Tikrit Journal of Veterinary Sciences (2024) 1(2): 19-25

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Science

Determination Effect of Colchicine on the Fertility of Adult Male Rats

Layth Luay Hassan[1] Buthina Abdulhameed Abdullah[1] Dakheel Hussein Hadree[1]

[1]Department of Pharmacology ,Physiology ,Biochemistry, College of Veterinary medicine, University of Tikrit, Tikrit , Iraq

ARTICLE INFO.

Article history:
-Received:
-Accepted:
-Available online:

Keywords:
Keywords: Rat , Colchicine , Fertility, Sexual Hormones, Sperm.

Corresponding Author:
Name: Layth Luay Hassan
E-mail: ll230098pve@st.tu.edu.iq
Tel:

ABSTRACT

This study was carried out to preparation of the chemical colchicine at a dose of (2.5 mg/kg body weight) , which, in the form of a powder in a sealed. And determines its effect on male fertility by evaluate the physiological bioassay of both sexual hormone and sperm parameters .

This study aimed at the determining the side effects of Colchicine and effectiveness on the sperm and the fertility of adult male rats. The experiment was carried out at Tikrit University's College of Veterinary Medicine in the animal house. Hence, 20 adult male rats and their weight was 300 gram were used for physiological bioassay challenge of the colchicine . The animals were split up into Two groups, with ten animals in each group for a duration of thirty days. First, the distilled water was administered as the control group, whereas oral colchicine (2.5 mg/kg body weight) was administered once a day to the second group.

The results of the study indicated that the group receiving colchicine alone had significantly lower levels of FSH ($P \leq 0.05$) (0.1280 ± 0.0316) when compared with control group (0.2390 ± 0.0119). Additionally, the group treated with colchicine alone revealed a significant decrease ($P \leq 0.05$) in the level of testosterone hormone (2.280 ± 1.080) compared with control group (4.363 ± 1.337) and a significant decrease ($P \leq 0.05$) in the level of LH (0.1120 ± 0.0103) compared with control group (0.2120 ± 0.0193).

The study found that the group receiving colchicine alone had significantly ($P \leq 0.05$) fewer sperm (3.500 ± 1.080) compared with control group (6.300 ± 1.337). Furthermore demonstrated a significant decrease ($P \leq 0.05$) in sperm viability in the colchicine-only group (71.500 ± 1.958) compared with control group (89.900 ± 1.792**) .** Additionally, significant decrease ($P \leq 0.05$) in sperm motility in the colchicine-only group demonstrated (43.330 ± 1.958) compared with control group(82.570 ± 1.792) and a significant increase ($P \leq 0.05$) in the sperm abnormality demonstrated in the colchicine-only group (46.400 ± 1.958) compared with control group (18.600 ± 1.792) .

Colchicine treatment had an impact on the fertility of adult male rats, resulting in decreased testosterone, FSH and LH levels as well as decreased sperm motility, count, and viability and increased sperm abnormalities.

Introduction

The formation of crystals of monosodium urate (MSU) is the cause of the chronic illness gout [1] .According to [1] gout usually manifests as an acute, self-limiting inflammatory monoarthritis that affects the lower limb joints. Colchicum autumnale seeds are the source of the alkaloid extract colchicine [2]. It is frequently used to treat Behcet's syndrome [3], familial Mediterranean fever with concomitant amyloidosis [4], and acute bouts of gouty arthritis [5]. Moreover, it is recommended for the treatment of skin conditions [6], coronary artery diseases [7] atrial fibrillation [8] and pericarditis [9].

According to [10], it has antimitotic effect by causing cellular mitosis to end during the cell cycle's metaphase and by upsetting the inflammatory pathway, which causes its anti-inflammatory action. In order to create irreversible tubulin-colchicine complexes, it binds to tubulin and activates nucleotide guanosine triphosphate (GTPase) , an enzyme that increases microtubule loss by depolymerizing it and stopping its elongation [11]. Furthermore, it has the ability to stop the meiotic divisions in mice [12].

The primary mode of action of the alkaloid colchicine is to prevent the production of microtubules and thus has an adverse effect on cells that go through both meiosis and mitosis By preventing the development of microtubules, colchicine inhibits the creation of spindles and may lead to abnormalities in both spermatogenesis and folliculogenesis [13]. While colchicine causes a variety of sperm analyses, ranging from azoospermia to oligospermia. Colchicine has conflicting effects on oocyte growth and function in women, much like it does on spermatagonesis [14]. Low sperm counts, motility, and abnormal sperm analysis were seen in 40–58% of patients treated to colchicine at a dose of 1-2 mg/day [15].

Aim of the study

Determination the effect of the Colchicine on the fertility of adult male rats .

Material and Methods

Preparation of laboratory animal

Twenty adult male rats used in the study obtained from animal house of veterinary medicine college \ Tikrit university. animals aged between (10-12) weeks and their weight was300gram. The experiment took place in the animal house of the College of Veterinary Medicine, Tikrit University, on 15/10/2023 until 15/11/2023. Animals were housed in plastic standard cages of dimensions 46*28*13 cm and it was cared for under ideal laboratory conditions of lighting, ventilation, and appropriate temperature(20-25 C^{o}). The floor of the cages was covered with sawdust and the floor of the cages was changed from two to three times a week to maintain cleanliness, with free access to water and food and exposed to artificial light for 12 hours per day, and then they were subjected to the experiment and the work was carried out.

Preparation of colchicine:

The preparation of the colchicine drug has been completed at a dose of 2.5 mg/kg body weight [16], which, in the form of a powder in a sealed. Sealed bag kept out of the sun, is the subject of the approved study in the current research phase. Following that, to calculate how much colchicine was given to each rat during the process, the weights of the rats were calculated at the beginning of the study. The average weight of one rat was 300 grams and the colchicine powder was 500 milligrams for every 60 kilograms. With proportionality, the therapeutic dose for one rat was 2.5 grams. After that, 500 grams of the therapeutic dose were taken and dissolved in 500 ml of phosphate buffer saline and 2.5 ml were given to each rat.

Experimental Design

Experimental animals were divided to two groups as follw:

- The first group (10 male rats) as control without treatment (normal saline) 2.5ml orally for 30 days.
- The Second group (10 Rats) treated with colchicine 2.5mg/kg for each male rat [16] given orally in dose 2.5 ml for each male rat for 30 days .

Blood Collection and Hormonal analysis

Serum separator tubes (SSTs) were used to prepare the blood for serum separation after it was drawn from treated group and control group, 24 hours after the treatment ended. After no more than fifteen minutes, the blood was allowed to clot. After five minutes of microfuge separation at 1500 RPM, the recovered sera were frozen at -20 ºC to be used for hormonal analysis (LH , FSH , Testosterone). The hormonal analysis done by serum testing using hormonal ELISA kit from Fine Care according to manufacturers instructions.

Epididymal tail suspension preparation and seminal analysis

The cauda epididymis was promptly removed after the conclusion of the therapy and placed in a Petri dish with 10 ml of warm normal saline at 37°C. It was then sliced longitudinally with a pair of fine-tipped scissors and squeezed using forceps. To do the microscopical examination on sperm characteristics, the cauda epididymis was cut into pieces (at least 200 cuts), releasing the sperms Esteves [17].

Statistical Analysis

Minitab software version 17.1 was used to analyze the data, and the ANOVA test was used. The Duncan's multiple range comparison of the means falls below the meaningful 0.05 threshold[18].

Results And Discussion

Hormonal Analysis Result

Testosterone levels decreased significantly ($p<0.05$) in the treated group (2.280 ± 1.080) in comparison to the control group(4.363 ± 1.337) after receiving colchicine treatment, as illustrated in table (1.1).

Follicular stimulating hormone (FSH) levels decreased significantly ($p<0.05$) in the treated group(0.1280 ± 0.0316) in comparison to the control group(0.2390 ± 0.0119) after receiving colchicine treatment, as illustrated in table (1.1).

Luteinizing hormone (LH) levels decreased significantly ($p<0.05$) in the treated group(0.1120 ± 0.0103) in comparison to the control group(0.2120 ± 0.0193) after receiving colchicine treatment, as illustrated in table (1.1).

Table (1.1) show the mean of testosterone, FSH and LH hormone levels

Groups	Testosterone ng/ml	Serum FSH mlU/ml	Serum LH mlU/ml
G1	4.363 ± 1.337 a	0.2390 ± 0.0119 a	0.2120 ± 0.0193 a
G2	2.280 ± 1.080 b	0.1280 ± 0.0316 b	0.1120 ± 0.0103 b

$=mean+/- standard deviation ($p\leq0.05$)

According to [19], the effect of colchicine on pituitary gland secretions, which also affect LH and FSH levels, may be explained by lower levels of these hormones. Additionally, [20] demonstrated that colchicine had a discernible effect on FSH and LH levels. Also, [21] observed that colchicine clearly affects both FSH and LH.

Seminal Analysis Results

This depiction displayed in table (1.2) the Sperm Count after treated with colchicine, showed treated groups(3.500 ± 1.080) decreasing Sperm Count compared with control group (6.300 ± 1.337) ,significantly ($p<0.05$).

This depiction displayed in table (1.2) the Sperm viability after treated with colchicine, showed treated group (71.500 ± 1.958)decreasing Sperm viability compared with control group (89.900 ± 1.792) ,significantly ($p<0.05$).

This depiction displayed in table (1.2) the abnormal Sperm after treated with colchicine, showed treated group (46.400 ± 1.958) increasing abnormal Sperm compared with control group (18.600 ± 1.792),significantly ($p<0.05$).

This depiction displayed in table (1.2) the Sperm Motility after treated with colchicine, showed treated group (43.330 ± 1.958) decreasing Sperm Motility compared with control group (82.570 ± 1.792),significantly ($p<0.05$).

Table (1.2) show the mean of Sperm Count, Sperm viability , Abnormal Sperm and Sperm Motility

Groups	Sperm count Million/ml	Sperm viability %	Abnormal Sperm morphology %	Sperm motility %
G1	6.300 ± 1.337 a	89.900 ± 1.792 a	18.600 ± 1.792 a	82.570 ± 1.792 a
G2	3.500 ± 1.080 b	71.500 ± 1.958 b	46.400 ± 1.958 b	43.330 ± 1.958 b

$=mean+/- standard deviation ($p<0.05$)

Discussion

The current investigation showed that therapy with colchicine resulted in a decrease in plasma testosterone levels. Our findings were consistent with those of [22], who demonstrated that toxicity to the male reproductive system lead to decreased sperm production or decreased testicular testosterone release, as well as potential alterations in the quality of sperm accessible for conception, also there is a connection between the rat's decreased body weight from colchicine intoxication, fat deposition, and a drop in testosterone levels.

Due to the fact that the toxicity of colchicine stems from its ability to bind to tubulin and disrupt the micro tubular network. According to [23], the impacted cells undergo poor protein assembly, decreased endocytosis and exocytosis, changed cell shape, decreased cellular motility, and mitotic arrest. Furthermore, sperm morphogenesis is a complicated process. Many microtubules are present in germ and sertoli cells, and these microtubules are necessary for the regular morphogenetic processes of spermatogenesis. According to [24], colchicine interacts with microtubules to cause structural defects in sperm. The sex hormones, including testosterone and estradiol, are synthesized in the brain and gonads, influencing behavior and gene transcription[25]. During puberty, follicle-stimulating hormone (FSH) and luteinizing hormone are synthesized and produced more readily when GnRH is released in a pulsing fashion from the hypothalamus toward the anterior pituitary gland, and GnRH neurons are the main regulators of puberty and fertility[26].This study showed that decreased levels of LH and FSH may explain the effect of colchicine on the secretions of the pituitary gland, which in turn affects the levels of LH and FSH , also, the study [13] showed that there is a clear effect of colchicine on levels of both FSH and LH. As noted by [21], there is a clear effect of colchicine on both FSH and LH. This is due to the relation between testosterone and follicle-stimulating hormone, which is important for the immature testis production [27].

Conclusion

Colchicine treatment had negative impact on the fertility of adult male rats, by decreasing testosterone, FSH, and LH levels as well as decreasing sperm motility, count, and viability and increased sperm abnormalities.

References

[1] Chen-Xu, M., Yokose, C., Rai, S. K., Pillinger, M. H. & Choi, H. K. Contemporary prevalence of gout and hyperuricemia in the United States and decadal trends: the National Health and Nutrition Examination Survey 2007–2016. Arthritis Rheumatol. 71, 991–999 (2019).

[2] Bertram GK, Masters SB, and Trevor AJ (2012). Basic clinical pharmacology. McGraw Hill., London, pp. 651-652.

[3] Yurdakul S, Mat C, Tüzün Y, Ozyazgan Y, Hamuryudan V, Uysal O, Senocak M, and Yazici H (2001). A double-blind trial of Colchicine in Behçet's syndrome. Arthritis Rheumatology, 44(11): 2686-2692.

[4] Hasbani G, Jawad A, and Uthman I (2019). Update on the management of Colchicine resistant Familial Mediterranean Fever (FMF). Orphanet Journal of Rare Diseases, 14: 244.

[5] Schlesinger N, Schumacher R, Catton M, and Maxwell L (2006). Colchicine for acute

gout. Cochrane Database Systemic Review, 2006: 4.

[6] Saxena S, Tandon S, Sardana K, and Bajaj S (2019). Herpetiform aphthous genital ulcers misdiagnosed as herpes genitalis in a young male and their effective response to colchicine therapy. International Journal of STD & AIDS, 30(13): 1340-1343.

[7] Vaidya K, Martínez G, and Patel S (2019). The role of Colchicine in acute coronary syndromes. Clinical Therapeutics, 41(1): 11-20.

[8] Siak J, Flint N, Shmueli HG, Siegel RJ, and Rader F (2021). The use of Colchicine in cardiovascular diseases: A systematic review. The American Journal of Medicine, 134(6): 735-744.

[9] Schenone hen Q, Wang Y, Zhang Y. Improvement of colchicine oral bioavailability by incorporating eugenol in the nanoemulsion as an oil excipient and enhancer. Int J Nanomed. 2011;6:1237–43.

[10] Leung YY, Hui LLY, and Kraus VB (2015). Colchicine-update on mechanisms of action and therapeutic uses. Seminars in Arthritis and Rheumatism, 45(3): 341-350.

[11] Bhattacharyya B, Panda D, Gupta S, and Banerjee M (2008). Anti-mitotic activity of Colchicine and the structural basis for its interaction with tubulin. Medicinal Research Reviews, 28(1):

[12] Liang JC, Hsu TC, and Gay M (1985). Response of murine spermatocytes to the metaphase arresting effect of several mitotic arrestants. Experimentia, 41:

[13] Arzu Yurci, Nurettin Türktekin(2021) , The role of colchicine treatment on reproductive outcome in women with Familial Mediterranean Fever, DOI: 10.32322/jhsm.896326 J Health Sci Med 2021; 4(3): 283-288.

[14] Holubcová Z, Blayney M, Elder K, Schuh M. Human oocytes. error-prone chromosome-mediated spindle assembly favors chromosome segregation defects in human oocytes. Science 2015; 348: 1143–7.

[15] Kaya Aksoy G, Koyun M, Usta MF, C¸omak E, Akman S. Semen analysis in adolescents with familial Mediterranean fever. J Pediatr Urol 2019;15:342.e1–342.e7.

[16] Rand Abdulateef Abdullah, Hana Khaleel Ismail and AL-Haaik Ammar Ghanim., (2022) impact of colchicine on histology of testis in rats12(3):250,259,September25,2022,DOI:https://dx.doi.org/10.54203/scil.2022.wvj31.

[17] Esteves, S., & Varghese, A. (2012). Laboratory handling of epididymal and testicular spermatozoa: What can be done to improve sperm injections outcome. Journal of Human Reproductive Sciences, 5(3), 233. doi:10.4103/0974-1208.106333.

[18] Duncan, D.B. (1955). Multiple Range and multiple F-Test. Biometrics, 11, 1-5.

[19] Said S. Elshama, Gaber M. G. Shehab, Ayman E. ElKenawy et al., (2013):Role of Nigella Sativa Seeds on modulation testicular toxicity of colchicine repeated use in adult albino rats.Life Science Journal 10(4): 1629-1639.

[20] Minoru INABA and Kunie KAMATA (1979) effect of colchicine on steroid secretion from rat adrenal gland . Japan J, pharmacol 29,631-638 accepted April 9,1979.

[21] Fernando sanchez‘, jose I Lara, lucinda cacicedo, franco sanchez franjose and ricardo Vazquez, (1991): inhibition by colchicine of immunoreactive vasoactive intestinal polypeptide release from anterior pituitary cells in culture , biomedical research 12 (2) 71-75.

[22] Dianne MC. Pathogenesis of Male Reproductive Toxicity. Toxicologic pathology, (2001), 29, 1, 64 –76.

[23] Filkenstein Y, Aks SE, Hutson JRet al.,(2010): Colchicine poisoning: the dark side of an ancient drug. Clin Toxicol. 48:407–414.

[24] Hess RA and Naki M (2000): Histopathology of male reproductive system inducedby the fungicide benomyl. Histol. Histopathil. 15:207-224.

[25] Imtithal A. Mohammed and Hala O. Adnan (2024):The Effects of Hormones on The Eyes and Blurry Vision in Rabbits,Kittens, Rats, and Human Egypt. J. Vet. Sci. Vol. 56, No. 1, pp. 241-255.

[26] Ibrahim Y. Alrawy and Souhayla Oneeis Hussain (2024) Impact of Both Growth Hormone and Gonadotropin Releasing Hormone on Puberty Based on Serum Progesterone and Insulin-Like Growth Factor-1 Level in Iraqi local Breed Ewe Lambs. Egypt. J. Vet. Sci. Vol. 56, No. 4, pp. 739-746.

[27] Maha Kh. Aljuboury and Hassan I. Mohamed (2024).Effect of Hydrocortisone on Spermatogenesis in Male Rats. Egypt. J. Vet. Sci. Vol. 56, No. 4, pp. 661-667.

تحديد تأثير الكولشيسين على الخصوبة في ذكور الجرذان البالغة

ليث لؤي حسن[1] **بثينة عبد الحميد عبدالله[1]** **دخيل حسين حدري[1]**

فرع الادوية , الفسلجة و الكيمياء الحياتية , كلية الطب البيطري , جامعة تكريت , صلاح الدين , العراق.

الملخص

في هذه الدراسة تم تحضير عقار الكولشيسين الكيميائي بجرعة دوائية 2.5ملغ \ كغم وزن الجسم والذي كان على هيئة مسحوق . وكذلك تم تحديد تأثير الكولشيسين على الخصوبة الذكرية بواسطة تقيم التحديات الفسيولوجية لكل من الهرمونات الجنسية واختبارات النطف.

الهدف من الدراسة هو تحديد التأثيرات الجانبية للكولشيسين وفعاليته على الحيوانات المنوية وخصوبة ذكور الجرذان البالغة. أجريت التجربة في كلية الطب البيطري , جامعة تكريت في البيت الحيواني. حيث تم استخدام 20 فأراً ذكراً بالغاً اوزانهم 300جرام لإجراء الاختبار الحيوي الفسيولوجي للكولشيسين. تم تقسيم الحيوانات إلى مجموعتين، كل مجموعة بها عشرة حيوانات لمدة ثلاثين يوماً. أولا، تم إعطاء الماء المقطر لمجموعة السيطرة، في حين تم إعطاء الكولشيسين عن طريق الفم 2.5 ملغم / كغم من وزن الجسم مرة واحدة يوميا للمجموعة الثانية..

حيث أظهرت الدراسة انخفاضا معنويا في مستوى الهرمون المنشط للجريبات في المجموعة المعالجة بالكولشيسين (0.0316 ± 0.1280) مقارنة مع مجموعة السيطرة (0.0119 ± 0.2390). كما ظهر انخفاضا معنويا في مستوى هرمون التستوستيرون للإباضة في المجموعة المعالجة بالكولشيسين (1.080 ± 2.280) مقارنة مع مجموعة السيطرة(1.337 ± 4.363). كما ظهر انخفاضا معنويا في الهرمون المحفز للإباضة في المجموعة المعالجة بالكولشيسين (0.0103 ± 0.1120) مقارنة مع مجموعة السيطرة (0.0193 ± 0.2120).

أظهرت الدراسة انخفاضاً معنويا في عدد الحيوانات المنوية في المجموعة المعالجة بالكولشيسين (1.080 ± 3.500) مقارنة مع مجموعة السيطرة (1.337 ± 6.300) . كما أظهر انخفاضاً معنويا في حيوية الحيوانات المنوية في المجموعة المعالجة بالكولشيسين (1.958 ± 71.500) مقارنة مع مجموعة السيطرة (1.792 ± 89.900). كما أظهر انخفاضاً معنويا في حركة الحيوانات المنوية في المجموعة المعالجة بالكولشيسين (1.958 ± 43.330) مقارنة مع مجموعة السيطرة(1.792 ± 82.570) وزيادة معنوية في عدد الحيوانات المنوية المشوهة في المجموعة المعالجة بالكولشيسين (1.958 ± 46.400) مقارنة مع مجموعة السيطرة(1.792 ± ,18.600) .

أظهر علاج الكولشيسين تأثيراً على خصوبة ذكور الجرذان البالغة، مما أدى إلى انخفاض مستويات هرمون التستوستيرون والهرمون المنبه للجريب ومستويات الهرمون الملوتن بالإضافة إلى انخفاض حركة الحيوانات المنوية وعددها وحيويتها وزيادة تشوهات الحيوانات المنوية.

الكلمات المفتاحية: الجرذان, الكولشيسين، الحيوانات المنوية، الهرمونات الجنسية، الخصوبة