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The effect of *Tribulus terrestris* on reproductive performance of male rats treated with amloidpine

تأثير نبات الحسك في الكفاية التناسلية لذكور الجرذان المعالجة بالاملوديبين

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ABSTRACT

This study was performed to determine the effect of *Tribulus terrestris* in genital male rats treated with amlodipine. Twenty four animals from adult male albino rats were divided into four groups (six animals per group), the first one is considered as a control group, the second group treated with 0.2 mg/kg of amlodipine , the third group where treated with 5 mg/kg of *Tribulus terrestris* and the fourth group is the mixed one in which the animals treated with both amlodipine (0.2 mg/kg) and the *Tribulus terrestris* (5 mg/kg). The experiment lasted for six weeks and then after the animals were sacrificed in order to the study of hormonal and pathological changes.

The results revealed no significant increase in the levels of the testosterone hormone in a group treated with *Tribulus terrestris* as well as the study showed some histological changes are important for *Tribulus terrestris* represented with the increase in the development of sperms compared with amlodipine group which showed some negative effects.

The study concluded that there was an effective reproductive efficiency of *Tribulus terrestris* in spite of the inability of the plant to minimize the negative effects of amlodipine.

Key words: - amlodipine, *Tribulus terrestris*, male rats, reproduction.

المستخلص:

هدفت الدراسة الحالية لمعرفة تأثير نبات الحسك في الكفاية التناسلية لذكور الجرذان المعاملة بالاملوديبين. حيث يعد نبات الحسك من النباتات العشبية المهمة في استخدامها كمحفز للجهاز التناسلي.

استخدمت في هذه الدراسة 24 حيوانا من ذكور الجرذان البيضاء البالغة وقسمت الى اربع مجاميع بواقع ست حيوانات لكل مجموعة ، حيث اعتبرت المجموعة الاولى كمجموعة سيطرة والمجموعات البقية كمجاميع معالجة حيث جرعت حيوانات المجموعة الاولى بالماء المقطر وعدت كمجموعة سيطرة وجرعت المجموعة الثانية 0.2 ملغم/كغم من الاملوديبين اما المجموعة الثالثة فجرعت بالاملودبين 0.2 ملغم / كغم و 5 ملغم/كغم من نبات الحسك والمجموعة الرابع جرعت فقط بنبات الحسك 5 ملغم / كغم واستمرت التجربة لمدة ستة اسابيع بعدها تم التضحية بالحيوانات لدراسة المعايير الهرمونية والتغيرات النسيجية.

اوضحت نتائج الدراسة زيادة في مستويات هرمون التستيستيرون للمجموعة المعالجة بنبات الحسك مقارنة بالمجاميع الباقية على الرغم من عدم وصول مستويات هذا الهرمون الى الاهمية المعنوية كذلك اوضحت الدراسة وجود بعض التغيرات النسيجية المهمة بالنسبة لمجموعة نبات الحسك حيث اوضحت وجود زيادة في عملية تطور الحيوانات المنوية مقارنة بمجموعة الاملوديبين حيث اوضحت بعض التاثيرات السلبية .

استنتج من الدراسة وجود تاثير فعال لنبات الحسك في الفعالية التناسلية على الرغم من عدم قدرة النبات على تقليل التأثيرات السلبية للاملوديبين.

كلمات المفتاح:- الاملوديبين ، نبات الحسك ، ذكور الجرذان ، الكفاية التناسلية.

INTRODUCTION

Tribulus terrestris is a tap rooted herbaceous perennial plant. It is a member of the *Zygophyllaceae* family, the annual herb found in many tropical and moderate areas of the world, including the U.S. and Mexico, the Mediterranean region, and throughout Asia [1].It is about 30 to 70cm high; it grows as a summer annual, has yellow flowers and satellite shaped carpel fruits [2].*Tribulus terrestris*, contains steroidal saponin, and act as a natural testosterone enhancer, *Tribulus terrestris* increases testosterone through increase in luteinizing hormone (LH) as well as there is good confidence that *Tribulus terrestris* is useful as a sexual enhancement herb[3].This plant has several advantages including antimicrobial, antibacterial, antioxidant and antitoxic activities used in the treatment of cardiovascular diseases, diabetes, tumors, articular pains and respiratory diseases [4]. In Iraq *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithontriptic and urinary anti-infective [5].*Tribulus terrestr*is increases body ability to produce muscular mass and physical strength. Furthermore, it causes production of red blood cells and improvement in circulation and oxygen transportation. Long-term use of *Tribulus terrestris* results in dilatation and improvement of coronary arteries without any side effects. [6]

Tribestan is a component of *Tribulus terrestris* which increases libido in addition to preventing infertility and menopausal disorders [7]. Dioscin present in *Tribulus terrestris* increases male sexual ability by increasing free testosterone levels and balancing levels of estrogen, progesterone and pregnenolone [8]. Since this plant contain protodioscin (PTN) and saponin which increases levels of testosterone and Luteotropic hormone (LH), it has been used for the treatment of sexual impotence in the traditional medicines of China and India from long ago [9].The aphrodisiac properties of *Tribulus terrestris* extract that contains protodioscin (PTN), a steroidal saponin that forms 45% (dry weight) of the extract was explored in castrated rats (10).

Administration of *Tribulus terrestris* to humans and animals improves libido and spermatogenesis , PTN is also found to increase the levels of testosterone, luteinizing hormone [11].

Besides, using *Tribulus terrestris* in addition to other herbal drugs has improved erection and sexual behavior in rat [12]. In another Animal studies in rats, rabbits and primates have demonstrated that administration of *Tribulus terrestris* extract can produce statistically significant increases in levels of testosterone, dihydrotestosterone and dehydroepiandrosterone. According to the findings of these studies showing *Tribulus terrestris* to increase sexual hormones, to improve sexual behavior and erection, it seems that this plant can be effective in spermatogenesis as well [13].

Amlodipine (as besylate, mesylate or maleate) is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle. Amlodipine does also act as functional inhibitor of Acid Sphingomyelinase (FIASMA) [14].

Amlodipine, ,has been shown to decrease sperm count in sperm suspensions collected from cauda Epididymis of rats[15] .In humans, long term treatment with amlodipine resulted in azoospermia in semen and few non-motile sperms in testicular sperm extraction. This indicates that long term use of amlodipine not only inhibits spermatogenesis but it also impairs sperm motility. Previous study has shown that long term treatment with amlodipine resulted in decreased serum testosterone level , and suppressed spermatogenesis as indicated by reduction in mean seminiferous tubular diameter and height of germinal epithelium [16].

The present study was designed to determine the effects of Tribulus terrestris on reproductive performance in male rate treated with amlodipine.

MATERIALS AND METHODS

Healthy Sprague Dawley male rats 12-14 weeks of age weighing between 150-200 gm. were used for present study. The animals were kept in plastic ideal cages in an animal house maintained under controlled atmospheric condition in College of Pharmacy at University of Kerbala. The animals were accommodated for a 10 days before the experiment. They were maintained in standard conditions at room temperature and relative humidity at 60± 5% for 12 hours light dark cycle. They have been given standard pellet diet and water was supplied *ad-libitum* throughout the course of study.

In the present study the animals were divided into four groups , six animals in each one. The groups are divided as the followings:-

1- Control group treated with distill water.
2- Amlodipine group treated with 0.2 mg/kg
3- Mixed group treated with amlodipine 0.2 mg/kg and *Tribulus terrestris* 5mg/kg (raw material from local market) suspended in distill water.
4- Herbal group treated with *Tribulus terrestris* (from local market) 5 mg/kg

All the animals drenched every day orally with their suitable dose for 6 weeks via a stainless steel intubation needle. The weights of animals were measured weekly to adjust the dose of drugs.

Laboratory tests:-

At the end of study all the animals were deeply anesthetized with chloroform and sacrificed , most of blood was withdrawn by cardiac puncture. Serum was separated from the blood by a centrifuge 5000 rpm for 5 minutes. Testosterone hormone levels was tested by Tosso-AIA apparatus .The testis of both sides were dissected out from each male, weighted by electronic balance to calculate the gonado somatic index (GSI).

Gonado-Somatic Index (GSI) =(Gonad weight/total body weight) ×100

Where Gonad weight= (weight of the right testis + weight of the left testis)/2 [17].

Tissue preparation:-

The organs were carefully made free from surrounding fat and connective tissue, washed briefly with tab water and immediately placed in 10% formal saline. The organs from one side of each animal were tested for histopathological changes. [18]

Statistical analysis:-

Results were expressed as mean ± SE. Statistical significance was calculated by using one way analysis of variance (ANOVA) by SPSS software version 12.0 ($p< 0.05$) was considered as significant. Values bearing different letters as superscripts showed significant differences ($p\leq 0.05$). [19]

RESULTS

According to body weight, there is weight gain in the third group in comparing with control group, while it was significantly decreased in second group in compare with control one (table 1).

The results of gonadosomatic index indicate non-significant increase in third group in compare with control and second group (table 2). As well as the testosterone level of the third group increase in comparing with control but it not reach to statistically significant levels, while the level in second group was decreased in comparing with control (table 3).

The histopathological pictures revealed that there is significant cellularity plus degenerative changes and parenchymal congestion with focal degeneration in seminiferous tubules of amlodipine treated group comparing with control (image 2). While the histopathological pictures of amlodipine and *Tribulus terrestris* treated group indicates relative increase in cellularity with few centrally located mature sperms and no significant degeneration seen (image 3).

Comparing with the fourth group there is a significant increase in spermatogenic activity with large number of mature sperms in this group (Image 4).

Table (1), represent the effect of amlodipine and *Tribulus terrestris* on body weight .

Groups	Weight gm.
Control group	259.6 ± 8.68 b
Amlodipine group	239.6 ± 4.44 d
Tribulus terrestris group	262.2 ± 6.68 a
Amlodipine and *Tribulus terrestris* group	245.8 ± 5.70 c

-Means with different letters refers to significant differences between groups.
Mean ± SE P≤ 0.05

Table (2), represent the effect of amlodipine and *Tribulus terrestris* on Gonadosomatic index

Groups	Gonadosomatic index
Control group	0.30 ± 0.04
Amlodipine treated group	0.36 ±0.057
Tribulus terrestris group	0.41 ± 0.037
Amlodipine and *Tribulus terrestris* group	0.41 ± 0.052

Mean ± SE P≤ 0.05

Table (3), represent the effect of amlodipine and *Tribulus terrestris* on testosterone

Groups	Testosterone ng/dl
Control group	235 ± 58.59
Amlodipine treated group	297.3 ± 54.96
Herbs treated group	435.3 ± 54.48
Amlodipine and herb treated group	224.3 ± 18.21

Mean ± SE P≤ 0.05

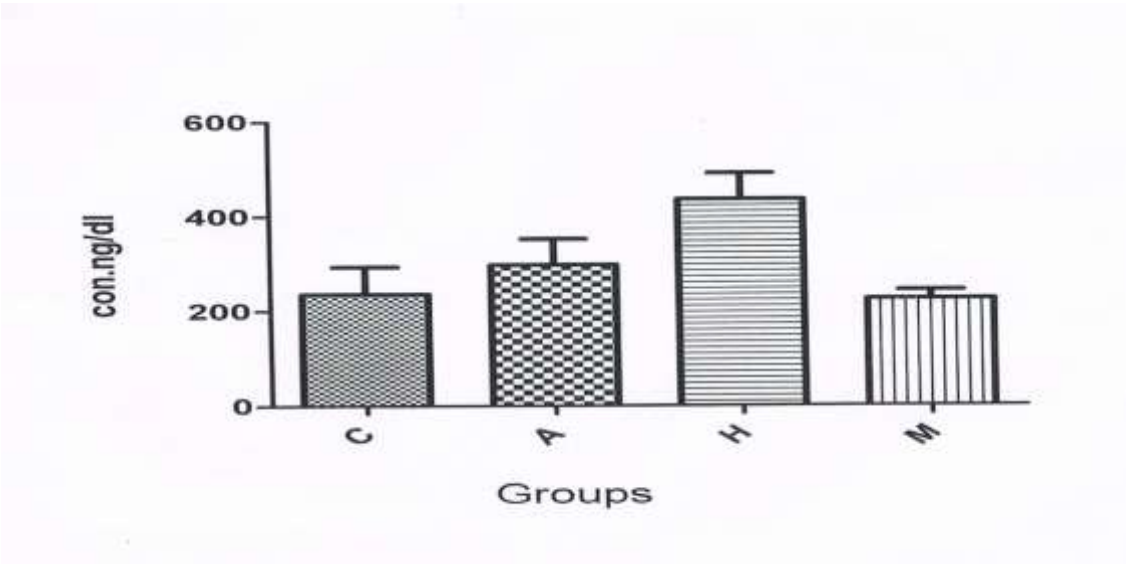

Figure (1), graphical representation of testosterone level C=control group, A= amlodipine group, H= *Tribulus terrestris* group, M= mixed treated group (amlodipine and *Tribulus terrestris*)

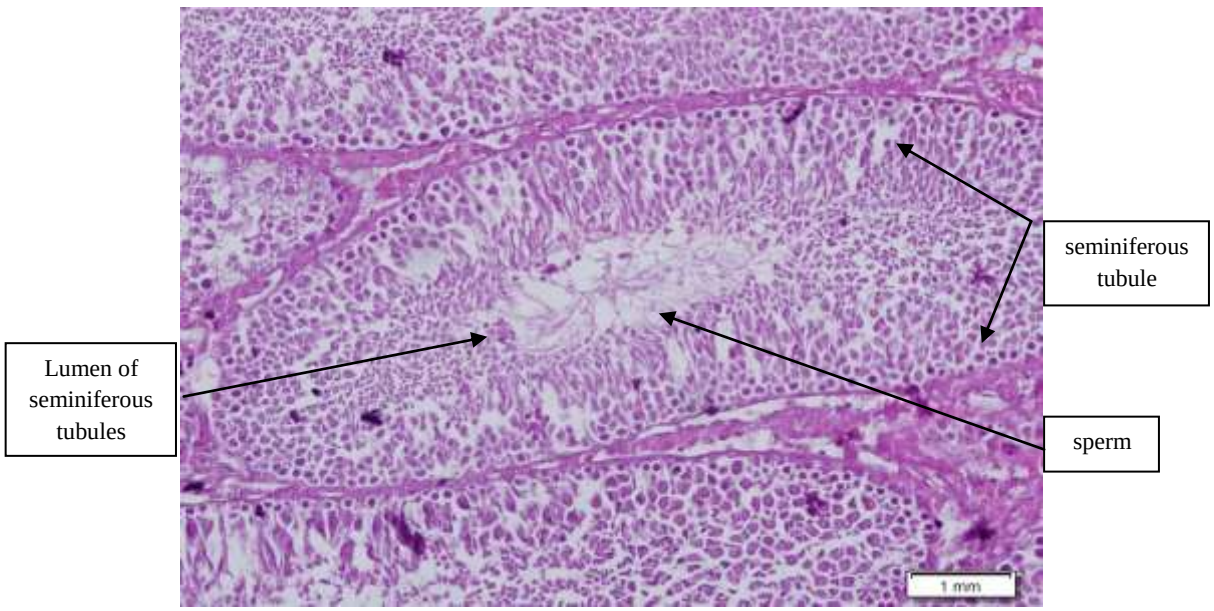

Image (1): transverse section of seminiferous tubules in control group show normal cellularity and normal spermatogenic action of the seminiferous tubules. (X20, H&E stain)

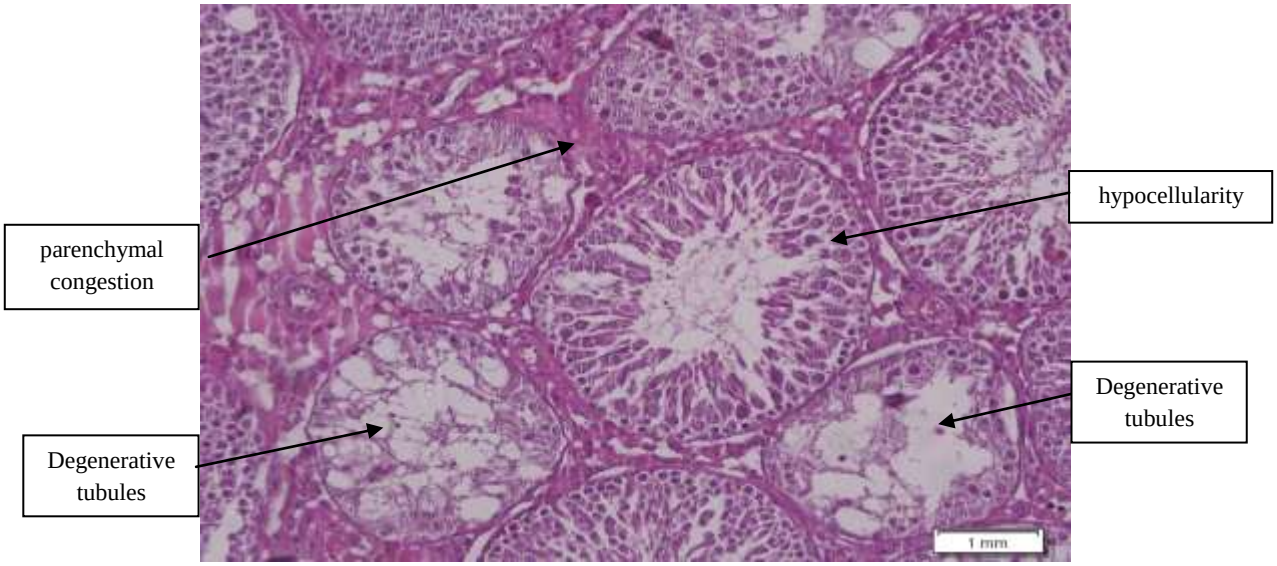

Image (2): transverse section of seminiferous tubules in amlodipine treated group show significant hypocellularity plus degenerative changes and parenchymal congestion and focal degeneration in some tubules. (X20, H&E stain)

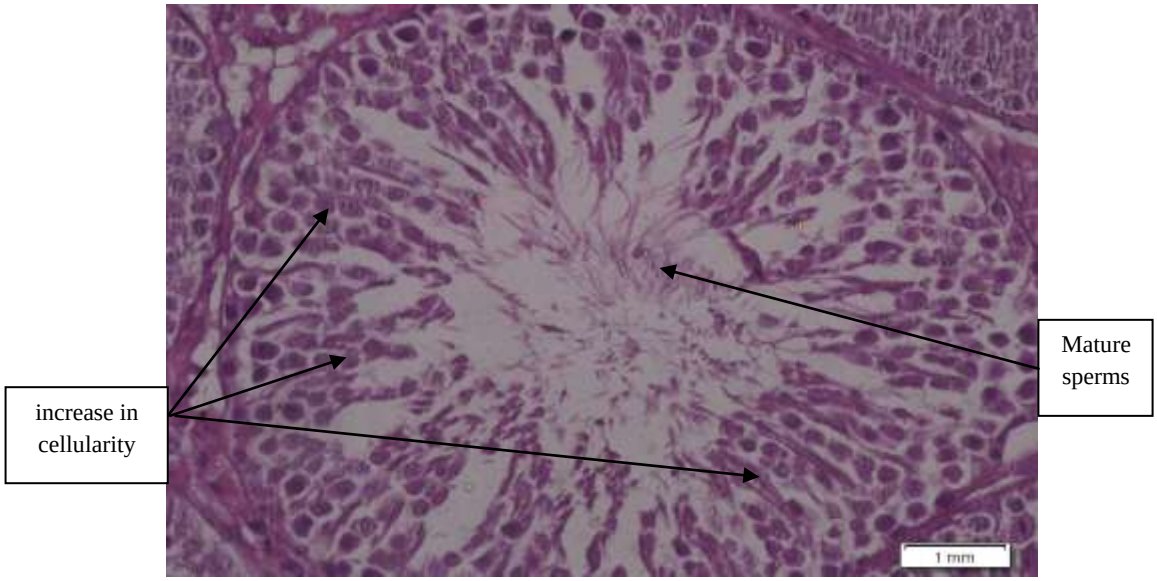

Image (3): transverse section in (amlodipine and *Tribulus terrestris*) treated group testis show relative increase in cellularity with few centrally located mature sperms no significant degeneration seen. (X20, H&E stain)

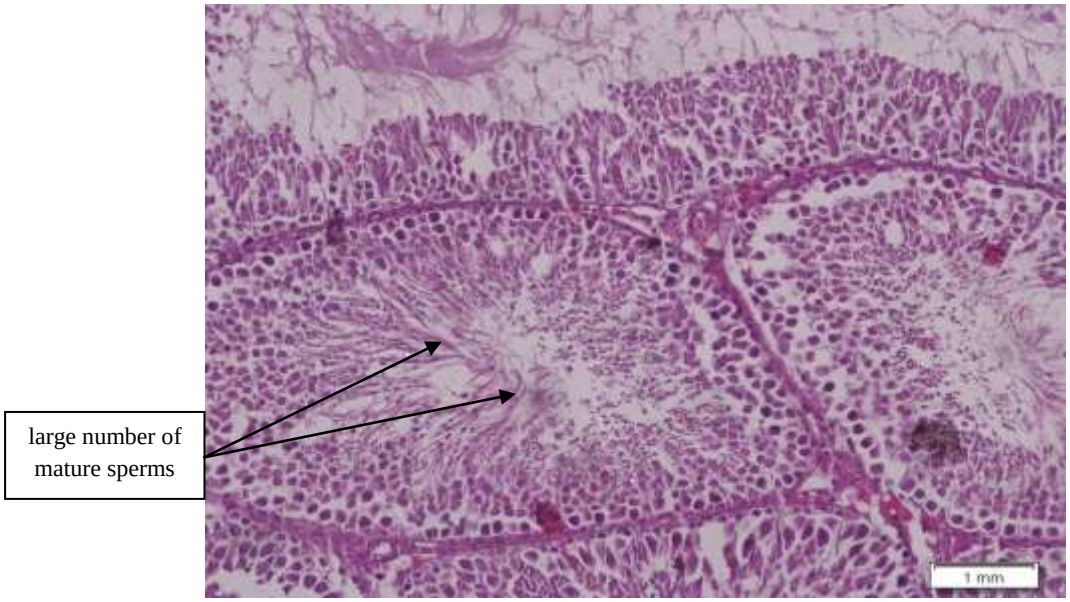

Image (4): transverse section in *Tribulus terrestris* treated group testis show significant increase in spermatogenic activity with large number of mature sperms.
(X20, H&E stain)

DISCUSSION

Tribulus terrestris is now being promoted as a booster for the purpose of increasing sex drive. Its use for this purpose originated from a Bulgarian study conducted in the 1970s, which found effects on free testosterone and luteinizing hormone in men belonging to infertile couples [20]. Besides, the group that has been treated with both (amlodipine and *Tribulus terrestris*) show no significant changing in testosterone and that is significantly become understood that the mechanism of action of the two drugs are different . Herb extract have direct hormonal activity , this hormonal modulation has been attributed to the presence of the steroidal saponin protodioscin in the plant extract .

It is believed that the hormonal effects of protodioscin are mediated through its metabolic conversion into dehydroepiandrosterone [21]**,** but another study say no direct hormonal effect of *Tribulus terrestris* demonstrated that *Tribulus terrestris* has no intrinsic hormonal activity, because it was unable to stimulate endocrine-sensitive organs in either male or female rats. They also demonstrated that the administration of *T. terrestris* to intact male rats for 28 days did not change serum testosterone levels and did not produce quantitative changes in the fecal excretion of androgenic metabolites [22]. Image (4) shows significant increase in spermatogenic activity in *Tribulus terrestris* these may be explained by the androgenic effect of herb , androgen have a major effect and role in the growth and differentiation of many tissues in addition to the organs of reproduction , androgen also responsible for pubertal development of testes . Administration of *Tribulus terrestris* preparation significantly increased the number of spermatogonia, spermatocytes, and spermatids in the testes of adult rats , they suggest an intensified DNA synthesis under the effect of protodioscin [23]. Image no. (2) of amlodipine treated group there is appearance of blood congestion they may be explained by amlodipine induce production of nitric oxide (NO) ,which lead to vasodilation effect on capillary vessels which may express the congestion, and amlodipine induce degenerative effect and significant hypocellularity as seen in same image, they may be explained by amlodipine has recently been described to down-regulate cell proliferation by two mechanisms: By inhibiting/reducing the expression of growth factors such as platelet-derived growth factor, transforming growth factor ß , and basic fibroblast growth factor ,and by inducing apoptosis, explained that amlodipine induced apoptosis by increasing the activity of apoptotic enzymes Cascade 3,7 and 8[24].

Amlodipine induced apoptosis by decreasing the expression of anti-apoptotic protein (Bcl-2) and proliferating cell nuclear antigen , Image (3) of mixed treated group there is no significant degeneration seen with few centrally located mature sperms may explained by that *Tribulus terrestris* protected the Sertoli cells by produce testosterone that plays a crucial role in the regulation of Sertoli cells tight junctions permeability barrier [25].

We used amlodipine drug among the other calcium channel blocker because of amlodipine is a long acting dihydropyridine calcium antagonist that inhibits transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It exhibits selectivity for the vascular smooth muscle cells than on cardiac muscle cells**.** Experimental facts support that amlodipine binds to dihydropyridine (DHP) as well as no dihydropyridine binding sites of voltage-dependent L-type calcium channels . It is several times more lipophilic than the first and second-generation DHP and occupies a well-defined position in the bilayer membrane. The calcium channel blockers are commonly associated with male infertility, long term administration of such drugs has been shown to suppress spermatogenesis [26].

Our study showed decrease in body weight of rats treated with amlodipine alone this may be related to that intracellular calcium ions play essential role in the regulation of cell growth, calcium channel blockers exhibit inhibitory influence on cell proliferation however this effect is more clear in malignant cell. Ca2+ channels are divided into ligand-dependent Ca2+ channels (LDCC) and voltage dependent Ca2+ channels (VDCC),VDCCs that are open in response to transmembrane voltage changes have been identified in various cell types, including testicular germ cells and somatic cells . CCBs cause drop in the weight of the testes and (gonado-somatic index) GSI and suppressed spermatogenesis. It has been shown that a strong correlation exists between testis weight and function (spermatogenesis) in mammals. In the absence of any known pathology, testis weight is highly related to daily sperm production[27].

As far as hormonal control of spermatogenesis is concerned, spermatogenesis depends on hypothalamic gonadotrophin releasing hormone (GnRH), pituitary gonadotropins, Luteinizing hormone (LH) and Follicle- stimulating hormone (FSH) . LH mediates its effects via testosterone and LH induced testosterone synthesis in the Leydig cells is dependent on calcium, so when used CCBs may cause disorder in this processes[28].

Tribulus terrestris is a traditional herbs have emerged in the past few years as an ‘instant’ treatment for sexual and erectile dysfunctions suggested to be effective in treatment of such dysfunctions by increasing serum LH and testosterone, and conversion of its phytochemical derivative, protodioscin to dehydroepiandrosterone (DHEA), The chemical structure of protodioscin is very similar to that of DHEA. Our observations on experimental group revealed that *Tribulus terrestris* administration resulted in increased body and testicular weight which were statistically significant when compared with the control. These findings agree with the findings of Gauthaman , who showed that treatment of castrated rats with *Tribulus terrestris* extract resulted in increased body and prostate weight. Gauthaman, while evaluating sexual effects of different doses of Tribulus terrestris on rats, observed a significant increase in body weight of treated rats which he presumed was due to androgenic effect of *Tribulus terrestris*, producing a stimulus to increase the appetite [29].

Testosterone has mainly two effects on the body firstly, the anabolic effect which mainly promotes protein synthesis, decreased nitrogen excretion, muscle growth and secondly, the androgenic effect which is responsible for the development and maintaining of the secondary sex characteristics, for example changes in hair distribution, physical changes, genital size, and sperm production [30]. Steroid hormones work by stimulation of receptor molecules in muscle cells, which activate specific genes to produce proteins. They also affect the activation rate of enzyme systems involved in protein metabolism, thus enhancing protein synthesis and inhibiting protein degradation (called an anti-catabolic effect), in case of GSI amlodipine cause decrease it however lengthening of treatment cause slightly increasing its values but not statistically as in our study, so testis weight is a sensitive early marker of gonadal injury or disorder, this variation may arise

from several factors including reactions to injury that mask a decrease in testicular weight like edema and, cellular infiltration, Leydig cell hyperplasia [31].

The study concluded that there is an effective reproductive efficiency of *Tribulus terrestris* in spite of the inability of the plant to minimize the negative effects of amlodipine and further detailed studies are warranted to prove such activity.

REFERENCES:-

1. Abeywickrama K, Bean GA. 1991. Toxigenic Aspergillus flavus and aflatoxins in Sri Lankan medicinal plant material. Mycopa-thologia. 113:187–190.
2.Phillips OA, Mathew KT, Oriowo MA. 2006. Antihypertensive and vasodilator effects of methanolic and aqueous extracts of Tribulusterrestris in rats. J Ethnopharmacol 104(3):351-5.
3.K. Gauthaman, A. P. Ganesan, and R. N. Prasad. 2003."Sexual effects of puncturevine (Tribulusterrestris) extract (protodioscin): an evaluation using a rat model". Journal of Alternative and ComplementaryMedicine. 9(2), 257-265.
4.Firas A, Bayati AL, Hassan F, et al. 2008. Antibacterial and antifungal activities of different parts of Tribulusterrestris L. growing in Iraq. J Zhejiang UnivSci B 9(2): 154–159.
5.S. H. Majeed, and M. J. Mahmood. 1988. Herbs and Medicinal Plants in Iraq between Traditional Medicine and Scientific Research. 1st Ed. Baghdad:Dar Al-Thaowra for Publishing 40.
6.Arsyad, KM. 1996. Effect of protodioscin on the quality and quantity of sperms from males with moderate idiopathic oligozoospermia. Medica 22:614-8.
7. Tomova, M. Tribestan. 1987. Pharmacy;37(6): 40-42.
8.CHEMEXCIL, 1992. *Tribulus terrestris* L. (N.O. Zygophyllaceae). Selected medicinal plants of India. A monograph of identity, safety and clinical usage. Bombay: Tata Press 323-6.
9. Koumanov F, Bozadjieva E, Andreeva M, Platonova E, Ankova V. 1982. Clinical trial of Tribestan. Exp Med . 4:211–5.
10.Anand R, Patnaik GK, Kulshreshtha DK, Dhawan BN. 1994. Activity of certain fractions of *Tribulus terrestris* fruits against experimentally induced urolithiasis in rats. Indian Journal of Experimental Biology. 32(8) 548-52.
11.Gauthaman K, Adaikan PG, Prasad RNV, Goh VHH, Ng SC. 2000. Changes in hormonal parameters secondary to intravenous administration of Tribulusterrestris extract in primates. International Journal of Impotence Research 12(2):6.
12. Sang-Won P, Chan-Ho L, Dae-Hee S, et al. 2006. Effect of SA1, a Herbal formulation, on sexual behavior and penile erection. Biol Pharm Bull 29: 1383-6.
13.Almaida SA, Teofilo JM, Franci JAA, Brentegani LG, Carvalho TLL. 2000. Antireproductive effects of calcium channel blocker amlodipine in male rats. Experimental and Toxicologic Patholology vol 52 (4) p 353-356.
14. Meacham RB. 2006.The Effect of Calcium Channel Blockers on Male Reproductive Potential. J Androl 52:311-8.
15.Kornhuber M, Trapp S, Pechmann S, Friedl A, Reichel M, Muhler C, Terfloth L, Groemer T, Spitzer G, Liedl K, Gulbins E, Tripal P. . 2011. Identification of Novel Functional Inhibitors of Acid Sphingomyelinase.PLoS ONE 6(8):e23852.
16.Latif R, Lodhi GM, Aslam M. 2008. Effects of amlodipine on serum testosterone, testicular weight and gonado-somatic index in adult rats. J Ayub Med Coll Abbottabad 20(4):8-10.
17.Franca LR, Suescun MO, Miranda JR, Giovambattista A Perello M, Spinedi E, et al. 2006. Testis structure and function in a nongenetic hyperadipose rat model at prepubertal and adult ages. Endocrinology 147:1556-63.
18. Gray P. 1953. Handbook of Basic microtechnique. Publisher Constable & Co., Ltd. UK
19. Paul R. Kinnear and Colin D. Gray. 2004 . SPSS made simple: release 12.0 publisher: Hove psychology
20.Gauthaman K, Adaikan PG, Prasad RN. 2002. Aphrodisiac properties of Tribulus terrestris extract (Protodioscin) in normal and castrated rats. Life Sci. 71:1385–96.

21.Du Plessis SS, de Jongh PS, Franken DR. 2004. Effect of acute in vivo sildenafil citrate and in vitro 8-bromo-cGMP treatments on semen parameters and sperm function. Fertil Steril. 81:1026–33.
22.Martino-Andrade AJ, Morais RN, Spercoski KM, Rossi SC, Vechi MF, Golin M, et al. 2010. Effects of Tribulus terrestris on endocrine sensitive organs in male and female Wistar rats. J Ethnopharmacol. 127:165–170.
23. Lai, Y.M., Fukuda, N., Su, J.Z., Suzuki, R., Ikeda, Y., Takagi, H., Tahira, Y. and Kanmatsuse, K. 2002. Novel Mechanisms of the Antiproliferative Effects of Amlodipine in Vascular Smooth Muscle Cells from Spontaneously Hypertensiv Rats.hypertens Res. 25(1):109-15.
24. Lan, L., Xinghua, X., Wenjuan, S and Liying, D. 2008.Effect of amlodipine on apoptosis of human breast carcinoma MDA-MB-231 cells. Journal of Medical Colleges of PLA. 23: 358-63.
25. Bauerle, H.D. and Seelig, J. 1991. Interaction of charged and uncharged calcium channel antagonists with phospholipid membranes. Binding equilibrium binding enthalpy, and membrane location. Biochemistry. 30: 7203–11.
26. Li LH, Wine RN, Miller DS, Reece JM, Smith M, Chapin RE. 1997. Calcium channel blockers in cultured rat seminiferous tubules: possible mechanisms. Toxicol Appl Pharmacol;144:105–19.
27. Saez JM. 1994. Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev. 5:574–626.
28. Bagatell CJ, Bremner WJ. 1996. Androgens in Men-Uses and Abuses. N Engl J Med 334: 707-14.
29. Gauthaman K, Adaikan PG, Prasad RN. 2002. Aphrodisiac properties of Tribulus terrestris extract (Protodioscin) in normal and castrated rats. Life Sci; 71: 1385-96.
30.Pope, H. G., Jr. & Katz, D. L. 2003, Psychiatric effects of exogenous anabolicandrogenic steroids. In: Wolkowitz, O. M. & Rothschild, A. J. (eds) Psychoneuro-endocrinology, the scientific basis of clinical practice. American Psychiatric Publishing, Inc, London,; pp. 331-358.
31.Bahrke, M.S., C.E. Yesalosk, and J.E. Wright. 1990 . Psychological and behavioural effects of endogenous testosterone levels and anabolic-androgenic steroids among males: a review. Sports Med;10: 303-337.