Plant Archives Vol. 21, Supplement 1, 2021 pp. 757-769 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.114

THE REPRODUCTIVE EFFECT OF LIPOSOMAL CIMETIDINE ON MALE MICE

Fatima Mofaq Abd-alhussein and Mohanad A. Al-Bayati
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad , Iraq
Email: fatimamuafaq22@gmail.com : aumnmumu@covm.uobaghdad.edu.iq

ABSTRACT

The experiment conducted to reformulation of cimetidine antacid into Nano-liposome form for the reduction of worsening effect on the male reproductive system. Nano Liposome encapsulated cimetidine was formulated according to Bangham thin-film, *in vivo* challenge of ordinary and Nanoliopsome formulated form, the forty male mice distributed into 4 groups ten for each according to treatment Control mice: Negative control: Pro liposome dosed mice (Esome): Positive control empty liposome 1% 0.1mg/10g body weight daily orally Cimetidine dosed mice (CIM): 20mg/Kg body weight daily orally Liposome Cimetidine dosed mice (CIMsome): 20 mg/Kg body weight daily orally for 38 days. the liposome cimetidine standardization outcome was driven multi-lamellar;1-3, multi-vesicles type, size ranged 21.6-213.3 nm. The loading efficiency and entrapment percentage of cimetidine liposome 76.13 ± 6.91% and 80.06 ± 5.76 % respectively. The results of body condition of in cimetidine liposome treated group did not affect significantly the animals performances. Hormonal level of cimetidine liposome dosed group produce improvement. The endpoint of cimetidine liposome dosed group was recovered values and corrected modification in the morphometric indices (testicular weight to body weight ratio, testicular volume) and stereometric analysis of testicular tissue;(quantitation of spermatogenic cells and Leydig cells indices). The turbidimetric analysis that associated with a recovered in the sperm viability and lessening in sperm abnormalities in the cimetidine liposome dosed compared with ordinary cimetidine dosed group The antioxidant activity of liposome cimetidine clearly appear on the genetic material, reducing DNA abnormalities. The Liposome Cimetidine was partial protected the tissue of post testicular blood testes barrier from cimetidine direct effect.

***Keywords*:** liposome, cimetidine, spermatogenesis, nanoparticles.

Introduction

For the first time in 1965, Banghum find out that phospholipid molecules could form spontaneous two-layer closed vesicles. Liposomes which is small spherical molecules with size ranged 5-500 nm had amphibious nature enable it to encapsulated hydrophilic and lipophilic drugs in tis aqueous core or by its bilayer phospholipid membrane (Greish, 2012). Liposomes had many advantages that made it a very useful tool in lipid-based Nano delivery system decrease the side effect to a certain extent. reduce the uptake of drugs from normal tissue. liposomes have low toxicity, antigenicity, prolonged releasing time of therapeutic agent, increase the solubility of the hydrophobic drug. increasing stability of the drug in *vivo*. (Yingchoncharoen *et al*., 2005). Liposomes provide selective passive targeting to tissue, in case of lipophilic drugs liposomes reduce the kidney excretion and prolonged the half-life. Cimetidine is one of the most potent H_2 receptor antagonists (Over the counter). Its block the histamine action in the basolateral parietal cell and reducing the acid secretion in the stomach. Also used in the treatment of erosive gastro-esophageal reflux and Zollinger-Ellison syndrome. Cimetidine has been interacting as dose-related inhibition with cytochrome P-450 mediated oxidation and enzyme inhibitor to many similar form of CYP including 1A2, 2C9, 2D6, 3A4 Clinically relevant inhibition of cytochrome 3A4 and 1A2 (Pino *et al*., 2019). Cimetidine had anti-androgen properties. Cimetidine adverse effect which loss of libido, impotence and gynecomastia, and explained to be related to anti-androgen effect, In the testicles, this drug has caused major tissue disorders in the seminal tubes, including reducing tubular diameter and epithelial area due to separation and loss of germ cells through apoptosis (Sasso-Cerri *et al*., 2002). This change appears to be the result of cimetidine interference on the tissues surrounding the tubules (França *et al*., 2000). studies have demonstrated that cimetidine causes testicular atrophy (Beltrame *et al*., 2017). The study was aimed at the reformulation of cimetidine into Nanoliposome for lessens the testicular tissue disruption and associated with the effect of the H2 receptor antagonist on the body condition and determine the sperm parameters, histopathological changes within the testicular tissue, and hormonal level.

Materials and Methods

Animal management and experimental design

Forty mature male mice weighing 25-35gm used in this experiment, the animal chosen randomly and divided into 4 groups for 38 days as follows**:** Control mice: Negative controlPro liposome dosed mice (Esome): Positive control empty liposome 1% 0.1mg/10g Bw daily orallyCimetidine dosed mice (CIM): 20mg/Kg Bw daily orallyLiposome Cimetidine dosed mice (CIMsome): 20 mg/Kg Bw daily orall.

Liposome Preparation and Standardization

Nano Liposome encapsulated cimetidine (Multi vesicles multi lamellar) was prepared according to Bangham thin-film procedure by phosphatidylcholine-cholesterol 1:1w/w (0.5-0.5) dissolved by chloroform-methanol 1:2 v/v (15 -5) ml. Examination of liposome cimetidine standardization outcome, the average size and lamellar were estimated by microscopy techniques this techniques are available for assessing micrometer liposome size which include scan type and transmission scattering technique. The Entrapment estimation laden liposome was measured the entrapment amount of cimetidine by estimated the non-entrapped volume and quantity of the drug. Absorbance curve and λ curve was take a look at spectrum in sequences of Wavelength 200-900 nm at 1% at 25 °C and attributed the best point curve absorbance (476)nm. Osmolartiy tolerance challenge and PH tolerance challenge was done according to (Al-Yasiri and Al-Bayati 2020, Jaafer *et al*., 2020).

Cumulative food intake: The Cumulative food intake was determined by an abstraction of the remaining weight of feed. (Westerterp, 2000).

Determination of body condition parameters

Body weight (gm) = b.w. after treatment –b.w. before. After 38 day of the treatment period finished**.** growth rate was performed to demonstrate weight gain per unit of time. (Gargiulo *et al*., 2014)

Euthanasia and blood collection

The animals were anesthetized and blood collected to obtain serum, the testis excised and cleaned from the fat tissue.

Testicular body weight indices the morphometric indices calculated: Testes weight and compared to body weight as following : Testicular weight. to body weight. ratio = Weight of testis (gm)X 100/Weight of animal (gm).The volume of testis was calculated by volumetric redual method according to: Volume of testis = V2 volume of testis after dip -V1 volume of testis before dip

Preparation of sperm suspension

Epididymis putted in 1 ml of normal saline at 37 °C in glass watch and minsed to 200 times with microsurgical scissor to free the spermatozoa. Turbidmeteric analysis Spectrophotometric method used to evaluate Lag time (sec.),Velocity of rapidly moving sperm fraction (Vrm) µ/sec, Motility index (SMI),Total sperm count, Fraction of rapidly moving sperm. Sperm viability % and Sperm morphology % according to (Cooper *et al*., 2010, Mohammed and Al-Bayati, 2014).

Daily sperm production The daily sperm yield of the male mice was calculated by a minor modification of the technique described by Joyce *et al*. (1993).

Evaluation of DNA abnormalities

Determination of DNA abnormalities done by cytochemical techniques using inductive dyes Acridine orange test (AO), Aniline blue (AB), and Tulidine blue (TB) (Talebi *et al*., 2012).

Determination of Testosterone, LH and FSH Level:

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.

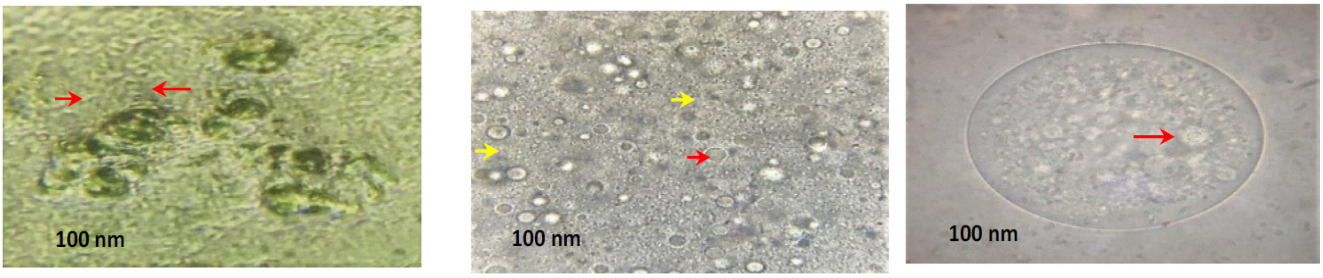
Result

000Liposome Standardization

Physical characteristic

Parameters		Liposome cimetidine
Size nm	Range	21.600 - 213.3
	Mean	69.912 ± 4.746
Entrapment	E%	80.060 ± 5.760
	EE%	76.130 ± 6.910
Lamellar	Range	1-3
	Mean	1.786 ± 0.261

Table 1: Physical characteristic of liposome entrapped cimetidine rang and size and lamellar, represented as mean ± SE, E: Entrapment %, EE Entrapment Efficiency %



The Light micrograph of cimetidine liposome denoted red arrow to the lamellar layers of multi - lamellar multi-vesicles liposome containing cimetidine

The Light micrograph of cimetidine liposome symbolized red arrow to the entrapped cimetidine liposome and yellow arrow represented the empty liposome

The Light micrograph of cimetidine liposome signified red arrow to the multi vesicles cimetidine liposome in aggregated

Fig. 1: The light micrograph of cimetidine liposome

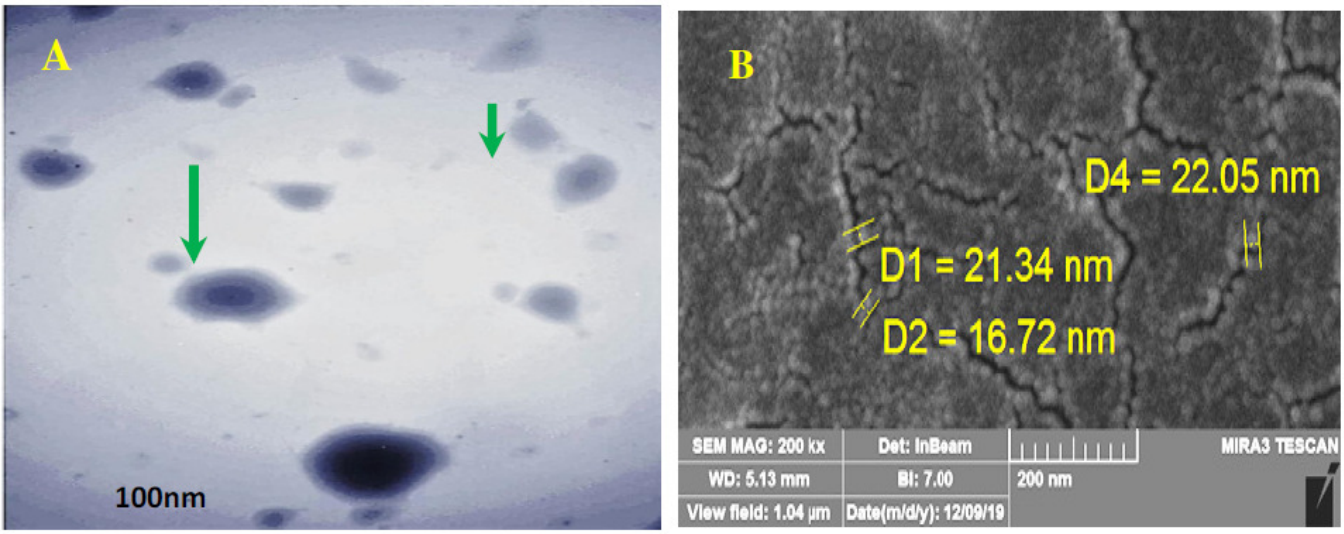


Fig. 2: Micrograph illustration transmission and scan electron microscope of liposome cimetidine A transmission micrograph and B and Scan micrograph mention size of liposome green arrow besides lamellar denoted..

Absorbance curve of liposome loading drug: The absorbance curve UV- visible result of liposome entrapped cimetidine

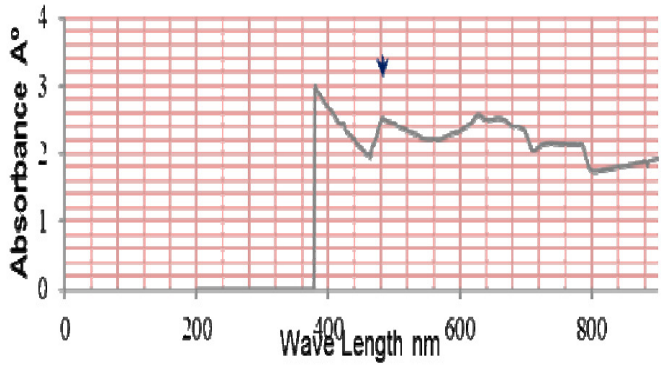


Fig. 3: Spectroscopic absorbance curve UV-visible of cimetidine liposome λ 476 nm

Liposome osmotolerance

The osmotolerance result of liposome type show no significant change between (0.45-0.85) as compared with the other osmo-change point and between the time of osmo-change exposure, the shading area was indicated that variation between times exposure and NaCl concentration.

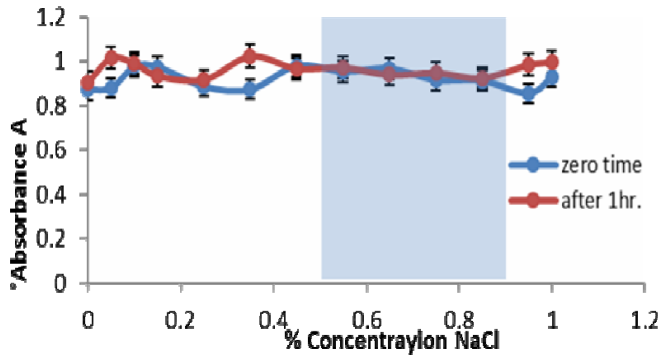


Fig. 4 : The osmo-tolerance in NaCl challenge solution of cimetidine liposome at zero and after one hour.

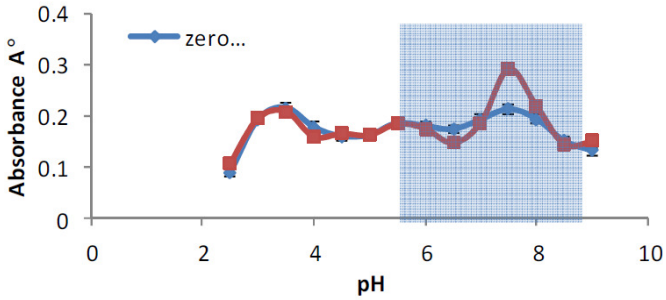


Fig. 5: The pH-tolerance in Acid-Base challenge solution of cimetidine liposome at zero and after one hour.

Cumulative food intake Impact of the cimetidine and liposome entrapped cimetidine treatments forms on cumulative food intake male mice: The results post 38 days with groups cimetidine, liposome entrapped cimetidine, and both positive and negative control; empty liposome and control, the results with cimetidine ordinary formula significantly ($p \leq 0.05$) reduced cumulative food intake as compared to the other groups. Whereas the liposome entrapped cimetidine treated group did not exhibit changes although the reduction in food intake at the final week of treatment with cimetidine did not reach statistical significance ($p > 0.05$) as compared with negative and positive control groups, the cumulative food intake amplification effects of the empty liposome as positive control between 2nd and 3rd weeks (14-28 days) were remarkably comparable to negative and liposome entrapped cimetidine (figure 6).

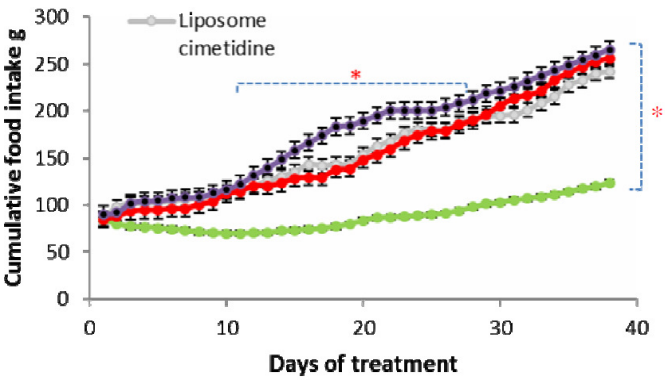


Fig. 6: Experimental data are presented as the mean ± SEM. *Dented the significant $p \leq 0.05$ changes within days of treatments. ** Dented the significant $p \leq 0.05$ changes between treated groups. The results with cimetidine ordinary formula significantly ($p \leq 0.05$) reduced cumulative food intake as compared to the other groups.

Body weight and Growth ratio

The body weight ameliorating liposome cimetidine (36.3±2.49) from reducing body weight effects of the cimetidine were significantly ($p \leq 0.05$) comparable to cimetidine (figure 7). At the end of the treatment period, the body weight changes of the cimetidine treated mice decreased by (-2.32±0.026) ($p > 0.05$). Liposome cimetidine (4.54±0.52) did have significant ($p \leq 0.05$) effects on body weight changes as

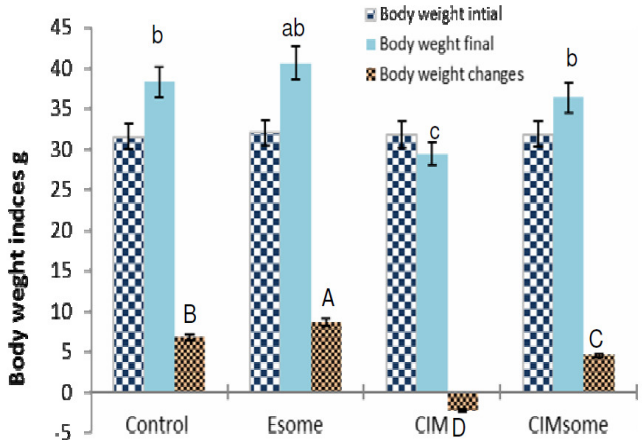


Fig. 7: Body weight and body weight changes of albino mice on a regular diet with cimetidine (CIM), Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control**.** Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p \leq 0.05$ between body weights. Capital litter denoted the significant $p \leq 0.05$ between body weight changes.

The mice growth rate in figure (8) showed ordered coincided with the body weight changes of significant values: control (0.178±0.004), empty liposome (0.226±0.039), cimetidine (0.061±0.002) and liposome entrapped cimetidine (0.119±0.017).

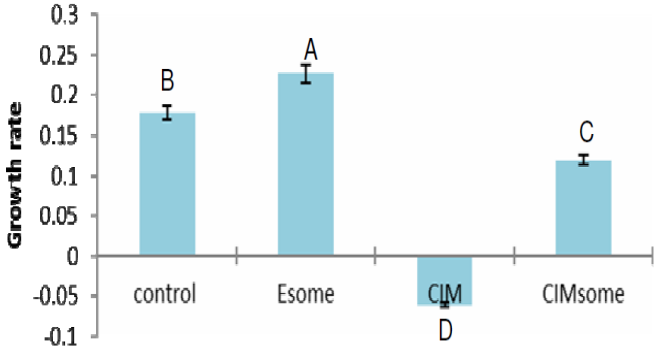


Fig. 8: Experimental data are presented as the mean ± SEM. Capital litter denoted the significant $p \leq 0.05$ between growth rates.

Testes to body weight ratio %

The testicular weight was recorded on 38 days of treatment, Generally, the testicular weights of CIM treated mice (0.062±0.007) were significantly ($p \leq 0.05$) lower as compared to other treated groups. However, the Esome treated mice (0.028±0.004), control (0.025±0.003), but the testicular weight of CIMsome (0.0262±0.0017) remained to keep the weight of the testicles ($p > 0.05$) in comparison to groups Esome and control during the whole study period. Testes to body weight ratio were significantly ($p \leq 0.05$) reduced in groups CIMsome (0.055±0.0042%) as compared with other groups, while no statistically significant ($p > 0.05$) changes were in testicular weight among other groups (Control 0.0653±0.0018, Esome 0.0689±0.0049 and CIMsome 0.055±0.0026) (figure 9).

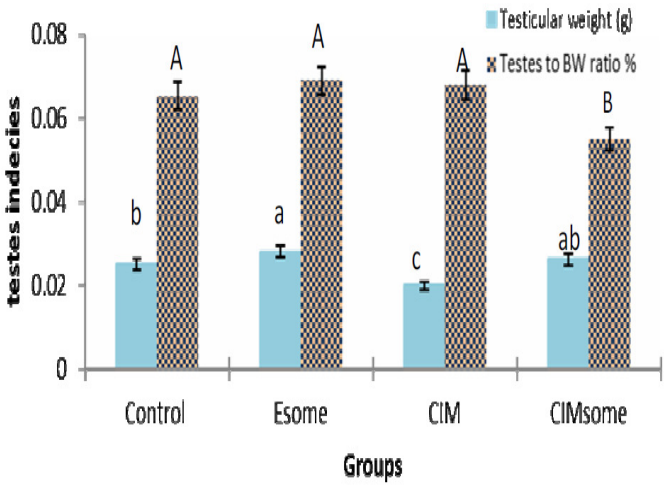


Fig. 9: Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p \leq 0.05$ between body weights. Capital litter denoted the significant $p \leq 0.05$ between body weight changes.

Testicular volume (cm^3) and dimension (cm)

Testicular volume was not affected ($p > 0.05$) by CIMsome (1.71±0.143) treatment with control but was increased significantly ($p \leq 0.05$) by treatment with CIM (1.17±0.221). Treatment with Esome (1.96±0.087).Exposure to CIM (1.44±0.125) resulted in a significant ($p \leq 0.05$) decrease in testis dimension, compared with control mice (1.78±0.058), and in males treated to CIMsome (1.77±0.201), testicular dimension was no significant ($p > 0.05$) changes as compared with control and Esome (1.86±0.115), although Esome was not statistically significant changing of dimension as compared with control (Figure 10).

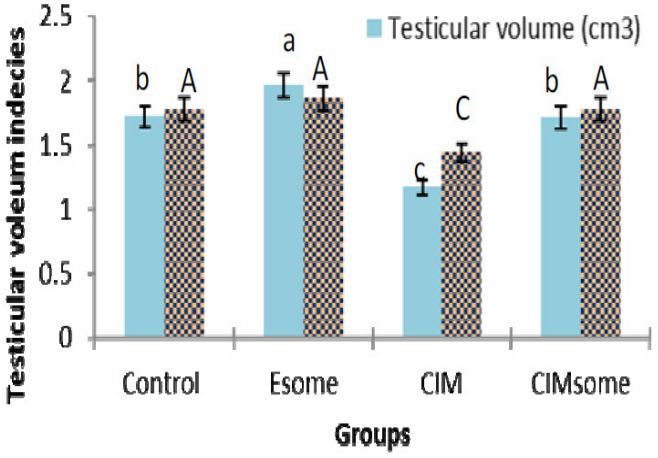


Fig. 10: Testicular volume and dimension of albino mice on a regular diet with cimetidine (CIM); Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control.

Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p \leq 0.05$ between testicular volumes. Capital litter denoted the significant $p \leq 0.05$ between testicular volumes.

Daily sperm yield

Daily sperm yield Esome ($60.19 \pm 7.04 \times 10^6$ cells/g/day) was higher than in control and CIMsome mice ($52.63 \pm 7.04 \times 10^6$ and $50.81 \pm 6.95 \times 10^6$ cells/g/day) (Figure 11). Whereas CIM treated mice was lower than other groups, this result specifies that the daily sperm yield of the treated mice was affected by the Cimetidine in testis yield and function.

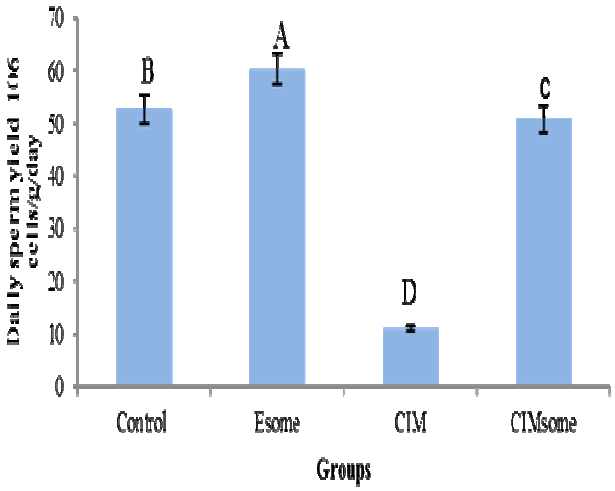


Fig. 11: The Daily sperm yield of albino mice on a regular diet with cimetidine (CIM), Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control.

Experimental data are presented as the mean ± SEM. Capital litter denoted the significant $p \leq 0.05$.

Hormonal levels (ng/ml)

Figure (12) illustrates plasma testosterone levels in treated mice after 38 days of a daily loading dose (20 mg per Kg Bw). Testosterone concentrations in serum had values parallel association with the levels of the LH in all groups except CIMsome, sequential significant ($p \leq 0.05$) lower deviations in serum testosterone levels in CIMsome (0.0268±0.0026 ng/ml) than other groups. Whereas, serum concentrations of LH (0.255±0.0574 ng/ml) testosterone levels (0.0925±0.0135) with CIM administration in male mice, there were increased significantly ($p \leq 0.05$) to as high as compared with other groups ng/ml. There were no significant ($p > 0.05$) changes between CIMsome (0.174±0.0331 ng/ml), Esome (0.190±0.0251 ng/ml) and control (0.186±0.0058 ng/ml) groups in LH levels. Profiles of serum FSH levels of male mice stimulated are clarified as follows. Serum FSH remained unchanged ($p > 0.05$) even with control, Esome, CIM and CIMsome (0.0106±0.0023 ng/ml, 0.0108±0.0047 ng/ml, 0.0110±0.0031 ng/ml and 0.0104 ±0.0029 ng/ml) respectively in males of mice.

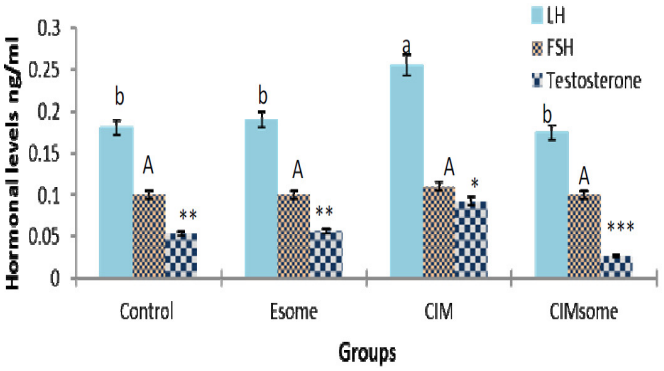


Fig. 12: Serum hormonal levels of luteinizing hormone (LH), testosterone and Follicular stimulating hormone of albino mice on a regular diet with cimetidine (CIM); Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control.

Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p \leq 0.05$ between LH levels. Capital litter denoted the significant $p \leq 0.05$ between FSH levels and the star's difference between testosterone levels.

Spermatogram indices

The percentages of abnormal spermatozoa are shown in figure (13). Morphological assessments of semen revealed a significant ($p \leq 0.05$) a higher percentage of spermatozoa with abnormal morphology in CIM treated groups 34.27±3.84 as compared with control (12.76±2.50) other groups. The significantly decreased ($p \leq 0.01$) incidences of sperm abnormality in groups Esome (2.17±0.63) and CIMsome (4.25±0.78) were detected as compared with other groups. Moreover, the percentage of sperm defects was significantly ($p \leq 0.05$) higher in the CIMsome group than Esome. The percentage of sperm viability significantly ($p \leq 0.05$) decreased in CIM treated groups (45.21±3.39). Whereas, the sperm viability in Esome treated mice (95.33±7.17) was showed significant ($p \leq 0.05$) predominant increase of viability as compared with other groups as well as no significant differences between CIMsome (88.59±6.02) and control groups (87.12±4.51).

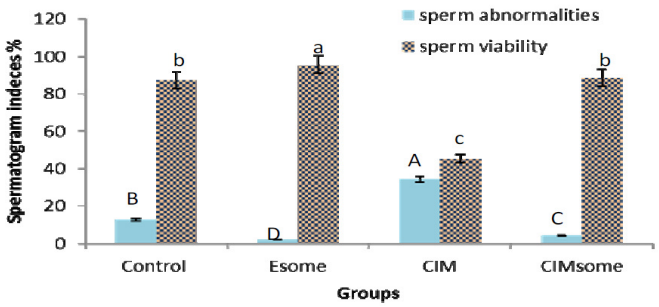


Fig. 13: Spermatogram indices; Sperm abnormalities and sperm viability of albino mice on a regular diet with cimetidine (CIM); Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control.

Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p \leq 0.05$ between sperm abnormality.

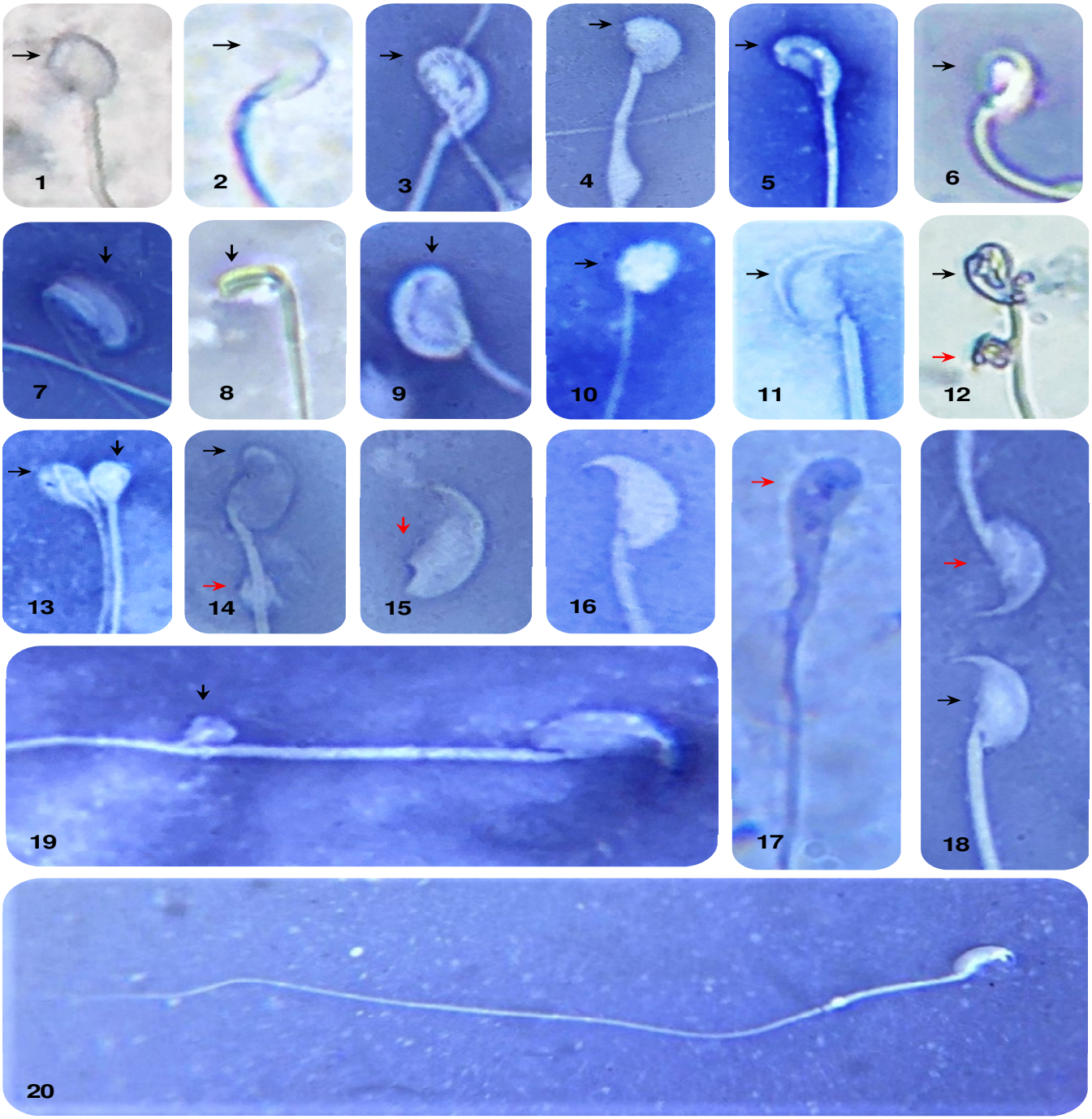


Fig. 14: Head deformity 1-6-7-13-14. Sperm head spike loss 7. Irregular and spick loss head 8. Micro and pend head sperm 9. pear-shape head sperm 10. Micro-irregular head sperm 11. Detached acrosome 12. Crooked head with vaculation 13. Micro head and spike loss 15. Tailless head 17. Long irregular pear-shape head.

Tail deformity 14. persist of cytoplasmic droplets 12-19. split of axial fiber (red arrow)

Normal sperm 16, Normal head of life sperm, 18. Dead sperm blue dye (red arrow) life sperm colorless (black arrow) 20. Normal sperm

Influence of the cimetidine and liposome entrapped cimetidine treatments forms on sperm motility analysis in male mice: The turbidimetric analysis of sperm motility results traced curve kinetic of sperm motion was displayed in control normal curve slop of Fraction of rabidly moving sperm A_1 and slope A_2 of maintenance sperm motility having a simple second distribution of slowly moving sperm, the calculated indices of sperm of motility behavior Lag time per 3.650±0.281sec, Fraction of rabidly moving sperms 0.113±0.013 $\times 10^6$ Sperm velocity µm/Sec 10.49±1.402 and Motility index percentage 1.330±0.011.The recording optical absorbance changes curve of sperm motility of cimetidine treated mice was showed shallower slope observed in recordings like that displayed in figure (14), as compared with other treated mice and significant ($p \leq 0.05$) lower values of Fraction of rabidly moving sperms 0.035±0.005$\times 10^6$, sperm velocity µm/Sec 3.620±0.139 and Motility index percentage 0.741±0.034 and longer Lag time 11.80±0.111 sec. The absorbance trace curve of CIMsome treated mice showed in figure (15) normal behavior of task in motility kinetic of movement path, and the Lag time simulation ($p > 0.05$) the control group and

Esome was 3.720±0.200 sec, whereas, others Fraction of rabidly moving sperms 0.166±0.038×10^6 Sperm velocity µm/Sec 15.55±1.091and Motility index percentage 2.020±0.222 significant (p≤0.05) higher than control and CIM treated mice.The mice treated Esome the sperms motility curve rise and shift to the right than other treated mice sperm curves, and factually that may refection on motility parameters Lag time per 3.890±0.014 sec, Fraction of rabidly moving sperms 0.186±0.027×10^6 Sperm velocity 16.83±0.250 µm/Sec and Motility index percentage 2.809±0.063 significant (p≤0.05) higher than others

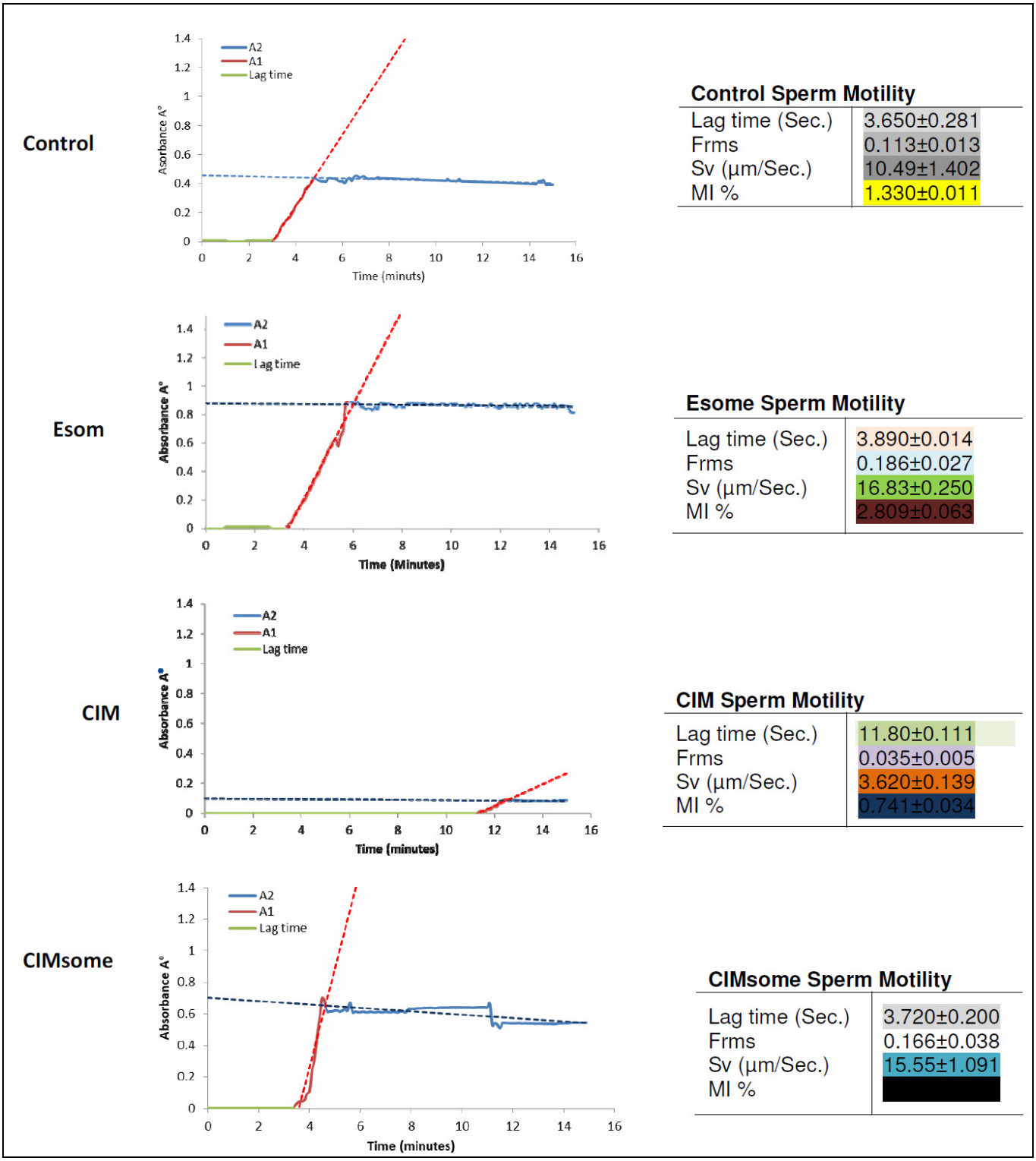


Fig. 15: Turbidimetric analysis of sperm motility; Lag time, Fraction of rabidly moving sperm (Frms), Sperm velocity (Sv) and Motility index (MI) of albino mice on a regular diet with cimetidine (CIM); Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control. Experimental data are presented as the mean ± SEM. Different color denoted the significant p≤0.05 between groups of certain parameters.

Turbidimetric trace of sperm motility:

Control regular tracing increment of motile sperm with obvious Frms and Lag time, Esome displayed amplification in trace curvature and line of Frms and short Lag time, CIM shallow curve of sperm motility and long Lag time, CIMsome showed a regular trace of sperm motility and Lag time

DNA Abnormality

Impact of the cimetidine and liposome entrapped cimetidine treatment forms on sperm DNA indices of abnormalities percentage in male mice: The DNA defect results of CIM treated mice was showed significant ($p\leq0.05$) higher values than other mice groups under exploring dyes aniline blue, acridine orange, and toluidine blue. Both Esome and CIMsome treated mice showed significant ($p\leq0.05$) lower DNA abnormality in all tested dyes as compared with the control group, whereas the toluidine blue dye test in Esome significant ($p\leq0.05$) higher than CIMsome figure(16).

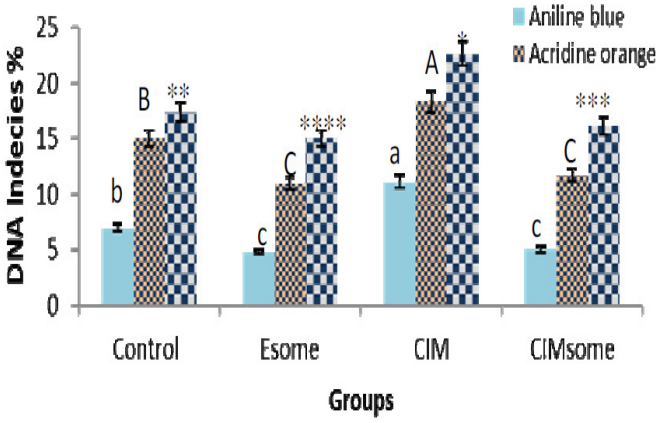


Fig. 16: DNA indices of abnormalities; Aniline blue, Acridine orange, and Toluidine blue of albino mice on a regular diet with cimetidine (CIM); Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control. Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p\leq0.05$ between aniline blue. Capital litter denoted the significant $p\leq0.05$ between acridine orange and the star's difference between toluidine

Leydig cells indices

The Leydig cells indices results of CIM (LC/ST 6.11±0.77, LC in CS/ST 5.04±0.83 and CS/ST 102.36±3.22) and CIMsome (LC/ST 6.95±0.52, LC in CS/ST 5.66±0.47 and CS/ST 117.87±9.58) treated mice was showed significant ($p\leq0.05$) lower values than other mice groups. CIMsome treated mice showed significant ($p\leq0.05$) higher Leydig cells indices than CIM treated groups, whereas, the indices of the Leydig cells in CIMsome statistically showed approximately no significant ($p>0.05$) as compared with the control group (LC/ST 6.91±0.37, LC in CS/ST 5.85±0.72 and CS/ST 123.75±4.69). The Esome (LC/ST 7.81±1.15, LC in CS/ST 6.09±0.99 and CS/ST 126.55±5.53) treated group showed significant ($p\leq0.05$) higher Leydig cells indices as compared with the control group, figure (17).

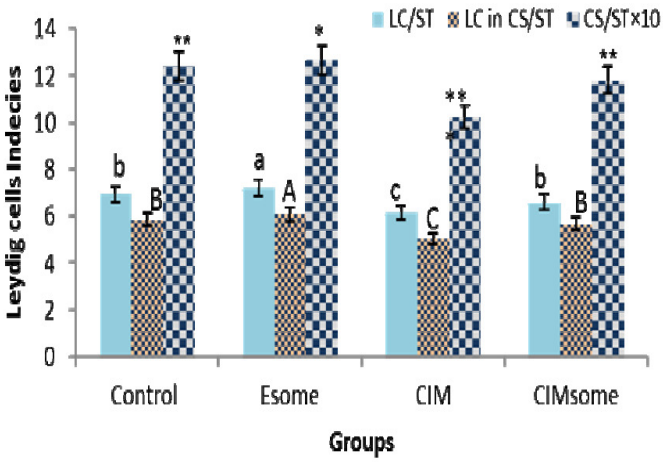


Fig. 17: Leydig cells indices; Leydig cells/Seminiferous tubules (LC/ST), Leydig cells in cluster/Seminiferous tubules (LC in CS/ST), and Leydig cells clusters/ Seminiferous tubules (CS/ST×10) of albino mice on a regular diet with cimetidine (CIM); Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control. Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p\leq0.05$ between LC/ST. Capital litters denoted the significant $p\leq0.05$ between LC in CS/ST and the star's difference between CS/ST×10.

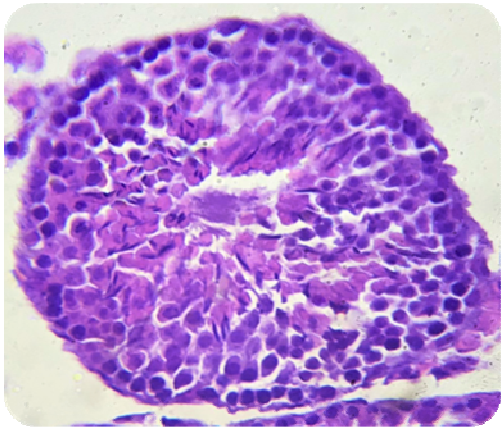
Quantitation of spermatogenic cells

The percentages of abnormal spermatozoa are shown in figure (18). The numerical amount of testicular cells yield revealed a significant ($p\leq0.05$) a lower Spermatogonia-Preleptoten (SPG-PL) in CIM treated groups (16.06±1.315) as compared with control (20.31±1.593) other treated groups. The significantly increased ($p\leq0.01$) in group Esome (22.07±0.844) as compared with other treated groups, whereas, CIMsome (91.95±1.820) were detected no significant ($p>0.01$) as compared with the control group. Moreover, the Preleptotine-Pachytene (PL-PCH) was significantly ($p\leq0.05$) lower in the CIM group (0.557±0.074) than control (0.832±0.039) and other treated groups, as well as the CIMsome, treated group (0.799±0.066) showed significant ($p\leq0.05$) increase as compared with CIM. Furthermore, there were no significant ($p>0.05$) between Esome (0.980±0.029) and control and between CIMsome and control. The Pachytene-Spermatid step 7 (PCH-ST7) and Spermatid step 7-step16 (ST7-ST16) significantly ($p\leq0.05$) decreased in CIM treated groups (1.127±0.197 and 0.314±0.085) respectively as compared with control and other treated groups. Whereas, Esome treated mice (PCH-ST7: 2.327±0.200 and ST7-ST16: 0.402±0.019) respectively was showed no significant ($p>0.05$) differences as compared with control as well as no significant ($p>0.05$) differences between CIMsome (PCH-ST7: 1.991±0.158 and ST7-ST16: 0.356±0.018) and control groups (87.12±4.51).

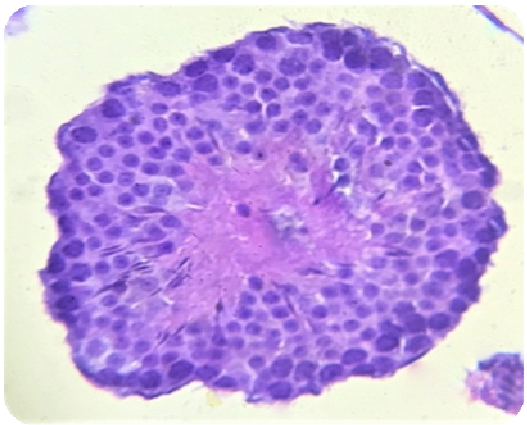
Table 2 : Spermatogonia-Preleptoten (SPG-PL), Preleptotine-Pachytene (PL-PCH), Pachytene-Spermatid step 7 (PCH-ST7) and Spermatid step 7-step16 (ST7-ST16) of albino mice on a regular diet with cimetidine (CIM); Liposome encapsulated cimetidine (CIMsome), empty liposome (Esome) treatment, and control. Experimental data are presented as the mean ± SEM. Small letters denoted the significant $p \leq 0.05$ between treatment groups.

Groups Cells	Control	Esome	CIM	CIMsome
SPG-PL	20.31±1.593 B	22.07±0.844 a	16.06±1.315 C	91.95±1.820 b
PL-PCH	0.832±0.039 Bc	0.980±0.029 ab	0.557±0.074 D	0.799±0.066 c
PCH-ST$_7$	2.115±0.206 Ac	2.327±0.200 a	1.127±0.197 D	1.991±0.158 c
ST$_7$-ST$_{16}$	0.395±0.008 Ac	0.402±0.019 a	0.314±0.085 D	0.356±0.018 c

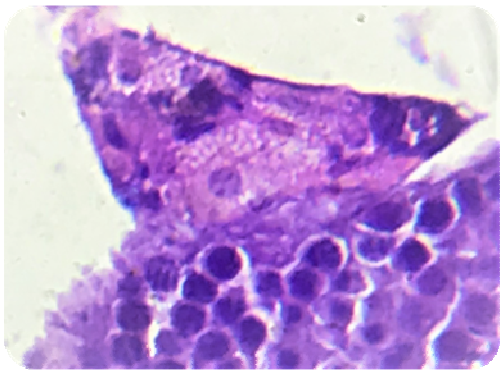
.



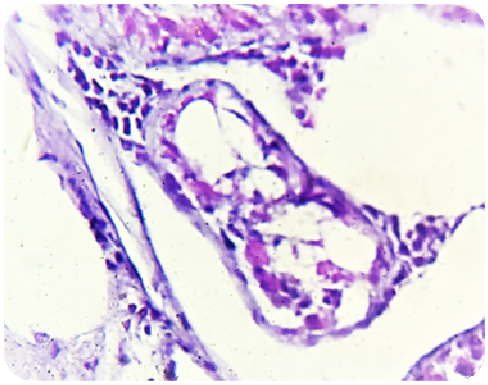
Histological cross section of testicular seminiferous tubules stage VI of *control* mice; indicates to normal architectural arrangement of seminiferous tubules containing chains of spermatogenic cells, E&S stain, X400



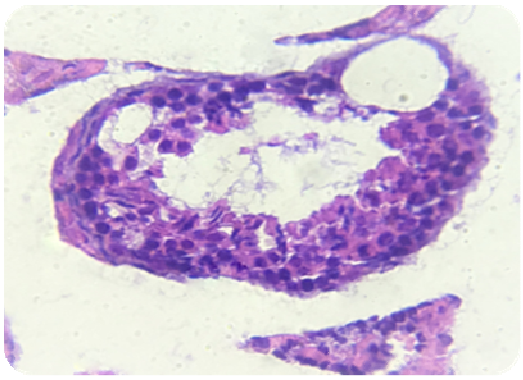
Histological cross section of testicular seminiferous tubules in *pro liposome* treated mice at stage VI; specifies hyper crowded normal spermatogenic cells chain with architectural specified organization of seminiferous tubules composed regular function spermatogenesis process, E&S stain. X400



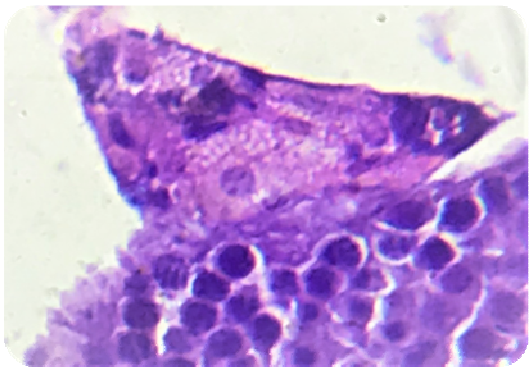
Histological cross section of mice testicular seminiferous tubules stage VI of *pro liposome* treated mice; point to hyper functionalized Leydig cells containing diffused cytoplasmic acidophilic granules, general appearance dominance of large leydig cells in forming clusters with fewer individuals cells, E&S stain, X 815



Histological cross section of mice testicular seminiferous tubules stage VI of Cimetidine treated mice; point at complete loss normal architectural arrangement spermatogenic cells with diffused absence of cell multiplication germ cells in primary and secondary spermatocyte, E&S stain, X 400



Histological cross section of mice testicular seminiferous tubules stage VI of Cimetidine treated mice; point at normal Leydig cells associated with partial derangement of al architectural building of germ cells with foci of vacuolation in seminiferous tubules, E&S stain, X 400



Histological cross section of mice testicular seminiferous tubules stage VI of *control* mice; indicates to functional Leydig cells containing cytoplasmic acidophilic granules, general appearance domination of clustered form with less individual cells arrangement, E&S stain, X 815

Fig. 18: Histopathological section

Discussion

The purpose of the usage of the CIM loaded liposome in dosed GIT formulation is to raise the bioavailability of the encapsulated pharmaceutics to the gastro-enteric tracts concerning both uptakes of liposome and time of residence. To achieve this project goal, liposomes with negative surface charges were set by negatively charged components and assessed. Thin lipid film-hydration technique was used to formulate liposomes. The modified Banghasome results provided an acceptable entrapment of 80.06 ± 5.76 with a particle size ranging from 21.6 - 213.3; In the aqueous medium, this shares a documented idea of the reality of fully integrated liposomes surrounded by the phospholipid and cholesterol membrane, and the spontaneous formation of liposomes resulting from the polar head reaction and the Van der Waals reaction. Due to the multi-lamellar single-cell liposomes having a large inner water core, they are ideal for encapsulating hydrophilic drugs, while the multilayer liposomes have more than two bi-fold structures and vary in diameter from a few hundred to hundreds of nanometers; It is suitable for packing hydrophobic medicine. Multiplatelet liposomes contain an effective amount of therapeutic dose optimized at an appropriate dose (Marasini *et al*, 2017, Laouini *et al*., 2012). The tolerance of CIMsome in *acidic* conditions in vitro that mimic the gastrointestinal medium followed the first-order releasing and destruction of CIM. In the acidic phase (pH 4), liposome remained steady in the multi-vesicular liposomes after 24 hours. In the simulated intra-intestinal condition pH 7.4 these pH results indicate the CIMsome is survived from the liposomes in a controlled sustained manner for over 24 hours of challenging periods. the influence of osmolarity tolerance on the encapsulation percent and efficiency was investigated showed tolerance at (0.45 to 0.85). These impact results collectively indicated to osmolarity was affected encapsulation efficiency of CIM in the multi-vesicular liposomes. the entrapment efficiency of the CIM for the multi-vesicular liposomes factors; solubility, the permeability of the CIM, and the osmolarity tolerance of the CIM ((Jaafer *et al*., 2020 Kesisoglou *et al.,* 2005). In this experimental trial, the Støa-Birketvedt (1993) attributed the reduced body weight due to the direct effect of cimetidine on the suppression of hunger, the Gastric acid secretion modulation by H2 receptor blocker may be endorsed in the functional appetite sensory regulation Walan and Strom

(1985). The Cimetidine might suppress the hunger pain sensations that promote a logically reduced food intake, The finding of reduced food intake derived decrease body weight agrees with reports of Støa-Birketvedt (1993) where attributed to reduce of appetite and weight gain in cimetidine dosed experiment model as well as possibility ability to reduce gastric acid medium followed by dropping the ability of intestinal absorption of nutrient coincided with (Walan and Strom 1985). Cimetidine it's a drug non-steroidal endorsed weak anti-androgenic, induces testicular tissue deformity in their spermatogenic cell maps arrangement in male mice after the oral dosed ordinary form (20 mg/Kg Bw.) and dysfunction of testicular somatic cells; Leydig cells (Pino *et al*., 2019 and, Liu *et al*., 2018). In preceding information and documented studies, it was established the cimetidine produce functional structure changes in the testicular built unit, decrement in the gross and morphometric testicular changes escorted weight and relative weight ratio, and reflexive decrease geometrical dimension and volume, associated with a reduction in spermatogenic cells yield and daily sperm yield. These associations agreed with the belonging of a bad prognosis on sperm function after 38 days of treatment. The results of CIM presumably attributed to causing increased autocoids mediator's iNOS, COX-II, and NF-κB (Koshimizu *et al*., 2013). The report of Kyriakis and Avruch (2001) and Bubici *et al*. (2006) attributed the oxidative and subsequent stress initiation of signaling kinases activation may derive transcription factor NF-κB. The activity response of NF-κB is induced injurious at cellular tissue, infection, and inflammation (Hoesel and Schmid, 2013), furthermore, It has a role in inducing programmed death apoptosis (Koshimizu *et al*., 2013 and Erl *et al*., 1999). The cimetidine may be endorsed in their effect with NF-κB induced reduction in spermatogenic activity and spermatogenic yield spermatozoa defects (Liu *et al*., 2018). In the treatment with cimetidine, reduction in the indices of the Leydig cells with a surge the levels of testosterone that may be caused by increased COX-II of cells in Leydig cells of the cimetidine treated group. Conversely, the hormonal levels of testosterone with decreased Leydig cell number. This elevation serum levels of testosterone presumably resulted from the amalgamation of effects: first, "An inductive effect caused by LH" and second COX-II adverse effect to Leydig cells, which were presumably deduced the inductive effect of LH is stronger proper than the adverse effect via by COX-II to yield elevated serum levels of testosterone. Some speculation may be due to increment testosterone levels via non-testicular origin like an adrenal gland. That coincided with the finding of Kurohmaru *et al*. (1990) an exertion of cimetidine effect is not directly related to lack of testosterone or gonadotropin-releasing effect.

The cimetidine treated mice may be increased NOS activity was documented by Liu *et al*. (2018), expression of iNOS were increased in Sertoli cells and other cells. These results presumably testicular cells are also affected by cimetidine, which was led to the promotion of inflammation in the testis and germ cell apoptosis. Several studies documented the cimetidine effect on sperm function test and testicular tissue defects directly derived the severity through dose-dependent manner (Aprioku *et al*., 2014 and Maya 2010). The peritubular testicular tissue is comprised of two somatic cell types located concentrically organized around the circumstance of seminiferous tubules (Al Bayaty, 2005) On the other hand, the cimetidine effect on the histological profile of the testes observed may be positively correlated to the endpoint of spermatogram results analysis: through disruption the normal style of architectural arrangement of the spermatogenic chain and sequential order of germ cells of seminiferous epithelium (Al-Nailey, 2010 and Hamid *et al*., 2011). The previous studies established the cimetidine induced adrenal enlargement that may be attributed to the increment of testosterone in the cimetidine treated group.

The cimetidine may target myoid cells and reduced P-mod S factor, emanating from the myoid cell, which may be reduced activity of Sertoli cell. The cimetidine was well known as a metabolic inhibitor associated with reduce. The antiandrogenic properties may be attributable to inhibition of dihydroxy testosterone binding and occupy to the androgenic receptor dihydroxy testosterone has a superior affinity for the androgenic receptors and abolishment the association-dissociation of these receptors.

This situation contradictory from the common tubular atrophy seen in deficiency of testosterone but the increment of cimetidine increasing testosterone concentration levels display abnormal spermatogenesis and secretion of cell drive and sequence of spermiogenesis development, and affects entirely tubules and does exist the tubule-tubule changeability in histometric profile (Franc□a *et al*., 1998).

Nano vesicles melt and entrapped in natural lipid bilayers or lamellae. The novelty of the Nano-encapsulation form for the drug to overcome removal of adverse effect belonging of traditional pharmaceutics, The Nano liposome one of the vesicles works as carriers to drug delivery at its targeted receptors sites. The reason for the encapsulation of cimetidine by liposome carrier preferred ideally fulfills two prerequisites. Started, it was well delivered the cimetidine at a defined period of exposure time and giving to the body mass requirements, and ended by it have channeled the dynamic active efficient amounts to the antacid action and achieved reduction of adverse effect as anti-androgen. These facts may be presumably occupied a major attribution to set the cimetidine improving their activity and overcoming the problem associated with bad chemical complex (Vyas *et al*., 2013). Both detrimental factors were assayed in cimetidine liposome was impacted directly on the cross the barrier and produced reduced obvious activity via liposome own physical and chemical properties that modulating Lipid solubility of drug molecules and modified re-pose size of chemical drug molecules (Barragan *et al*., 2005). That managed reduced the entry liposome cimetidine to testicular-Blood barrier redistribution to a major target of receptor sites biotransformation and increase bioavailability in systematically following treatment (Wei and Ba, 2020).

The liposome-encapsulated cimetidine increase the potency and efficacy on their effect out of testicular tissue or pre tubular tissues. Furthermore, Liposome as one of role in increase enhancement of cimetidine solubility and bioavailability, reduce toxicity and protection from adverse effect, stability enhancement of stability, improving redistribution macrophages in tissue, slowing or sustained drug release, and drug protection from chemical and physical degradation (Barragan *et al*., 2005).

The results referred to testosterone hormone reduction may be direct effects of inhibiting of P450 microsomal enzyme inhibitor induce the testosterone reduction via

increase dysfunction of conversion their precursor to an active hormone despite the phosphatidylcholine of liposomes also acts as a hepato-protective. These reduced of testosterone in cimetidine liposome treated group was not shared drop of testosterone effect on the other functional properties of the reproductive system may be presumably due to direct effect release pituitary-Leydig cells-system controlling testosterone, as well as the histological section was not presented the disruption in the spermatogenic tissue and peritubular cells containing Leydig cells. Furthermore, the entire sperm function test was set approximately to normal or ordinary values (Naeem, 2017). In another word, some attribution of decrease and severity of reduction of testosterone presumably derived from the phenomena of longer time-circulating cimetidine liposomes were shown to reduce the time required for the absorption of cimetidine and hence the period of staying liposome extend with their extended circulation time directly modulated t½ of cimetidine. "These were called stealth liposomes" and elasticity for passive (Lu *et al*., 2014). The suggested long-circulating cimetidine liposomes may be proved like liposomal behavior kinetic as dose-independent, without saturable conduct, that promotion log-linear kinetics, increased bioavailability, and direct increase efficacy (Zylberberg and Matosevic, 2016 and Van Slooten *et al*., 2001).

Conclusion

The successful composed of entrapped cimetidine in Nano form of liposome small multi lamellar type and efficient entrapment with tolerated in certain gradient osmolartiy and series of pH. The Liposome Cimetidine was partial protected the tissue of post testicular blood testes barrier from cimetidine direct impairment of spermatogenesis and that reflected in the quantitative of spermatogenic cells and testicular weight and velum with drive normalized the daily sperm yield and hold DNA integrity.

References

Al Bayaty, M.A. (2005). Quantitation of Leydig cell in testicular biopsies of gossypol treated rats. *The Iraqi Journal of Veterinary Medicine*, *29*(1), 179-189.

Al-Nailey, K.G.C. (2010). Study of the protective effect of Nigella sativa against cimetidine induced reproductive toxicity in male mice. AL-Qadisiya J. Vet. Med. Sci., 9: 55-62.

Al-Yasiri, R.M. and Al-Bayati, M. (2020). Attenuation dexamethasone adverse effect by nano-green tea phytosome encapsulated dexamethasone on rat leydig cells in vitro: Part I. *Eurasian Journal of Biosciences*, 14(1): 2171-2177.

Aprioku, J.S.; Ibeachu, C. and Ijeomah, M.A. (2014). Protective Effect Of Ascorbic Acid On Cimetidine-Induced Reproductive Toxicity In Male Wistar Rats. Journal of Pharmacology and Toxicology, 9: 46-54

Bailey, S.A.; Zidell, R.H. and Perry, R.W. (2004). Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint?. Toxicologic pathology, 32(4): 448-466.

Cònsol, G.; Moles, A.; Ricart-Jané, D.; and Llobera, M. (2005). An in situ perfusion protocol of rat epididymal adipose tissue useful in metabolic studies. Journal of lipid research, 46(8): 1803-1808.

Crewson, P. (2006). Applied statistics handbook. AcaStat Software, Leesburg.

Dhule, S.S.; Penfornis, P.; Frazier, T.; Walker, R.; Feldman, J.; Tan, G. and Pochampally, R. (2012). Curcumin-loaded γ-cyclodextrin liposomal nanoparticles as delivery vehicles for osteosarcoma. Nanomedicine: Nanotechnology, Biology and Medicine, 8(4): 440-451.

Erl, W.; Hansson, G.K.; de Martin, R.; Draude, G.; Weber, K.S. and Weber, C. (1999). Nuclear factor-κB regulates induction of apoptosis and inhibitor of apoptosis protein-1 expression in vascular smooth muscle cells. *Circulation research*, 84(6): 668-677.

Franca, L.R.; Parreira, G.G.; Gates, R.J. and Russell, L.D. (1998) Hormonal regulation of spermatogenesis in the hypophysectomized rat: quantitation of germ cell population and effect of elimination of residual testosterone after long-term hypophysectomy. J Androl, 19: 335–342.

Gargiulo, S.; Gramanzini, M.; Megna, R.; Greco, A.; Albanese, S.; Manfredi, C. and Brunetti, A. (2014). Evaluation of growth patterns and body composition in C57Bl/6J mice using dual energy X-ray absorptiometry. BioMed research international, 2014.

Gargiulo, S.; Gramanzini, M.; Megna, R.; Greco, A.; Albanese, S.; Manfredi, C. and Brunetti, A. (2014). Evaluation of growth patterns and body composition in C57Bl/6J mice using dual energy X-ray

Hoesel, B. and Schmid, J.A. (2013). The complexity of NF-κB signaling in inflammation and cancer. *Molecular cancer*, 12(1): 1-15.

Jaafer, N.S.; Balqees, H.A. and Al-Bayati, M.A. (2020). Preparation and standardization of liposomes encapsulated newcastle disease vaccine in unilamellar and multilamellar forms. *Plant Archives*, 20(1): 978-982.

Joyce, K.L.; Porcelli, J. and Cooke, P.S. (1993). Neonatal goitrogen treatment increases adult testis size and sperm production in the mouse. Journal of andrology, 14(6): 448-455.

Kesisoglou, F.; Zhou, S.V.; Niemiec, S.; Lee.; J.W.; Zimmer-mann, E.M. and Fleisher, D. (2005). Liposomal Formulations Of Inflamma-Tory Bowel Disease Drugs: Local Versus Systemic Drug Delivery Inaratmodel. Pharmaceutical Research. 22(8): 1320- 1330.

Koshimizu, J.Y.; Beltrame, F.L.; de Pizzol, J.P.; Cerri, P.S.; Caneguim, B.H. and Sasso-Cerri, E. (2013). NF-kB over expression and decreased immuno expression of AR in the muscular layer is related to structural damages and apoptosis in cimetidine-treated rat vas deferens. *Reproductive Biology and Endocrinology*, 11(1): 1-10.

Kurohmaru, M.; Sinha-Hikim, A.P.; Mayerhofer, A.; Bartke, A. and Russell, L.D. (1990). Golden hamster myoid cells during active and inactive states of spermatogenesis: correlation of testosterone levels with structure. Am J Anat; 188: 319–327.

Kyriakis, J.M. and Avruch, J. (2001). Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiological reviews*, 81(2): 807-869.

Laouini, A.; Jaafar-Maalej, C.; Limayem-Biouza, I.; Sfar, S.; Charcosset, C. and Fessi, H. (2012). Preparation, Characterization and Applications of Liposomes: State of the Art. J. of Colloid Set. Biotechnol. 1: 147-168.

Levin, R.M.; Hypolite, J.A. and Wein, A.J. (1984). Clinical use of the turbidimetric analysis of sperm motility: an update. *Andrologia*, 16(5): 434-438.

Liu, X.; Jia, Y.; Chong, L.; Jiang, J.; Yang, Y.; Li, L. and Zhou, L. (2018). Effects of oral cimetidine on the reproductive system of male rats. *Experimental and therapeutic medicine*, 15(6): 4643-4650.

Lue, Y.; Sinha Hikim, A. P.; Wang, C.; Leung, A. and Swerdloff, R.S. (2003). Functional role of inducible nitric oxide synthase in the induction of male germ cell apoptosis, regulation of sperm number, and determination of testes size: evidence from null mutant mice. *Endocrinology*, 144(7): 3092-3100.

Marasini, N.; Ghaffar, K.A.; Slwarczynski, M. and Toth, I. (2017). Liposomes as a Vaccine Delivery System. Micro- and Nanotechnology in vaccine Development. P: 221-239

Mathur, V.; Satrawala, Y. and Rajput, M.S. (2014). Physical and chemical penetration enhancers in transdermal drug delivery system. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm, 4(3).

Maya, W.C. (2010). Sperm count. Do we need a new reference

Mbaeri, T.U.; Orakwe, J.C.; Nwofor, A.M.; Oranusi, K.C. and Mbonu, O.O. (2013). Accuracy of Prader orchidometer in measuring testicular volume. Nigerian journal of clinical practice, 16(3): 348-351.

Mohammed, A.I. and Al-Bayati, M.A. (2014). Comparative study among watermelon crud extract, citrulline and lycopene on some reproductive indices in male mice. *International Journal of Research*, *3*(6): 2307-2083.

Napoli, C.; Paolisso, G.; Casamassimi, A.; Al-Omran, M.; Barbieri, M.; Sommese, L.; ... and Ignarro, L. J. (2013). Effects of nitric oxide on cell proliferation: novel insights. *Journal of the American College of Cardiology*, 62(2): 89-95.

Oguntola, J.A. (2010). The Dose-Dependent Testiculoprotective and Testiculotoxic Potentials of Telfairia occidentalis Hook f. Leaves Extract in Rat.

Oliveira, M.F.; Kycia, S.W.; Gomez, A.; Kosar, A.J.; Bohle, D.S.; Hempelmann, E. and Ferreira, S.T. (2005). Structural and morphological characterization of hemozoin produced by Schistosoma mansoni and Rhodnius prolixus. Febs letters, 579(27): 6010-6016.

O'Neil, E.; Burton, S.; Horney, B. and MacKenzie, A. (2013). Comparison of white and red blood cell estimates in urine sediment with hemocytometer and automated counts in dogs and cats. Veterinary clinical pathology, 42(1): 78-84.

Pino, M.A. and Azer, S.A. (2019). cimetidine, StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing

Ramalho-Santos, J.; Amaral, S.; and Oliveira, P.J. (2008). Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. Current diabetes reviews, 4(1): 46-54.

Sakamoto, J. and Hashimoto, K. (1986). Reproductive toxicity of acrylamide and related compounds in mice effects on fertility and sperm morphology. Archives of toxicology, 59(4): 201-205.

Støa-Birketvedt, G. (1993). Effect of cimetidine suspension on appetite and weight in overweight subjects. *British Medical Journal*, *306*(6885): 1091-1093

Szebeni, J.; Di Iorio, E. E.; Hauser, H.; and Winterhalter, K. H. (1985). Encapsulation of hemoglobin in phospholipid liposomes: characterization and stability. Biochemistry, 24(12): 2827-2832.

Talebi, A. R.; Vahidi, S.; Aflatoonian, A.; Ghasemi, N.; Ghasemzadeh, J.; Firoozabadi, R. D.; and Moein, M. R. (2012). Cytochemical evaluation of sperm chromatin and DNA integrity in couples with unexplained recurrent spontaneous abortions. Andrologia, 44, 462-470. value?. *Arch. Esp. Urol*, *63*(2): 133-138.

Walan, A.; and Ström, M. (1985). Metabolic consequences of reduced gastric acidity. *Scandinavian Journal of Gastroenterology*, *20*(sup111): 24-29.

World Health Organization, and Cooper, T. G. (2010). WHO laboratory manual for the examination and processing of human semen.