg/B.W)
- The Fourth group G4 (5 rats) was treated with Diclofenac. (1.4 mg/Kg/B.W)
- The fifth group G5 (5 rats) was treated with Melatonin + Diclofenac. (0.7 mg/Kg/B.W +0.07 mg/Kg/B.W)
- The sixth group G6 (5 rats) was treated with Melatonin + Diclofenac. (1.4 mg/Kg/B.W +0.07 mg/Kg/B.W)

Histological Study of Testis

After the treatment was finished, the animals were killed by cervical displacement, testicles and epididymis removed, and connective tissue and fat were also removed. Histological Sections were produced for the histological investigation in accordance with Luna (1968) (11) and as follows:

1. Fixation To determine the figures, size, cell, and copies, the samples were promptly fixed in 10% buffered neutral formalin for 48 hours.
2. Cleaning The samples were repeatedly rinsed with water after fixing in order to eliminate a high fixation ratio.
3. By running the samples through Ethanol Nursing Ascending Nursing (70%, 80%, 90%, 96%, and 100%), dehydration was obtained. To extract the water from the samples, the samples in

each race were left in the solution for an hour and a half.
4. clearing up. The deletion was accomplished by passing the tissue via xylene.
5. Intrusion After the samples were placed in the Xylene and Para plast mixture of wax paraffin at 54–56, they were utilized for 15 minutes in an electric oven. After that, the samples melted paraffin for two to three hours, with fresh paraffin being added every hour.
6. After embedding, the tissue block is taken out of the heating furnace and placed in a little container containing freshly melted paraffin. The tissue block is implemented by chopping paraffin.
7. Dividing. The tissue-containing paraffin block was repeatedly sliced with a microtome as the paraffin solidified. Usually, sections between 5 and 10 µm thick were obtained using the steel blade.
8. Putting up Mayer's albumin was used to adhere the tissue sections to a glass slide.
9. Discolorations The sections of histology were stained using the Bancroft method.

Results and discussion

The study of Histopathological Examination

The interstitial tissues and seminiferous tubules make up the testis. Seminiferous tubules contain male germ cells, myoid cells, and sertoli cells; leydig cells are located in the spaces between adjacent seminiferous tubules. (12).

Group 1 Control Testis

The testicular tissue was formed by seminefrons tubule (snts) , each tubule is formed by different stage spermatic development, the spermatogenia were located on the basement membrane , the primary spermatocyte were one row adjacent the spermatogonia , the primary spermatocyte transformed into secondary spermatocyte 2_3 rows , then the secondary spermatocyte transformed into spermatids by a process spermieogensis , the center of snts containing spermatozoa , the interstitial c.t containing groups of leydig cells (fig 1).

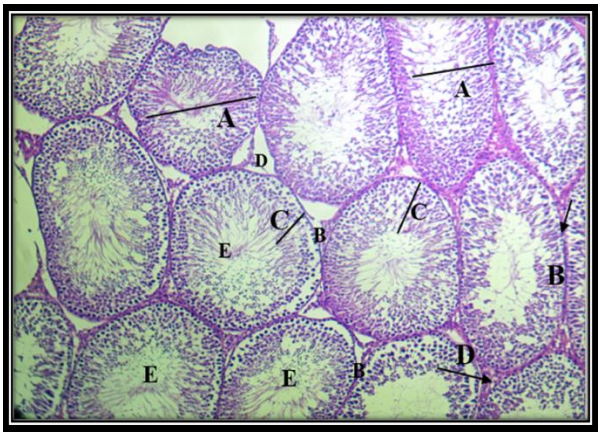

Figure 1:- Testicular tissue, seminefrouns tubule(A), basement membrane (B), different stage of spermatic development (C), leydig cell(D), spermatogonia (E) (H2E X 10).

Each S.N.Ts was unsheathed by (b.m) spermatogonia were small cells located on the B.M. primary spermatocyte were greater in size with basophilic spherical nuclei , sertoli cells were in between primary spermatocyte, secondary spermato cyet were smaller in size and greater in number , spermatids were present in grIups or cluster. Spermatozoa were on bundle like in the center of each s.n.t leydig cells were present in groups in between the S.N.Ts (fig 2).

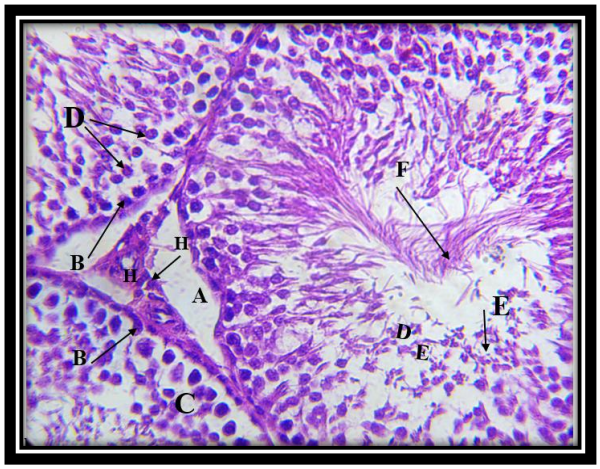

Figure (2):- s.n.ts, basement membrane (A), Spermatogonia (B), primary spermatocyte (C), secondary spermatocyte (D), spermatid(E), spermatozoa (F), leydige cells (H) (H2E X 40).

Groups 2 Diclofenac received dose (0.7 mg/Kg/B.W)

The testis was surrounded by capsule which appeared dissociated from the parenchyma of testis most of the lumens of S.N.Ts appeared with few degenerated spermatozoa and creating tubule had degenerated spermatocyte (fig 3).

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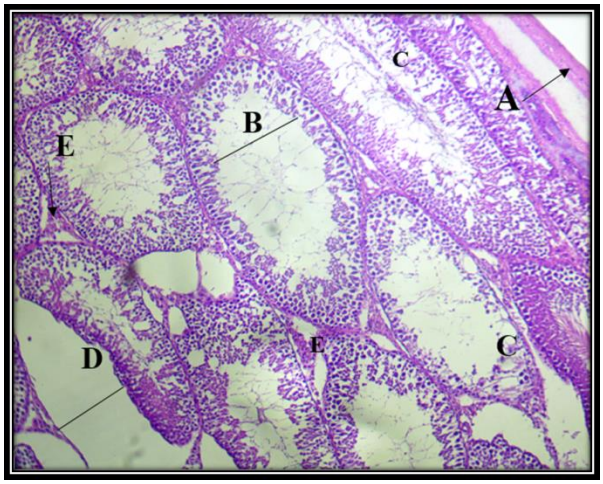

Figure (3):- Testis, detached capsule(A), S.N.Ts (B), spermatocyte degeneration (C), spermatogenic cell loss(D), leydig cells(E) (H2E X 10).

The S.N.Ts had hypertrophic primary spermatocyte and degeneration of creatin secondary spermatocyte most of spermatozoa were disappeared from the lumen of S.N.Ts , basement membrane was thick in creatin areas , degeneration of leydig cells also demonstrated (fig 4).

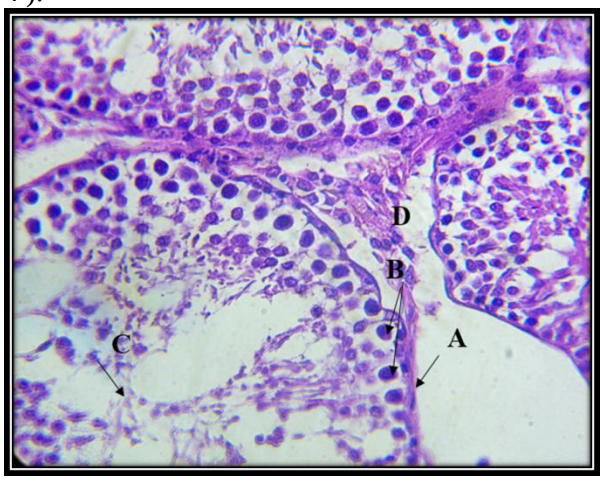

Figure (4):- Thick B.M.(A), primary spermatocyte hypertrophy (B), spermatozoa degernation (C), leydig cell degeneration (D) (H2E X 40).

Groups 3 Melatonin received dose (0.07 mg/Kg/B.W)

The parenchyma of testicular tissue was occupied eith crowded s.n.ts and the lumen of those tubule were engorged eith whole stage of spermatogenic development and spermatozoa were present in the center of lumens of s.n.ts (fig 5).

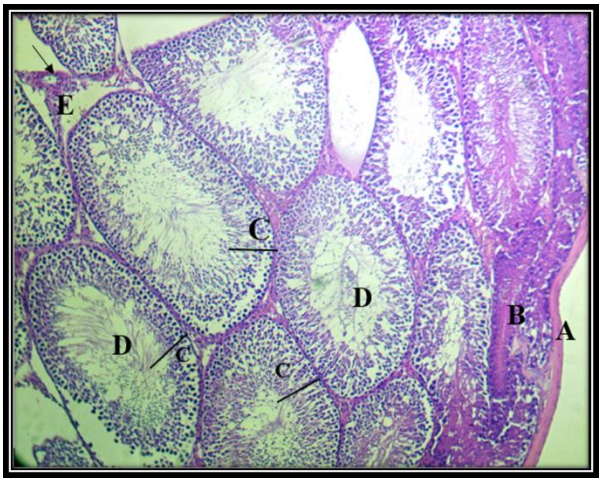

Figure (5):- Testicular tissue, capsule (A), subcapsular blood vessels (B), s.n.ts with engorgement with different stage of spermatic development (C), spermatozoa (D), leydig cells (E) (H2E X 10).

The S.N.Ts were occupied with well-organized of different types of spermatocyte , sertoli cells were demonstrated and great cells with spermatozoa which embedded in the cytoplasm of sertoli cells , leydig cells were loadged in the interstitial c.t (fig 6).

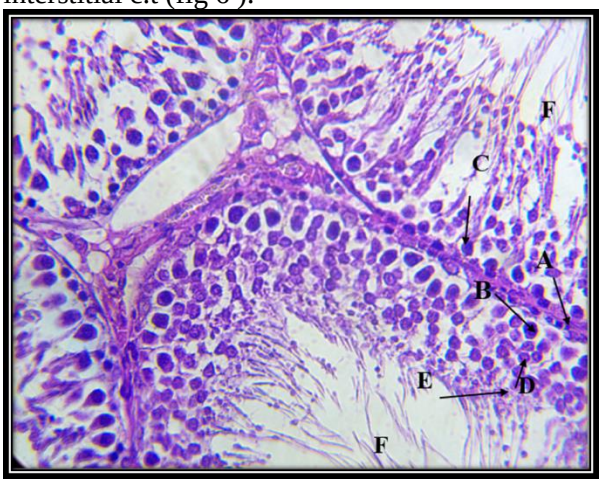

Figure (6) :- Thick B.M.(A), primary spermatocytes(B), sertoli cells (C), secondary spermatocytes(D), spermatid clusters(E), spermatozoa(F) (H2E X 40).

Groups 4:- Diclofenac received dose (1.4 mg/Kg/B.W)

The capsule of the testis was formed by collagen bundules with present of vacuole in the capsule . , subcapsular blood vessel were congested blood , most of the S.N.Ts were containing degeneration of the most spermatozoa , also disappearance of spermatozoa were degenerated in other lumens of tubule (fig 7)

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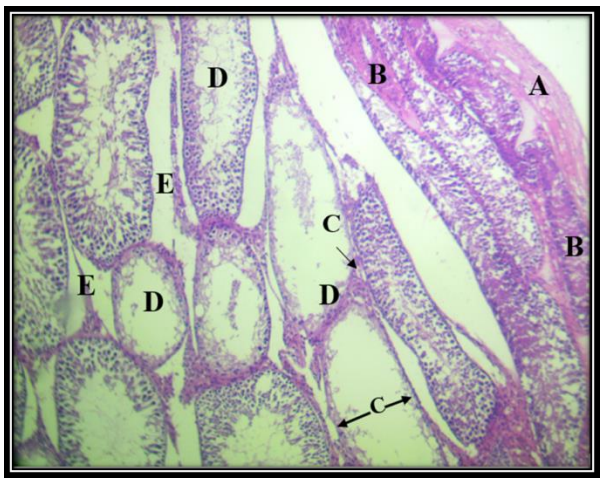

Figure (7): - : Testis capsule with vacuolation(A), sub capsular congested of blood vessels(B), spermatocyte degeneration(C), spermatozoa disappearance(D), lydige cell atrophy (E) CH2E X (10).

Creating lumen of S.N. Ts were containing degenerated primary and secondary spermatocyte , also spermatids were santy and present in clumps other S.N.Ts had spermatocyte , leydige cells were degenerated and detached from each other (fig 8).

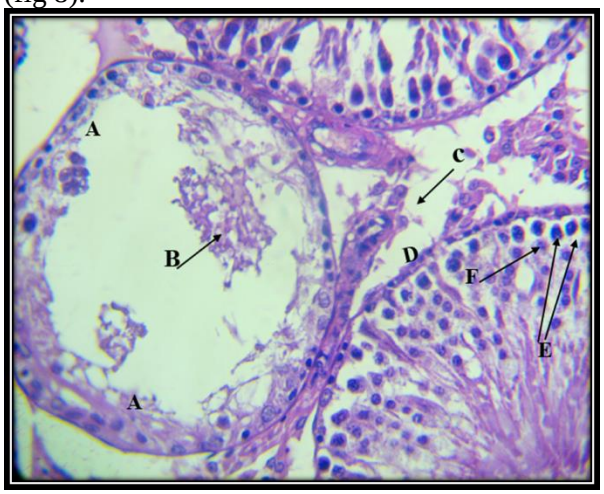

Figure (8):- S.N Ts with degenerated primary and secondary spermatocytes (a), clump of spermatid (b), degenerated lydgie cell (C), Thick B.M. (d) primary spermatocyte (E), secondary spermatocyte (F) (H2E X 40).

Lumen of S.N.Ts were evacuated from the different type of spermatocyte and remainants of cellular debris were present in the lumen of s.n.ts , leydig cells were degenerated and scattered in the inrtstitial c.t (fig 9).

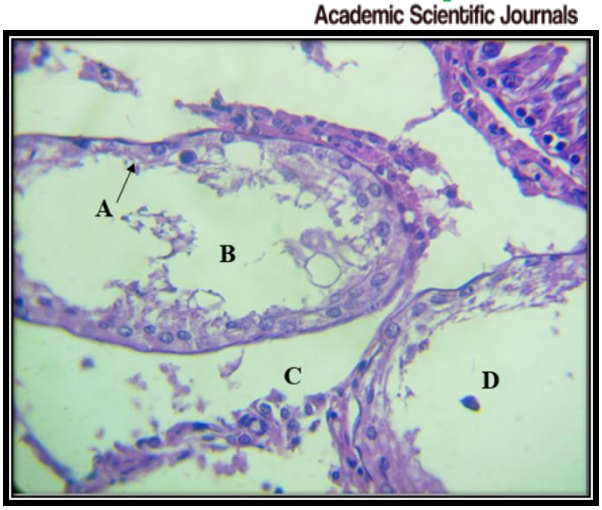

Figure (9):- S.N.Ts with spermatocyte degenerate (A), cellular debris (B), leydig cell degeneration (C), spermatozoa disappearance (D) (H2E X40).

Groups 5:- Diclofenac received dose10mg\kg + Melatonin received dose (0.7 mg/Kg/B.W +0.07 mg/Kg/B.W)

The parenchyma of testis was occupied by s.n.ts and these tubule were containing different stage of spermatocyte , spermatozoa were few in creatin tubule, interstitial c.t was wide with present of atrophied leydig cells , blood vessels in the subcapsular region were hyperaemic (fig 10).

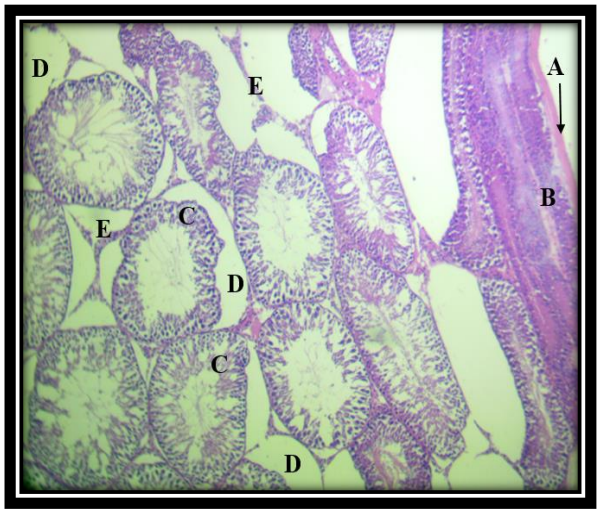

Figure (10):- : Testicular tissue and capsule(A), hyperaemia in the subcapsular blood vessel(B), s.n.ts with varying stages of spermatic development(C), broad interstitial c.t(D), leydige cell atrophy (E) (H2E X 40).

The lumen of s.n.ts had multiple spermatogonia, primary spermatocte was scattered and appeared hypertrophic with basopillic spherical nuclei , most of secondary spermato cyte were dissociated from thr primary spermatocyte, spermatids were in groups at the periphery of the center of lumen of s.n.ts spermatozoa were scanty and surrounded by testicular fillerate (fig 11).

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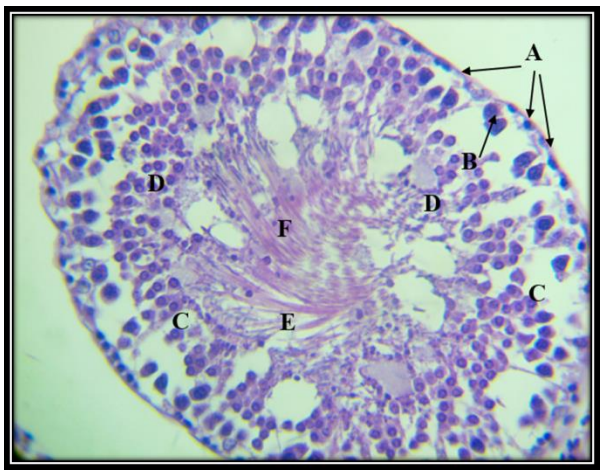


Figure (11):- : S.N.Ts, spermatogenic (A), hypertrophic primary of spermatocyte (B), secondary spermatocyte (C), cluster of spermatid (D) scanty of spermatozoa (E), homogenized fillerate (F) (H2E X 40).

Groups 6:- Diclofenac received dose 20mg\kg + Melatonin received dose (1.4 mg/Kg/B.W +0.07 mg/Kg/B.W)

The paracnchyma of tetis was covered by thick dense capsule. The subcapsular blood vessel were congested with hemolyzed blood , the s.n.ts were contaninig the different types of spermatocyte , the center of s.n.ts lumen was containing few spermatozoa , the basement membrane wasthick, certain lumens of s.n.ts had testicular fillerate (fig 12).

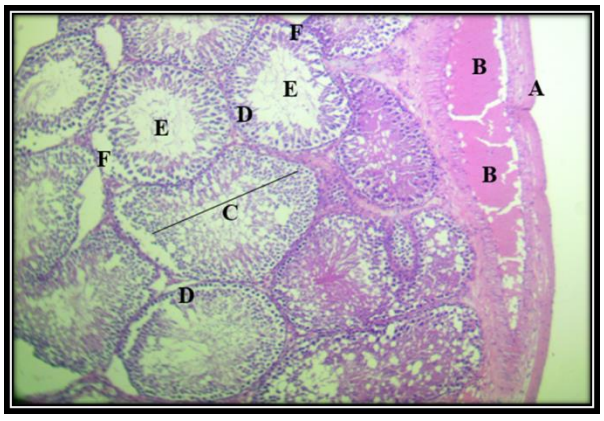
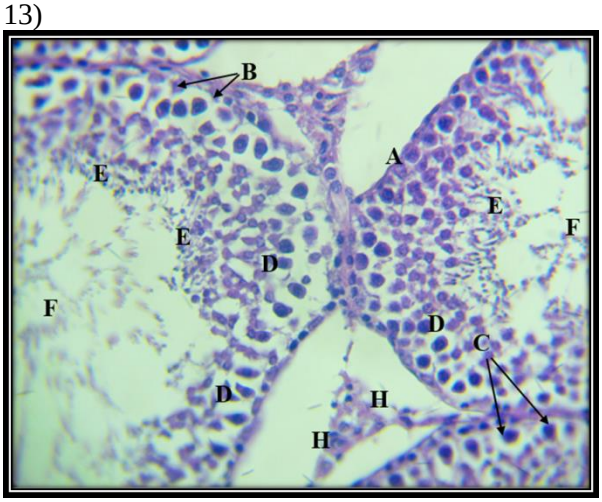


Figure (12):- Testis, capsule(A), subcapsular blood vessels with blood hemolysis (B) s.n.ts with spermatic development(C), thick B.M (D), spermatozoa of few number (E), leydige cells (F) (H2E X 10).

B.M ensheath the s.n.ts was thick , primary spermatocyte had great basophilic spheric nuclei , secondary spermatocyte were smaller in size and greater in number , spermatid were hyperplastic , certain spermatozoa were degenerated groups of lydig celld were present in the interstitial c.t , groups of those cells were mostly atrophied (fig 13)



Figure(13):- B.M. (A), spermatogenia (B), hypertrophic primary spermatocyte (C), secondary spermatocyte (D), cluster of spermatid (E), degenerated of spermatozoa (F), groups of leydige cells (H) (H2E X 40).

Diclofenac is an anti-inflammatory properties drug; its use has been associated with a disruption in the antioxidant enzyme system, hence inducing oxidative stress (OS) (13). Chronic administration of DF induces oxidative stress, leading to the generation of free radicals that might traverse the bloodstream and result in malfunction of testicular tissues. The substantial variations in the endogenous levels of these markers are undoubtedly linked to the complete destruction of cells inside the seminiferous tubules of the testes in the DF group.

The overproduction of free radicals due to an imbalance in the enzymes that regulate the antioxidant system has been observed to adversely affect sperm cells and testicular health (14). Administration of melatonin following DF therapy in the DF + Mel group exhibited cell regeneration, despite persistent indications of disturbance in the histoarchitecture of the testicular tissue due to DF.

Melatonin is an effective free radical scavenger. Consequently, melatonin can mitigate the toxicity of numerous environmental and chemical agents that induce oxidative stress (15). It influences gonadotropin-releasing hormone (GnRH) stimulators by modulating the production of hormones associated with the hypothalamus-pituitary-gonadal axis, thereby influencing the secretion of LH and FSH (16).

Melatonin, as an antioxidant, could restore the adverse effects of unilateral testicular injury on morphometric, spermatogenic, and oxidative parameters (17). Melatonin safeguards against male infertility by enhancing antioxidant defense

and preventing apoptosis through direct effects on male reproductive system cells.

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Tikrit Journal of Veterinary Sciences (2025) 4(1): 28-35
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تقييم دور الميلاتونين لحماية الأنسجة الخصية من الآثار الضارة للديكلوفيناك في الفئران الذكور

ريم باسل ارحيم ، سهام عجمي وادي

فرع الادوية والفسلجة والكيمياء الحياتية، كلية الطب البيطري، جامعة تكريت، العراق، تكريت

الملخص

تبحث هذه الدراسة في التأثير الوقائي للميلاتونين ضد تلف الخصية الناجم عن ديكلوفيناك في الفئران. تم استخدام ثلاثين فئران في هذه الدراسة، والتي استمرت للشهر. مقسمة إلى 6 مجموعات، تحتوي كل مجموعة على 5 فئران. تلقت المجموعة الضابطة G1 (مركبة معالجة) محلول ملحي طبيعي (0.1 مل/يوم). المجموعة الثانية 5) G2 فئران) عولجت مع ديكلوفيناك. (50 ملغ كجم) ، تم علاج المجموعة الرابعة 5) G4 فئران) مع ديكلوفيناك (20 ملغ) ، المجموعة الخامسة عولج 5) G5 فئران) مع الميلاتونين + ديكلوفيناك (10 ملغ +50 ملغ) ، وتم التعامل مع المجموعة السادسة 5) G6 فئران) مع الميلاتونين + ديكلوفيناك. (20 ملغ +50 ملغ). جميع المجموعات التي تلقاها عن طريق الفم لمدة 30 يومًا. تشير النتائج إلى أن الصوديوم Diclofenac يستحث خللًا كبيرًا في الإنجاب في الفئران الذكور ، من خلال الفحص النسيجي ، وكذلك عد خلايا Leydig ، التي تتميز بتنكس الخلايا المنوية وخلايا Leydig. توضح الدراسة الحالية أن إدارة ديكلوفيناك تقلل بشكل كبير من تعداد خلايا Leydig ، مما يشير إلى وجود تأثير ضار على وظيفة الخصية. وعلى العكس من ذلك ، يبدو أن الميلاتونين يدعم تكاثر خلايا Leydig ، مع إدارتها وحدها مما يؤدي إلى عدد الخلايا مماثلة لمجموعة التحكم. والجدير بالذكر أن الجمع بين الميلاتونين وديكلوفيناك (10 ملغ/كغ) أدى إلى عدد خلايا لايديج مرتفع بشكل ملحوظ ، مما يشير إلى دور وقائي أو محفز محتمل للميلاتونين ضد الضرر الناجم عن ديكلوفيناك. ومع ذلك ، بجرعة ديكلوفيناك أعلى (20 ملغ/كغ) ، أدى الجمع مع الميلاتونين إلى تحسن معتدل فقط في أعداد خلايا ليديج. يبدو أن إدارة الميلاتونين تتخفف من بعض هذه الآثار الضارة ، خاصة في الجرعة المنخفضة من ديكلوفيناك (10 ملغ/كغ) ، مما يشير إلى إمكاناته كعامل وقائي ضد سمية الإنجاب الناجم عن ديكلوفيناك. هناك ما يبرر المزيد من الدراسات لاستكشاف الآليات الكامنة وراء هذه الآثار الوقائية وتحسين بروتوكولات العلاج.

في الختام ، يمكن تخفيف آثار السمية الإنجابية لـ Diclofenac هذه الآثار على الأنسجة الخصية عن طريق العلاج بالميلاتونين.

الكلمات المفتاحية: الميلاتونين، ديكلوفيناك، أنسجة الخصية، ذكور الجرذان