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Reverse side effect of nifedipine on Leydig cells male rabbits in vitro by liposome containing nifedipine and sodium nitroprusside

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ABSTRACT

The purpose of this study was to look into the effect of nifedipine on the activity of Leydig cells. reduction by liposomes containing nifedipine and sodium nitroprusside by measuring the functional characteristics of Leydig cells in vitro. There were two main groups created; the first received treatment with regular nifedipine - sodium nitroprusside , and the second received treatment with liposomally encapsulated nifedipine - sodium nitroprusside. Nifedipine concentration was in 20 µl and SNP concentration was in 20, 40, 60, 80, and 100 µl, respectively. A positive control group and a negative control group served as additional controls.

The results of Leydig cells revealed that nifedipine had a negative effect on inhibiting the activity of Leydig cells in vitro, steroidogenesis. It was also discovered that SNP had a positive stimulatory effect on Leydig cells at low concentrations.

The current study found that liposomes containing nifedipine - sodium nitroprusside reduced nifedipine negative side effects on leydig cells and steroidogenesis, while also improving steroid activity in Leydig cells.

1. Introduction

Nifedipine is a highly successful pharmaceutical compound that acts as a calcium influx antagonist and is an effective treatment for mild to severe hypertension and cardiovascular disease. Nifedipine inhibits L-type calcium channels, which are voltage-dependent channels that relax smooth muscle in the vascular system (among other things), as well as having negative inotropic and chronotropic effects on the heart. Calcium channel blockers (CCBs) such as Nifedipine have also been shown in studies to reduce male fertility [1] .

Antihypertensive drugs have recently been added to the list of medications that have been shown to impair male fertility. Nifedipine and other calcium channel blockers have been shown in vitro to have anti-fertility effects, but not in vivo [2].

Steroidogenesis is a complex multistep process for the biosynthesis of steroid hormones from cholesterol. The testes and adrenal glands are the primary organs for steroidogenesis in all males. Steroidogenesis in the testis is restricted to Leydig cells, where cholesterol is converted to testosterone, According to recent research, nifedipine inhibits the process of steroid formation within Leydig cells and has a clear effect on fertility in general [3].

Sodium nitroprusside (SNP), a well-known arterial and venous vasodilator, is used in clinical practice to lower blood pressure. SNP was discovered by Playfair in 1849, and Johnson used it on a patient for the first time in 1922. It was shown to be both safe and effective when

given intravenously to patients with severe hypertension in 1955. It quickly gained popularity as a fast-acting medication beneficial to lower intraoperative hypertension, induce hypotension to reduce surgical blood loss, decrease afterload, and increase cardiac output in heart failure after being successfully used as an intraoperative antihypertensive in 1970. Cardiovascular surgery, hypertensive crises, heart failure, vascular surgery, pediatric surgery, and other emergencies are all clinical applications. This proactive role is played by nitric oxide [4]. In males, SNP is important for steroidogenesis, erectile function, sperm capacitation, and acrosome reaction.

SNP also regulates the interaction of Sertoli cells with germ cells and the maintenance of the blood-testis barrier [5].

Nanotechnology is one of the most important modern medical technologies because it improves treatment efficacy while reducing side effects. Liposomes, also known as lipid vesicles, are synthesized artificially by enclosing an aqueous core within one or more phospholipid bilayers ranging in size from nanometers to hundreds of micrometers. Liposomes were discovered in the 1960s and have since become a useful tool in drug delivery, membrane science, and artificial cell synthesis due to their resemblance to cells and natural vesicles, as well as their ease of formation, operation, and modification [6].

Cell cultures are used in pharmaceutical, medical, and biological sciences. The ethical and financial limitations of in vivo models drive the urgent need for a replaceable cell model that is more closely related to the characteristics of organisms and can be used for high-throughput drug screening [7]. This study aimed to remove the side effects of nifedipine by using the liposome containing nifedipine and sodium niroprusside.

2. Materials and Methods

This study was carried out at the University of Baghdad-College of Veterinary Medicine department of Physiology, 'Biochemistry,' and Pharmacology; the experimental design protocol's ethical database was based on the authority of lab animal regulatory management. [8] "standardized guidelines for the care and use of laboratory animals in research and teaching in Iraqi scientific institutions.

Design of Experiment

The experiment was divided into two parts: the first was for the preparation of liposome-loaded NIF-SNP and the second was for the standardization of the physical and chemical properties of liposome NIF-SNP. A second part involves the challenge of liposome NIF-SNP on cultured leydig cells. As shown in **diagram 2.1** and **2.2**. The experiment was designed as a double-blind placebo grouping of Leydig cells culture strategy to investigate the liposome containing Sodium Nitroprusside and Nifedipine for attenuating the adverse effect on the function of Leydig cells *in vitro*.

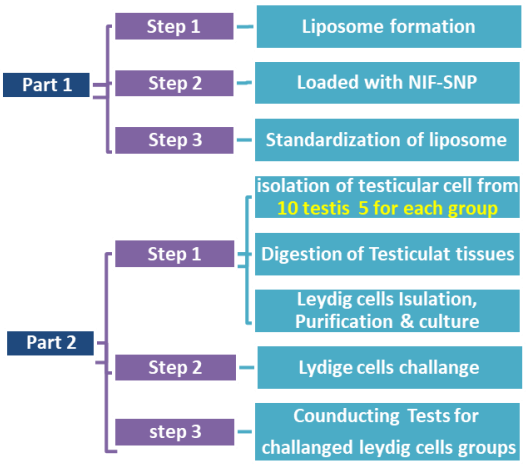

Diagram 2.1 Expermintal designs of liposome preparation , Lydige cells tissue culture and challanged groups.

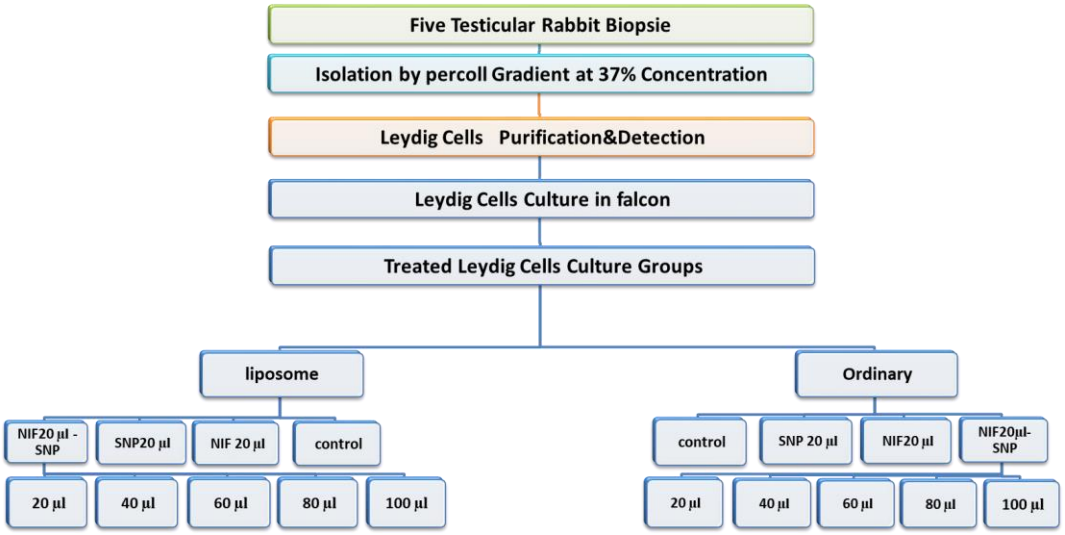

Diagram 2.2 : Experimental design & challenge grouping of Leydig cells.

Liposome Preparation

5ml of methanol was added to a glass tube, followed by 10ml of chloroform added to the methanol-containing tube. Five minutes were spent stirring the mixture. Lecithin was added to the mixture and vortexed for five minutes until completely mixed. Cholesterol was added to the mixture and vortexed for five minutes. The mixture was then stirred with vacuum air using the vacuum device until the liquid dried and the liposome was formed, as shown in diagram 2.3 This procedure took between 30 minutes and two hours.

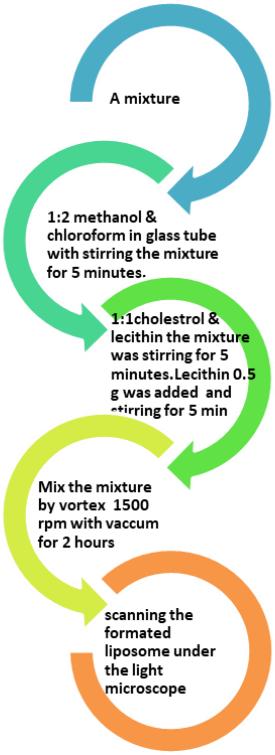

Diagram 2.3 The protocol of liposome preparation

Liposome containing SNP-NIF Standardization

FESEM Assay The Field Emission Scanning Electron Microscopy (FESEM)[9].

TEM Assay The Transmission Electron Microscopy (TEM) [10].

XRD The X-Ray Diffraction (XRD) Assay [11].

FTIR Fourier-Transform Infrared Spectroscopy [12]. **Zeta potential** The stability of a nanoparticle dispersion was tested using conventionaland nano-lipid NIF-SNP [13].

The Leydig Cells Culture

Testicular tissue sample preparation: the biopsies of testes of adult rabbits were collected after immediate surgical removal of the testicle in the stem cell lab at the College of Veterinary Medicine/University of Baghdad, and then the biopsies were reserved in cold DMEM solution .The testis was dissected at the midline, the testis was excised, the Testicular layers were removed (scrota, tunica vaginalis, and tunica albuginea), then epididymis dissected along with the last layer and the testicular tissue was gently excised and cut longitudinally in two halves as testicular tissue digestion and separation. The cells were then counted and their vitality was measured, after which the cells were incubated, and then the treatment of the groups was carried out based on [14, 15, 16].

The Leydig Cell Number

The cells number was determined by adding 50µl of Leydig cell culture into a hemocytometer and counting under the microscope at 100X , The cell number was calculated according to the following equation [17].

$$Number\ of\ cell's = \frac{Total\ cells\ counted}{Number\ of\ boxes\ scounted} \times Dilution\ factor\ \times 10{,}000$$

The Leydig Cell Steroidogenesis

Leydig cell steroidogenesis was challenged aginst NIF-SNP concentration then againts testosterone concentration. The incubated NIF-SNP treated Leydig cells 100×106 cells/ml in Falcon culture plates to activate steroidogenic function by LH 100 ng/ml. Leydig cells were harvested and centrifuged at 100g for 5 min, and the supernatant layer was stored at -18˚C [18]. The level of hormones was measured using the Copas device; the cobase 411 analyzer is a fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis. The analyzer is available as a rack or disk sample handling system achieved in Bashaer Al-Harthiya Laboratory[19].

3. Results and Discussion

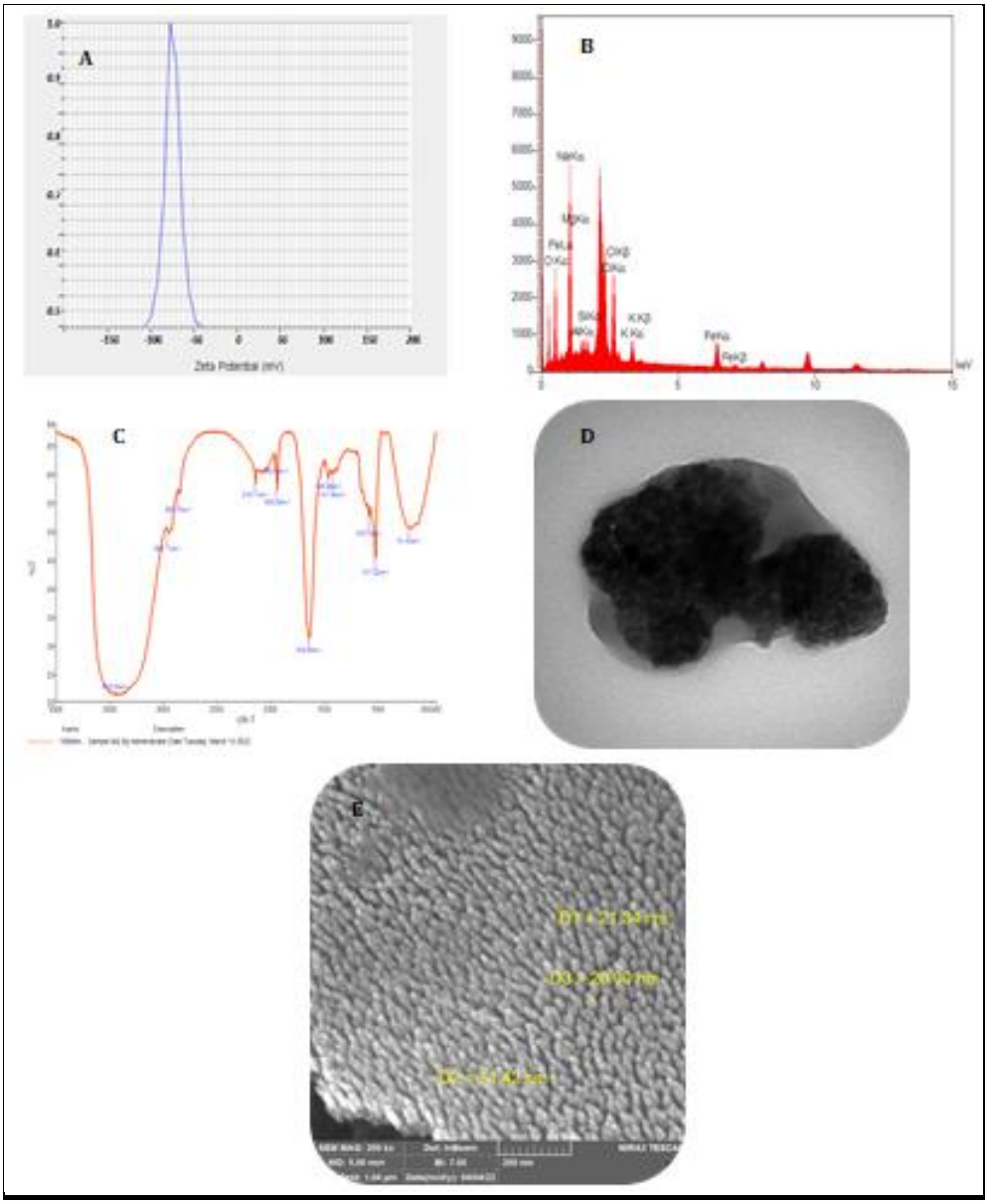

Figure 3.1 The liposome standrization (A) Zeta potential (B) FTIR (C) XRD (D) TEM (E) FESEM.

Results

Leydig Cell Number

After the treatment and incubation of the grouped Leydig cells with Liposome containing NIF&SNP in concentrations of (20,40,60,80, and 100 µl) versus ordinary NIF & SNP (20,40,60,80,100 µl) and NIF in the concentration of 20 µl, SNP 20 µl, with and without liposome, the number of cells was calculated using a hemocytometer stained with trypan blue dye, as shown in diagrams 3.1 ,3.2, and figure 3.1.

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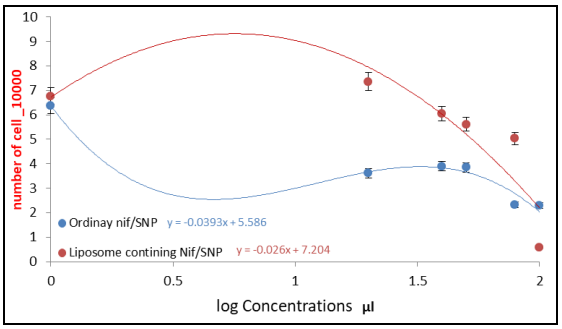


Diagrams 3.1 The difference in the number of Leydig cells in groups treated with synergism SNP&NIF with and without liposome. These results showed the inhibitory effect of nifedipine while showing the stimulating effect of SNP. They also showed the effect of synergism in the liposome and the removal of the side effect of nafdipine by SNP.liposome containing NIF&SNP and ordinaryNIF&SNP.

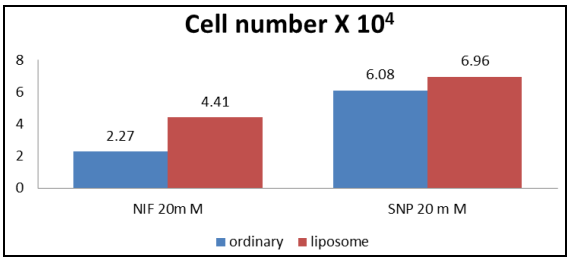


Diagram 3.2 The difference in the number of Leydig cells in groups treated withNIF, SNP, in the concentration of 20 µl with and without liposome.These results showed the inhibitory effect of nifedipine while showing the stimulating effect of sodium natroprusside.

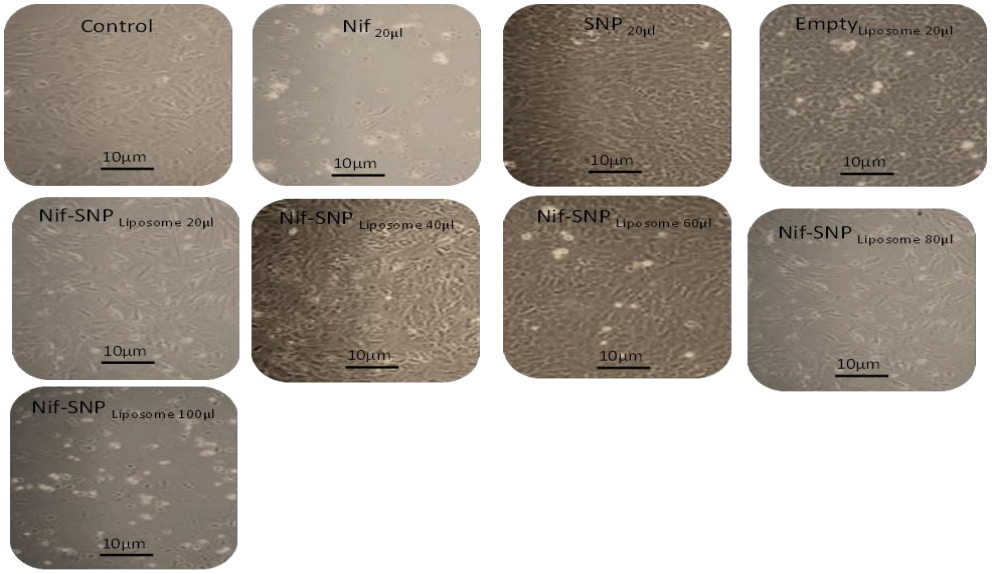


Figure 3.1 Leydig cells concentration in different challenged groups .These results showed the inhibitory effect of nifedipine while showing the stimulating effect of sodium natroprusside.They also shows the effect of synergism in the liposome and the removal of the side effect of NIF by SNP liposome containing NIF&SNP and ordinary NIF&SNP

Steroidogenic Activity Test

As shown in diagrams 3.3 and 3.4, the effect of liposome continuing NIF&SNP on testosterone concentration of steroidogenic process was minimized at the significance of (p 0.01) of NIF adverse effects in testosterone concentration of steroidogenic process. When compared to the other groups, NIF treatment resulted in a significant (p 0.05) decrease in testosterone concentration.

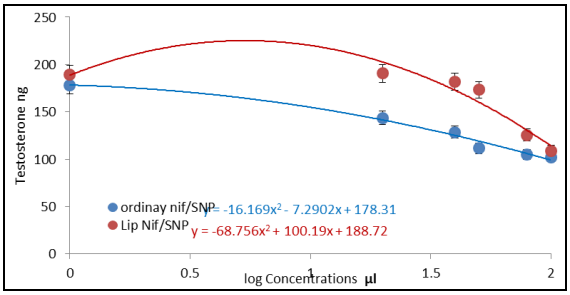
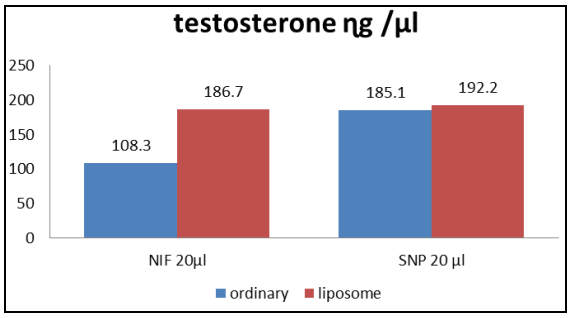


Diagram 3.3 the difference in the concentration of testosterone ηg /ml . These results showed the inhibitory effect of nifedipine while showing the stimulating effect of sodium natroprusside.They also showed the effect of synergism in the liposome and the removal of the side effect of nafdipine by sodiumnatroprusside liposome containing NIF&SNP and ordinary NIF&SNP



Diagrams 3.4 the effect of NIFand SNP with and without liposome in the concentration of 20 µl on the concentration of testosterone ηg /ml. These results showed the inhibitory effect of nifedipine while showing the stimulating effect of sodium natroprusside.

Discussion

The FESEM image of the liposomes containing NIF-SNP as shown in Figure 3.1 indicated spherical like shapes in the submicron size range The FESEM data showed liposomes surface aggregated into Polygonal scale-like shapes ranging in diameter (21.34,26.00 nm). This result agrees with [20] The quantitative assay was used to analyze the size and shape of liposomal by Transmission Electron Microscopy(TEM) The examination showed the for formation of liposomes and their probable decomposition with the materials used in the study NIF-SNP. The shape of the liposome appeared as a spherical bilayer shape consisting of two layers loaded with the NIF- the imaging field ranged between (300 – 50 nm) as shown in Figure 3.1 This agrees with the result found by [21].

The results of Fourier Transform Infrared (FTIR) as shown in Figure 3.1 the results of the examination liposome containing NIF-SNP, showed that this may indicate that SNP and NIF were loaded completely on the liposome and did not undergo chemical changes during the loading process. This is agreed with the results found by [22].

XRD Test results of NIF&SNP showed that the This may clearly indicate that the encapsulation process of led to a de-crystallization and reduction of crystals size and, This may be due to the effect of the continuous vortex process during encapsulation this is agreed with the result found by [23].

The zeta-potential results of the liposome containing NIF-SNP as shown in Figure 3.1 that it was more than -30mV about (-74.6 mV),This may clearly indicate that the NIF-SNP encapsulated by liposome is stable in structure, has little sedimentation and disintegration, and is also fast-acting, When the zeta potential of a nanoparticle is between 10 and +10 mV, it is may be said to be about neutral, however when it is larger than +30 mV or lower than 30 mV, it is said to be strongly cationic or anionic. Zeta potential can indicate a nanoparticle's propensity to penetrate membranes because the majority of biological membranes have a negative charge. Cationic particles typically exhibit more toxicity due to the breakdown of cell membrane this agree with [24].

The results of the numbers of Leydig cells showed a clear decrease in the numbers of Leydig cells that were treated with NIF in the concentration of 20µl. The number of cells was (2.27 ± 0.09 X 10^4 /ml) compared with the results of the control negative group, whose result was (6.38 ± 1.21 X 10^4 /ml). This result indicates the collateral damage of nifedipine, as it contributed to a decrease in the number of leydig cells, that is, it may lead to an increase in opoptosis. This may be due to the oxidative stress exerted by nifedipine as a calcium-blocker on Leydig cells. The decrease in the number of live cells necessarily leads to a decrease in testosterone secretion, and this result agrees with that found by [25].

However, the result of liposomes containing NIF was (4.4 ± 0.25 X 10^4/ml) compared to the control positive group (6.77 ± 2.12 X 10^4 /ml). This may indicate that liposomes reduced the effect of oxidative stress on Leydig cells and thus slightly reduced the programmed cell death [1].

The results of SNP at a concentration of 20µl showed that the number of cells was preserved, and there was no clear decrease as it was (6.08 ± 1.35 X 10^4) and the synargasm result of liposome countining NIF-SNP 20 µl was (7.35 ± 0.25 X 10^4), as shown in digrams 4.6 and 4.7.This may clearly indicate that sodium nitroprusside may abolish the apoptosis-inducing effect of nifedipine and reverse it by indicating that NO may up-regulate the expression of PPARGC1A and its downstream factors through the cGMP pathway, thereby decreasing granulosa cell apoptosis as mentioned in [26].

4. Conclusion

This study entitled Eliminating the negative effects of nifedipine steroidogenesis on rabbit Leydig cells in vitro by using Liposomes encapsulated nifedipine - sodium nitroprusside approved that:

1. Nifedipine has a negative effect on the

Leydig cells, as it inhibits the activity of Leydig cells.
2. liposomes have a role in increasing the effectiveness of treatment and reducing side effects and improving the integrity of the cell membrane.
3. Sodium nitroprusside has a positive and stimulating effect on Leydig cells at low concentrations less than 80µl.
4. Sodium natroprusside and nifedipine encapsulated in liposomes have improved the steroidal activity of Leydig cells by reversing the side effects of nifedipine.

5- References

[1] Ebiya RA, Montaser MM, Darwish SM. Downregulated StAR gene and male reproductive dysfunction caused by nifedipine and ethosuximide. The Journal of Basic & Applied Zoology. 2016;76:42-51.
[2] Morakinyo AO, Iranloye BO, Adegoke OA. Antireproductive effect of calcium channel blockers on male rats. Reproductive medicine and biology. 2009;8:97-102.
[3] Toor J, Sikka S. Rep Dev Toxicol. Elsevier; 2017.
[4] Hottinger DG, Beebe DS, Kozhimannil T, Prielipp RC, Belani KG. Sodium nitroprusside in 2014: A clinical concepts review. Journal of anaesthesiology, clinical pharmacology. 2014;30(4):462.
[5] Dutta S, Sengupta P. The role of nitric oxide on male and female reproduction. The Malaysian Journal of Medical Sciences: MJMS. 2022;29(2):18.
[6] Sun W, Wang F, Zhang B, Zhang M, Küpers V, Ji X, et al. A rechargeable zinc-air battery based on zinc peroxide chemistry. Science. 2021;371(6524):46-51.
[7] Shi X, Zuo Y, Zhai P, Shen J, Yang Y, Gao Z, et al. Large-area display textiles integrated with functional systems. Nature. 2021;591(7849):240-5.
[8] Prochaska JH, Göbel S, Keller K, Coldewey M, Ullmann A, Lamparter H, et al. Quality of oral anticoagulation with phenprocoumon in regular medical care and its potential for improvement in a telemedicine-based coagulation service–results from the prospective, multi-center, observational cohort study thrombEVAL. BMC medicine. 2015;13:1-11.
[9] Sarkar N, Bose S. Liposome-encapsulated curcumin-loaded 3D printed scaffold for bone tissue engineering. ACS applied materials & interfaces. 2019;11(19):17184-92.
[10] Baxa U. Imaging of liposomes by transmission electron microscopy. Characterization of nanoparticles intended for drug delivery. 2018:73-88.
[11] Petersen ALdOA, Campos TA, Dantas DAdS, Rebouças JdS, Da Silva JC, De Menezes JP, et al. Encapsulation of the HSP-90 chaperone inhibitor 17-AAG in stable liposome allow increasing the therapeutic index as assessed, in vitro, on Leishmania (L) amazonensis amastigotes-hosted in mouse CBA macrophages. Frontiers in cellular and infection microbiology. 2018;8:303.
[12] Severcan F, Sahin I, Kazancı N. Melatonin strongly interacts with zwitterionic model membranes—evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2005;1668(2):215-22.
[13] Zhao L, Temelli F. Preparation of liposomes using a modified supercritical process via depressurization of liquid phase. The Journal of Supercritical Fluids. 2015;100:110-20.
[14] Marin V, Kaplanski G, Gres S, Farnarier C, Bongrand P. Endothelial cell culture: protocol to obtain and cultivate human umbilical endothelial cells. Journal of immunological methods. 2001;254(1-2):183-90.
[15] Chen G-R, Ge R-S, Lin H, Dong L, Sottas CM, Hardy MP. Development of a cryopreservation protocol for Leydig cells. Human reproduction. 2007;22(8):2160-8.
[16] Steinberger A, Klinefelter G. Sensitivity of Sertoli and Leydig cells to xenobiotics in in vitro models. Reproductive Toxicology. 1993;7:23-37.
[17] Milon A, Knapczyk-Stwora K, Pawlicki P, Duliban M, Gorowska-Wojtowicz E, Kotula-Balak M, et al. Effect of estrogen-related receptor silencing on miRNA protein machinery expression, global methylation, and deacetylation in bank vole (Myodes glareolus) and mouse tumor Leydig cells. Theriogenology. 2019;139:178-90.
[18] Slater CH, Schreck CB. Testosterone alters the immune response of chinook salmon, Oncorhynchus tshawytscha. General and comparative endocrinology. 1993;89(2):291-8.
[19] Herold DA, Fitzgerald RL. Immunoassays for testosterone in women: better than a guess? : Oxford University Press; 2003. p. 1250-1.
[20]Rushmi ZT, Akter N, Mow RJ, Afroz M,

Kazi M, de Matas M, et al. The impact of formulation attributes and process parameters on black seed oil loaded liposomes and their performance in animal models of analgesia. Saudi pharmaceutical journal. 2017;25(3):404-12.
[21] Jeon S, Yoo CY, Park SN. Improved stability and skin permeability of sodium hyaluronate-chitosan multilayered liposomes by Layer-by-Layer electrostatic deposition for quercetin delivery. Colloids and surfaces B: biointerfaces. 2015;129:7-14.
[22] Ji H, Zhao W, Yu Z. Interaction mechanism of three egg protein derived ACE inhibitory tri-peptides and DPPC membrane using FS, FTIR, and DSC studies. Food Chemistry: X. 2022;15:100366.
[23] Eswaran M, Tsai P-C, Wu M-T, Ponnusamy VK. Novel nano-engineered environmental sensor based on polymelamine/graphitic-carbon nitride nanohybrid material for sensitive and simultaneous monitoring of toxic heavy metals. Journal of Hazardous Materials. 2021;418:126267.
[24] Acar M, Solak K, Yildiz S, Unver Y, Mavi A. Comparative heating efficiency and cytotoxicity of magnetic silica nanoparticles for magnetic hyperthermia treatment on human breast cancer cells. 3 Biotech. 2022;12(11):313.
[25] Mardihusodo HR, Rizaldi F, Hakim L. The Effect of Nifedipine on Apoptosis, Necrosis and Viability of Germinal Epithelial Cells in The Contralateral Testicle and Plasma Testosterone Levels in Male White Rats (Rattus Novergicus, Wistar Strain) After Unilateral Testicular Torsion. Indonesian Journal of Urology. 2019;26(2):166-73.
[26] Zhang J, Ye R, Grunberger JW, Jin J, Zhang Q, Mohammadpour R, et al. Activation of autophagy by low-dose silica nanoparticles enhances testosterone secretion in leydig cells. International Journal of Molecular Sciences. 2022;23(6):3104.

عكس التاثير الجانبي للنفدبين على خلايا لايدج الماخوذة من ذكور الارانب في المختبر بواسطة استخدم الايبوسوم الحاوي على نفدبين و نتروبروسيد الصوديوم

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الملخص

ان الهدف من هذه الدراسة هو النظر في تأثير الجانبي النفدبين على نشاط خلايا ليديك في المختبر. كما هدفت ايضا إلى دراسة تاثير نتروبروسيد الصوديم على خلايا لايدك وازالة التاثير السلبي للنفدبين بواسطة الايبوسوم الحاوي على نفدبين و نتروبروسيد الصوديوم .تم إنشاء مجموعتين رئيسيتين؛ تلقى الأول العلاج باستخدام النفدبين نتروبروسيد الصوديوم العادي، والثاني تلقى العلاج باستخدام النفدبين نتروبروسيد الصوديوم المغلف باليبوسوم. كما كان النفدبين موجودًا في 20 ميكرولتر وكان نتروبروسيد الصوديوم موجودًا في 20 و40 و60 و80 و100 ميكرولتر على التوالي. بالإضافة مجموعة مراقبة إيجابية ومجموعة مراقبة سلبية كعناصر تحكم إضافية. كشفت نتائج خلايا لايدك أن النفدبين كان له تأثير سلبي على نشاط خلايا لايديك في المختبر، وبالتالي له تاثيرات جانبية سلبية على عملية تصنيع الستيرويد. واثبتت الدراسة أيضًا ان نتروبروسيد الصوديوم كان له تأثير تحفيزي إيجابي على خلايا لايدك بتركيزات منخفضة لكنه كان له تاثير سمي في التاراكيز العالة اكثر من 80 مايكرو لتر، وجدت الدراسة الحالية الايبوسوم المحتوي على نفدبين – نتروبروسيد الصوديوم قللت من التأثيرات السلبية للنفدبين على خلايا كما حسنت أيضًا نشاط الستيرويد في خلايا لايدك.