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Determination of the Effect of Nano-Lipid Colchicine and Ordinary on Sexual Hormones in Adult Male Rats



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Abstract

HIS STUDY AIMS to reduce the harmful effects of the drug colchicine on male hormones using lipid nanotechnology after loading colchicine onto the Nano- lipid (SNL) and confirming the loading by adopting several tests, including Efficiency of entrapment and loading of SNL, Scan and transmission electron microscope, Fourier transform infrared spectroscopy (FTIR), And X-ray diffraction (XRD).

The study included 60 adult male rats were used in this study. The animals were split up into six groups, with ten animals in each group. First group, distilled water was administered as the control group, whereas oral colchicine (3 mg/kg body weight) was administered once a day. the second group The SNL was administered orally to the third group once a day at 2ml. whereas the fourth group (30 rats) was divided into three subgroups (10) rats for each subgroup treated with Nano-Lipid colchicine depending on the different concentrations of the drug. The doses were: The first subgroup with (1.5 mg/kg. body weight), the second subgroup with (3 mg/kg. body weight) and the third subgroup with (6 mg/kg. body weight).

The duration of treatment was 30 days after completion. the samples were taken blood, and hormone levels were examined. A decrease in LH hormone and FSH hormone and Testosterone hormone levels was observed in the groups that were dosed with colchicine, while in the groups that were dosed with colchicine loaded with Nano lipid there was a significant increase in hormone levels, especially when the increased concentration of Nano -lipid loaded with colchicine.

The current study demonstrated that Nano lipids reduce the undesirable effects of colchicine and improve its action.

Keywords: Colchicine, Nano-Lipid, Sexual Hormones, Fertility.

Introduction

Gout is a chronic disease caused by monosodium urate (MSU) crystal deposition. Gout typically presents as an acute, self-limiting inflammatory mono arthritis that affects the joints of the lower limb [1]. Colchicine is an extract alkaloid originated of Colchicum autumnal seeds [2]. Colchicine is commonly prescribe for the administration of acute of gouty arthritis [3]. Behcet's syndrome familial Mediterranean fever with associated amyloidosis [4, 5]. The Colchicine is prescribed to the management of the pericarditis [6], fibrillation atrial [7], artery coronary diseases [8], and skin disorders [9].

Colchicine has anti-inflammatory action by inhibiting the stopping of cell mitosis during

metaphase of the cell cycle and disrupting the inflammatory pathway to induce its antiinflammatory action [10]. Colchicine attaches to tubulin to form irreversible tubulin-colchicine complexes and induced guanosine triphosphate GTPase enzymatic action to enhance the loss of the microtubule causing its depolymerization and cessation of its elongation [11]. Also, it can stop the meiotic divisions at murine [12]. Long-term colchicine treatment is effective in preventing diseases, but longer therapy introduces the problem of potential adverse effects, especially those related to microtubular systems. For most of the treated patients thus the issues of possible chromosomal abnormalities and defective spermatogenesis and hormone levels, about prolonged colchicine therapy, are of great concern[13].Recently, a new

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technology has emerged, which is nanotechnology for loading medications. Its goal is to reduce the harmful effects of medications, and one of this technology is Nano lipid [14].

Nanoparticles (NPs) and other colloidal drugtransmission systems change the kinetics, drug distributed in the body. The reported of the Nanostructured lipid carriers (NLCs) is alternate system to liposome, emulsions, solid lipid nanoparticles (SLNs) and micro particles because of numerous advantage the [15]. Nanostructured lipid carriers (NLCs) provide sustained release of drugs and target them to the site of action [16]. This system has treatment of diseases due to its modulation with drug efficacy and sustained for longer periods. Nanocarriers exhibit improved pharmacokinetics and biodistribution of therapeutic agents due to their higher surface area to volume ratio, thereby minimizing toxicity through their preferential accumulation at target sites [8, 17]. To improve drug availability, drug ability, and bioactive drug researchers employed production. have nanoparticles, Nano cargoes, and biocompatible materials produced from chemical conjugation and organic processes [18].

The aim of the study: The purpose of this study was to reformulate and optimize colchicine based on a Nano lipid delivery system. And reducing the side effects of Colchicine, to probability protect of the activity and levels of the sexual hormones and probability protect of the fertility of adult male rat.

Material and methods

Animals

For the investigation, sixty mature male rats were utilized., as acquired from Tikrit University's Animal House in Veterinary Medicine College. The animals were between 10 and 12 weeks old, and they weighed an average of 325 grams, ranging from 300 to 350 grams. The experiment was conducted from October 15, 2023, to November 15, 2023, in the animal house of the College of Veterinary Medicine, Tikrit University. The animals were kept in standard plastic cages measuring 46 by 28 by 13 cm, with proper lighting, ventilation, and temperature control (20 to 25 C). In order to preserve hygiene, the cages' floors were coated in sawdust, replaced two or three times a week, allowed unrestricted access to food and water, and had artificial lighting .

Sixty adult male rats were grouped into four major groups, as shown in Fig. (1)

The first group (10 rats) was negative control without treatment (normal saline only)

The second group (10 rats) was positive control and treated with colchicine only (3 mg/kg .body weight) [17].

The third group (10 rats) was treated with Nanolipid only.

The fourth group (30 rats)was treated with Nano-Lipid Colchicine.

The 4th treated group was 30 adult male rats divided into three subgroups, with ten male rats for each subgroups, depending on the different concentrations of the drug doses explained in Fig. 3.1. The doses were:

The first subgroup	(1.5 mg/kg .body weight).
The second subgroup	(3 mg/kg. body weight).
The third subgroup	(6 mg/kg .body weight).

Ethical approval

As instructed by the Ministry of Higher Education and Scientific Research in Iraq, ethical approval was acquired in accordance with No. 7/18/44/46 on 3/14/2019, and scientific and humane approaches were followed in ethical handling with animals [19].

General experimental design

The plan was designed in two major experimental parts Fig. (2).

Part one: Nano-Lipid colchicine preparation by the solvent diffusion method and standardization.

Part two: Determining the effect of Nano-Lipid Colchicine and ordinary on physiological function of reproductive system In adult male Rat.

The parameters were :

Hormonal assay (LH, FSH, Testosterone).

Part One : The Preparation and Standardization of Nano-Lipid

Protocol for preparation of Nano-lipid structure

Fig. (3) illustrates the production process of Nano lipid synthesis. The formulation was prepared by solvent diffusion method [18].

There were two types of the lipid phase:

Lipid form: A lipid dispersion is created by overtaxing at 1500 rpm for 30 minutes after 100 mg of stearic acid and 100 mg of glycerin monostearate are dissolved and then distributed at 800 rpm using 2 ml of castor oil.

Developing lipid phase dissolution: 100 mg of phosphatidyl cholin was used to dissolve the developing lipid phase, and 800 rpm of loaded and distributed colchicine were needed for 30 minutes. After that, the dissolved form is combined with the lipid form, mixed for an hour at 800 rpm, and refrigerated for the entire night at 8 °C until needed. Before using, blend for thirty minutes at 800 rpm [20].

Blood Collection

Within 24 hours of the therapy ending, blood was drawn from all rat groups (experimental and control). On day thirty following the start of the blood collection process, blood was extracted by cardiac puncture using 3 ml disposable syringes. The blood was processed for serum separation using a serum separator tube (SST), with an average volume of around 1.5 ml. Within a maximum of fifteen minutes, the blood was permitted to clot on its own. Before being utilized for hormonal assay, the separated serum was frozen in a deep freezer at -20 °C using a microfuge for five minutes at 1500 RPM, than hormonal analysis done by serum testing using hormonal ELISA kit from Fine Care according to manufacturers instructions.

Statistical Analysis

Data were analyzed by Minitab program system ver, 17 and ANOVA test were applied. The means compared by Duncan's multiple range under the level of significant (0.05),[21].

Results

Part one: SNL Colchicine standardization and characterization

The results of SNL loaded Colchicine standardization was expressed as determination the identity formation and size variation as well as determined functional group and crystallization features, the results showed as follows:

The micrograph of SNL Colchicine and conventional:

Under varying magnification, the light micrograph displayed the globular-like structure of the Colchicine nano-lipid in loaded phase. The light microscope's optical field revealed aggregated form in various sizes of the nano-forming particles that derived together in configuration and composition of the overall pattern.

Transmission and scanning electron microscopy

The electron microscope scan and transmission are determined by the morphological type of SNL Colchicine. A scan of the Nano-Lipid revealed finely separated, spherically shaped vesicular assemblies in varying sizes. The picture of the transmission mode portrayal showed the small size of the structured Nano lipid in addition to the tense density that indicated the drug loaded and the fitting structural type of the Nano lipid as displayed in Fig. (4).

Absorbance curve, & max peak and Calibration curve of SNL

The Λ max peak of the Nano- Colchicine and ordinary were estimated in the UV-visible spectrum at 37 °C. The results in the plotted graphs displayed clearly the Λ max peak absorbance. These showed

the peak to be significantly to Λ max (350) nm for concerning tested compounds. The plotted absorbance curve approximately coincided at Λ max and portions of curves.

Efficiency of entrapment and loading of SNL Colchicine percentage

Table (1) listed the loading and entrapment efficiency of Colchicine for each of the five patches. Colchicine's loading % and amount were 75.2 ± 8.3 and 79.04 ± 4.96 in the SNL, respectively.

Size of SNL Colchicine

The Nano-metric scale particle size was measured at SNL 85.7-300 nm and summarized as shown in Table (2).

Fourier Transform Infrared (FTIR)

Fourier transform infrared spectroscopy (FTIR) was screen wave band concerning molecular structures and interactions

X-ray diffraction (XRD)

X-ray diffraction (XRD) it is a useful tool to determine the physical nature of nanoparticles, it is a rapid analytical technique used primarily to measure atomic spacing. It provides accurate quantitative information about atomic arrangements at interfaces, determines the crystalline phases present in samples, and provides information about the size of unit cells along with physical properties [22]. The degree of crystallinity of the nanoparticles was evaluated by measuring the geometric scattering of X-ray crystalized using Xray diffraction. Findings XRD Fig.(5). depicts the SNL-diffraction pattern, which has prominent peaks that show the colchicine's crystalline structure. The Nano-lipid structure of drug showed higher crystallization than the conventional type due to the wide range order of their distribution.

Part two: Bioassay challenge between SNL Colchicine and Ordinary Colchicine in rat.

The hormonal Level

Testosterone Hormone:

This depiction displayed in Fig. (6) the level of hormone Testosterone after treated, showed groups 4 and 6 increment levels of hormone. The SNL group displayed a higher level than the conventional (group2) significantly (p<0.05).

The FSH and LH hormonal Levels:

This depiction displayed in Fig. (7) the level of hormone FSH after treated, showed groups 4 and 6 increment levels of hormone. The SNL group displayed a higher level than the conventional (group2) significantly (p<0.05).

The LH hormonal Level

This depiction displayed in Fig. (8) the level of hormone LH after treated, showed groups 4 and 6 increment levels of hormone. The SNL group displayed a higher level than the conventional (group2) significantly (p<0.05).

Discussion

The current investigation showed that therapy with colchicine resulted in a decrease in plasma testosterone levels. Our findings corroborated those of [23], who demonstrated that damage to the male reproductive system can lead to decreased testosterone release from the testis, decreased sperm yield, and possibly even lower-quality sperm that are 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. Additionally, repeated use of colchicine can cause testicular toxicity as shown by [24].

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 deal with the, which in turn affects the levels of LH and FSH , also, the study showed Arzu and Nurettin, [13], that there is a clear effect of colchicine on levels of both FSH and LH. As noted by Fernando et all[27], 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[28].

The results of our investigation showed that the adverse effects of colchicine decreased in the groups where we applied Nano-lipid technology. In recent years, nanotechnology has gained much attention within the scientific community in many countries [29],[30]. There could be a number of causes for this, most likely being, It has been observed that NLCs and other Nano particulate systems increase oral drug bioavailability through M-cells of Peyer's patch intracellular uptake [14]. Drug diffusion across the gastrointestinal barrier will be increased because drug release from nanoparticles is effective (high surface area) and passive diffusion will continue as long as the concentration gradient is maintained. Temporary opening of tight junctions or spaces between two neighboring intestinal epithelial cells, as a result of highly lipophilic surfactants' ability to enhance Para cellular absorption[31]. increased retention and absorption due to the nanoparticles' adherence to the intestinal underlying epithelium [32] lengthens the period of time a food spends in the stomach and upper small intestine because it is lipidic, which improves absorption.

Conclusion

Colchicine-containing structured Nano lipid was effectively created in this work, offering a stable formulation. The main conclusions and findings from our investigation are an increasing in the levels of sexual hormones, S.N.L colchicine exhibited decreasing side effects of colchicine compared to ordinary colchicine in various sexual hormone levels tests, including the Testosterone, LH, and FSH, There was a noticeable increase in the levels of sexual hormones after administration of S.N.L colchicine, compared to the low levels caused by ordinary colchicine.

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Conflict of Interest: The authors declared that no conflict of interest.

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Fig.1. The experimental design of groups



Fig.2. The general experimental design



Fig. .3. The Structural Nano-Lipid colchicine preparation protocol.



Fig. 4. Scan and transmission electron microscope(A) Scan electron microscope(B) TABLE 1. The SNL Colchicine loaded profile and loading efficiency percentage

SNL	Loading %	Loaded efficiency %
SNL Colchicine	79.04 ± 4.96^{a}	75.72 ± 8.3^{a}

n= $\overline{5}$ samples, Data presented \pm SE of mean, SNL: structure Nano-Lipid Colchicine

TABLE 2. The size of SNL carrying Colchicine

SNL	Nano-lij	Nano-lipid's size in nm	
	Range size	Mean ± SE	
Nano-lipid Carrying Colchicine	100-350	210 ± 38.47	

n= 5 samples, Data presented \pm SE of mean, SNL: structure Nano-Lipid

TABLE 3.Functional groups peak m⁻¹ of conventional and SNL Colchicine

Peaks of Colchicine	Peaks of SNLColchicine m ⁻¹	Functional groups
3437.15	336.71	Hydroxyl group
3005.15	2807.08	Methyl (CH3)
2020.23	1647.21	Alkenyl C=C
2850.70	1530.20	Vinyl C-H
1739.79	1423.47	
1647.21	1315.45	Aromatic C-H
1425.18	1157.29	
1165	1029.90	



Fig.5. Conventional, Show coupling between conventional and particles SNL Colchicine



Fig.6. Level hormones Testosterone both Ordinary Colchicine and SNL Colchicine pharmaceutics forms. n: 10 rats



Fig..7. Level hormones FSH both Ordinary Colchicine and SNL Colchicine pharmaceutics forms. (n: 10 rats).



Fig.8. Level hormones LH both Ordinary Colchicine and SNL Colchicine pharmaceutics forms. n: 10 rats

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تحديد تأثير الكولشيسين الدهنى النانوي على الهرمونات الجنسية في ذكور الجرذان البالغة

ليث لؤي حسن 1 ، بثينة عبد الحميد عبدالله 1 و دخيل حسين حدري 1

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الخلاصة

هدفت الدراسة إلى تقليل التأثيرات الضارة لدواء الكولشيسين على الهرمونات الذكرية باستخدام تقنية النانو الدهنية بعد تحميل الكولشيسين على الدهون النانوية (الدهون النانوية المهيكلة) وتأكيد التحميل من خلال اعتماد عدة اختبارات منها كفاءة الانحباس وتحميل الدهون النانوية المهيكلة ، المجهر الإلكتروني الماسح والنافذ، مطيافية تحويل فورييه للأشعة تحت الحمراء (FITR)، حيود الأشعة السينية (XRD).

تم استخدام 60 من ذكور الجرذان البالغة في هذه الدراسة من البيت الحيواني في كلية الطب البيطري جامعة تكريت، تم تقسيمها الى اربع مجاميع بواقع(10حيوانات في المجاميع الثلاثة الاولى) بينما في المجموعة الرابعة استخدمت 30 من ذكور الجرذان، وزعت إلى 3 مجاميع بواقع (10) ذكور، خلال فترة التجربة تم إعطاء المجموعة الأولى الماء المقطر كمجموعة سيطرة، في حين تم إعطاء المجموعة الثانية الكولشيسين الطبيعي عن طريق الفم (3 ملغم / كغم من وزن الجسم) مرة واحدة في اليوم بينما أعطيت الدهون النانوية المركبة عن طريق الفم (3 ملغم / كغم من وزن بمعدل 2 مل، في حين تم التعامل مع المجموعة الرابعة حسب تقسيمها مع الكولشيسين الدهني النانوي اعتمادا على التراكيز المختلفة للدواء وكانت الجرعات: المجموعة الفرعية الفركية عن طريق الفم روزن الجسم)، المجموعة التراكيم ال التراكيز المختلفة للدواء وكانت الجرعات: المجموعة الفرعية الفرعية الأولى به الكولشيسين الدهني النانوي اعتمادا على الفرعية الثالثة بروزن الجسم)، المجموعة الفرعية الفرعية المركبة من وزن روز المعمر عد الثلثة مرة واحدة يومياً

كانت مدة العلاج 30 يوما بعد الانتهاء تم جمع عينات الدم وفحص مستويات الهرمونات، حيث اظهرت النتائج انخفاضا معنويا في مستويات الهرمون اللوتيني والهرمون المنبه للجريب وهرمون التستوستيرون في المجموعات التي تم تجريعها بالكولشيسين، بينما في المجموعات التي تم تجريعها بالكولشيسين المحمل على الدهون النانوية حدثت زيادة كبيرة في مستويات الهرمون، خاصة عند زيادة تركيز الكولشيسين المحمل على الدهون النانوية.

الكلمات المفتاحية: الكولشيسين، الدهن النانوي ، الهرمونات الجنسية، الخصوبة.