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A comparative study between the effect s of *Arctium lappa L.* leaves extract and Pentoxifylline on DNA of sperms rats treated with Gentamicin

Hassanin D. Alwan[1], Huda F. Hasan[1]*, Muhammad-Baqir M-R Fakhirldin[2]

[1]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

[2]Department of Clinical Reproductive physiology, High Institute of infertility Diagnosis and ART, Al Nahrain University, Baghdad, Iraq

Abstract:

This study were designed to make comparison between the influence alcoholic extract of *Arctium lappa L*. leaves and Pentoxifylline on treatment the adverse effect of gentamicin on DNA of sperms. Thirty five male rats were divided into 5 groups: the 1st group (T1) was made as negative control group, the 2nd group (T2) was given distilled water and gentamicin 5mg/kg **(**positive control group), the 3rd group (T3) was given a dose 600 mg/kg of *Arctium lappa L* and gentamicin, the 4th group (T4) was given dose 100mg/kg of pentoxifylline and gentamicin, the 5th group (T5) was given a dose 300mg/kg of *Arctium lappa* extract with dose 50mg/kg of pentoxifylline and gentamicin. The results of DNA damage% and agglutination % in T3, T4 and T5 appeared an important increase as compared with T2. Testosterone level in T3 and T5 showed an important arise when made comparison with T2 and T4. The section of testis in (T2) showed several destructions in the testis and sever necrosis of the seminiferous tubules, while the section of testis in (T3) showed slight odema in the interstitial tissues and decrease degenerative changes of the somniferous tubules. The section in (T4) showed odema in the interstitial tissues and blood vessels congestion, while the section in (T5) group showed development in tissue of testis, there is healthy spermatids and mild changes in the testis when compared with section of rat testis treated with pentoxifylline only. In this study concluded the *Arctium lappa* leaves extract and pentoxifylline have the ability to lessen the adverse action of gentamicin on DNA of sperms and *Arctium lappa* leaves caused improvement the action of pentoxifylline in improvement fertility.

Keywords: *Arctium lappa*, pentoxifylline**,** DNA damage, fertility

دراسة مقارنة بين تأثير مستخلص اوراق الأرقطيون والبنتوكسيفيلين على الحمض النووي للحيوانات المنوية في الجرذان المعاملة بالجنتامايسين

حسنين داخل علوان[1]، هدى فلاح حسن[1]*، محمد باقر محمد رشاد فخر الدين

[1]فرع الفسلجة والادوية البيطرية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

[2]المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب، جامعة النهرين، بغداد، العراق

الخلاصة:

هدفت الدراسة الى المقارنة بين تأثير المستخلص الكحولي لأوراق نبات الارقطيون ودواء البنتوكسيفلين على تعزيز الحمض النووي وعلاج تأثير الجنتامايسين السلبي على الحيوانات المنوية في الجرذان. تم استخلاص اوراق الارقطيون بواسطة المستخلص الكحولي . قسمت ذكور الجرذان (35) الى خمس مجاميع

*Email: dr.hudaalqaraghuli@yahoo.com

وكانت فترة العلاج 30 يوم : تركت المجموعة الاولى (T1) من دون اي علاج (مجموعة السيطرة السلبية)، المجموعة الثانية (T2) معالجة بالماء المقطر عن طريق الفم وبالجنتامايسين (5ملغم/كغم) وزن الجسم عن طريق الخلب ، المجموعة الثالثة (T3) معالجة بجرعة يومية من المستخلص النباتي (600 ملغم / كغم) عن طريق الفم والجنتامايسين (5ملغم/كغم)، المجموعة الرابعة (T4) معالجة بجرعة يومية من البنتوكسيفلين (100 ملغم/كغم) عن طريق الفم والجنتامايسين(5ملغم/كغم) ، المجموعة الخامسة (T5) معالجة بجرعة يومية من مستخلص نبات الارقطيون (300 ملغم/كغم) وبدواء البنتوكسيفلين(50 ملغم/كغم) والجنتامايسين (5ملغم/كغم). اظهرت نتائج نسبة ضرر الحمض النووي ونسبة التكتلات في المجاميع T5, T4,T3 انخفاض معنوي بالمقارنة مع مجموعة T2. في حين اظهرت المجاميع المعالجة T5 T3, افضل زيادة لمستوى هورمون التيستيستيرون بالمقارنة مع T4, T2. المجاميع أوضحت المقاطع النسيجية لخصى الجرذان المعالجة T2 تلف الانسجة و ونخر في الانابيب المنويةو تساقط للطبقة الجرثومية، اذمة واحتقان الاوعية الدموية ، في حين أضهرت مقاطع الخصى في (T3) اذمة خفيفة في النسيج الخلالي وقليل من التغيرات التنكسية في الانابيب المنوية. اوضحت المقاطع النسيجية في المجموعة (T4) اذمة في النسيج الخلالي مع احتقان الاوعية الدموية في جين تميزت المجموعة(T5) ب تحسن في انسجة الخصية بالاضافة الى تغيرات خفيفة في الخصية بالمقارنة مع انسجة من خصى الفئران المعالجة ب البنتوكسيفلين والجنتامايسين فقط. من هذه الدراسة نستنتج الى امكانية المستخلص الكحولي لاوراق نبات الارقطيون ودواء البنتوكسيفلين على تقليل الاثار الجانبية للجنتامايسين في الحمض النووي ، من جهة اخرى قدرة المستخلص الكحولي على تحسين دور البنتوكسيفلين في تعزيز الخصوبة.

Introduction:

Male sexual dysfunction frequently treatment by medical drugs that have adverse effect, the herbal plants are chief basis of drugs and display a vital starring effect in the benefit of the world's population, [1].Therefore, several medicinal drugs used with drugs to potentiate effect and minimize adverse effects of the drugs [2]. *Arctium lappa* products have been charity in the a voiding of numerous pathological circumstances of animals since they are commonly rich with antioxidants [3]. *Arctium lappa L* . is widely acted as a antipyretic and diuretic also for gout, hepatitis , hypertension and other inflammatory disorders[4]. Furthermore, *Arctiumlappa L* has ability to heal the infertility without or fewer side effect . [5]

Many drugs used to treat and enhance fertility with elevate performance of reproductive system in male e.g pentoxifylline have many side effect and acute toxicity [6]. Pentoxifylline is a methyxanthin resulting in the alike the caffeine which makes the break of cyclic adenosine monophosphate (cAMP). This lead to cell glycolysis and production energy (ATP) that stimuli the sperms movement appearances [7, 8]. Antibiotics indicated to heal many diseases such as infectious diseases and it's have several adverse effects and may be cause decrease in fertility [9]. Gentamycin is classified as aminoglycocide antibiotic treating systemic infections and other neonatal sepsis occured by gram negative microorganism. Gentamycin has poisonous effects like nephrotoxicicy and ototoxicity because the generation of free radical and oxygen species [10]. This study was performed to decrease the negative outcome of gentamicin on DNA of sperms by exhausting *Arctium lappa L*. alcoholic extract and Pentoxifylline.

Materials and Methods:

Extraction leaves of *Arctium lappa L* :

The *Arctium lappa L* leaves powder was carried with 70% ethyl alcohol to make the extraction. One hundred fifty grams of the plant powder were put in one liter flask with 700 milliliter of alcohol. The hole of flask was enclosed by a Teflon, after this step the flask was positioned on magnetic stirrer hot plate. The extraction temperature was 45c°. Then the solution left to moving for seventy two hrs. and then filtered via sterilized gauze to acquire free of rough particulars, then solution sieved by Whitmann filter. The remains was transferred in hygienic and germ-free petridish and saved at 45c° temperature in incubator until dehydration after that saved in deep freezing [11].

Experimental Animals:

Male rats about 250gm body weight and 35 in number were obtained from the National Center for Pharmaceutical Control Baghdad. Rats were kept in plastic cages 60x20x20 cm in the lap room.

Classic rat food (marketable nourish pellet) and water was spontaneously obtainable. Accommodation environments were preserved at light /dark cycle (14/10 hours) and 28±2c° The brood of cages was altered every 7 days.

Experimental designs:

Thirty five male rats were divided into five groups equally and the period of treatment was 30 days:

The animals of 1st group (T1) were port without treatment **(**negative control group).

The animals of 2nd group (T2) were given distilled water orally by gastric tube with gentamicin (5mg/kg) B.W given intra peritoneal (positive control group).

The animals of 3rd group (T3) were given a dose of (600 mg/kg) B.W. of *Arctium lappa L* orally by gastric tube and given gentamicin (5mg/kg) B.W daily.

The animals of 4th group (T4) were given a dose (100mg/kg) B.W of pentoxifylline given orally by gastric tube and gentamicin (5mg/kg) daily.

The animals of 5th group (T5) were given a dose orally of (300 mg/kg) B.W of *Arctium lappa* extract with a dose orally (50mg/kg) B.W of pentoxifylline and gentamicin (5mg/kg) B.W daily.

Blood collection

After the finish of the experiment, diethyl ether was used to anesthetiz the rats and the blood specimens were achieved via cardiac puncture by disposable syringes. Samples of blood put in conical plastic test tubes and centrifuged at 2500 RPM for 15 minutes, and serums specimens were separeted and put in tubes and stored in freezer at (-18ċ) till use.

Serum testosterone: Levels of the testosterone hormone were measured by Radio-immunoassay (RIA) kit (Invitrogen)®, after the samples treating with I^{125} (Labeled testosterone tracer), then by Gamma Counter the joining between I^{125} with testosterone hormone were measured in ng/ml unit. The analyses were achieved in Radio Active Isotope Clin. Laboratory at Harithyeh Street- Baghdad.

Semen Collection: After sacrified rats in the five groups by merciful way, the testis were separated from left epididymis. The caudal epididymis were removed from the testis, clear with filter paper and put in glass of watch with one ml of warm sodium citrate 2.9% , after this step the micro scissor was used to cut epididymis about 200 pieces [12].

Assessment of DNA damage of sperm: Sperm chromatin integrity was evaluated by acridine orange fluorescence. After semen collection, 0.1ml of sperms were taken and assorted with 0.1 ml of tyroid solution and then centrifugation for 15 min , then one drop was taken from the precipitate and put on the sterilized glass slide on the similar day of the investigation, then it was fixed overnight in a prearranged coronoys solution [13]. Within following day, it was deried at room temperature and the slides staining by acridine orange solution (10ml of 1% AO in distilled water added to amixture of 40 ml of 0.1 citric acid and 2.5ml of 0.3M Na2HPO4, 7H2O) for 5 minutes, the slides were slightly rinsed with distilled water and observation under fluorescence microscope sperms chromatin grade was evaluated by using the technique described by[14]. The percentage sperm with standard DNA were measured by calculating at minimum 200spermatozoa under a fluorescence microscope in total magnification 400x, with excitation at 450-490 nm. Sperm with typical, integral double-stranded DNA marked green and those abnormal ones exhibited red or carroty fluorescence were assessed **.**

The percentage of normal DNA spermatozoa = normal DNA (Green color)/ total No .of spermsx100

Sperm agglutination: The percentage of sperm agglutination (movement of spermatozoa branch to each other heads to heads, tails to tails or in mixed) were estimated according to the following formula which recorded by [15]:

Agglutinated sperm (%): No. of agglutinated sperms/ Total number of sperms x100.

Histopathological changes*:* Afterward the finish of experiment, rats were sacrificed and cervical dislocation were done to animals, testes and epididymis were excised and cleared off the involved fat and connective tissue. Histological sections were prepared according to [16].

Statistical analysis: SAS [17] is the Statistical Analysis System- was used to result of changed factors in parameters of study. Least important difference LSD test was used to significant compare between means in this study and the analysis is one way, mean±SE, different capital letters mean significant ($P<0.05$) within the same columns.

Results and discussion:

The results of DNA damage % of rats in (T3, T4 and T5) appeared significant decrease ($p< 0.05$) in mean values (11.5±0.93,10.8±0.73,10.1±1.01) respectively as compared with positive control treated group (66.34±1.6), as in (table 1), in addition to that appeared in images that obtained from

immunofluorescence microscope in Figures-1,2,3 and 4. These results might be attributed to capability of *Arctium lappa* in the management the abnormality in DNA by the antioxidant possessions, this results agreed with results recorded by [18], when mentioned that the effect of *Arctium lappa L* extract as antioxidant was recognized by observing the signs power of superoxide dismutase (SOD) in relative to 5, 5-dimethyl-1- pyrroline-N-oxide (DMPO)-OOH. Furthermore, the extract performance by enhancing glutathiones (GSH), and by dropping malondialdehyde (MDA), for this reasons the *Arctium lappa L* had raised abilities in free radicals scavenge [19]. The Pentoxifylline and gentamicin treated group showed inhibition in DNA abnormality in comparison with animals given gentamicin, this effect of Pentoxifylline may be regarded to it act as anti-inflammatory effect with phosphodiesterase suppressor that improves the intracellular cAMP, reduces free radicals anion and depress TNF-α, which is in charge for DNA breakdown, furthermore, the Pentoxifylline is saves the tissues from lipid peroxidation by free radicals which is responsible for reducing the H2O2, this action is regarded to the declining of the progress of endoperoxides as a result from the high cAMP intensities that prevent the cyclooxygenase by the arachidonic acid pathway, this result similar with results that recorded by [20], the a cut DNA damages in positive control group may be regarded to gentamicin was recognized to enhance the peroxidation of the lipid , ROS formation and by falling enzymes that act as antioxidant lead to oxidative and DNA degradation, the results agreed with results reported by [11].

Table 1- The percentages of DNA damage% and agglutination of sperms% after 30 days of the treatment in different groups.

Parameters / Groups	DNA damage%	Agglutination%
T1(animals without treatment)	10.60±0.81A	2.30±1.65A
T2(distilled water and Gentamicin (positive control) treated group)	66.34±1.60B	23.60±0.41B
T3(extract of *arctium lappa* L. and gentamicin treated group)	11.5±0.93A	2.10±0.71A
T4(animals given pentoxifylline drug and gentamicin)	10.8±0.73A	2.0±1.80A
T5**(**extract of *arctium lappa* L with pentoxifylline treated group)	10.1±1.01A	2.5±1.04A

*** The data are expressed as mean±SE, different capital letters mean significant difference (p<0.05) between groups within the same column.**

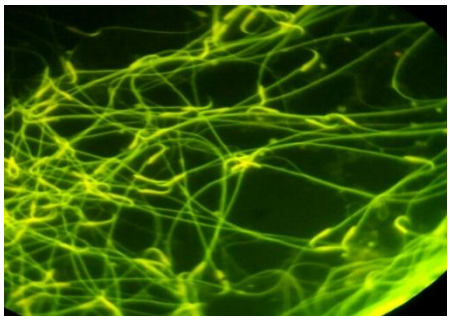

Figure 1- Sperm head under magnification power displaying green fluorescence as normal with intact DNA in group treated with extract of *Arctium lappa L* and gentamicin for 30 days of administration

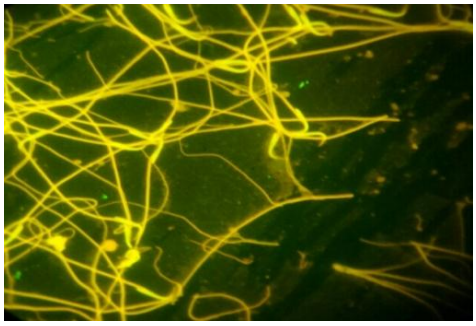

Figure 2- Sperm head under magnification power displaying green fluorescence as normal with intact DNA: group given extract of *Arctium lappa* L with pentoxifylline and gentamicin for 30 days of administration

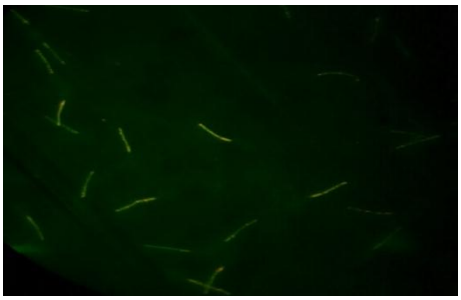

Figure 3- Sperm head under magnification power displaying green fluorescence as normal with intact DNA in group treated with pentoxifylline and gentamicin for 30 days of administration.

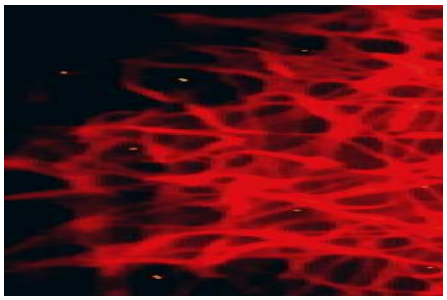

Figure 4- Sperm head under magnification power displaying red fluorescence as abnormal with intact DNA in group treated with distilled water gentamicin for 30 days of administration

The outcomes of the of the agglutination percentage revealed a significant decrease ($p<0.05$) in group T3, T4 and T5 in mean value (2.10±0.71, 2.0±1.80 and 2.5±1.04) respectively in comparison with T2 group (23.60±0.41) however, there was no a significant differences as compared with T1 group as listed in Table-1. The inhibition in % of agglutination in T3 possibly is result from the ingredients of *Arctium lappa L* extract which contain alkaloid that enhance the development of tissue vessels in the reproductive organs in addition, to the mixes of important constituents in *Arctium lappa*L. leaves extract assisted in developments of a sexual purpose [21]. Furthermore, the extract of plant have a high performance such as inhibition the inflammatory process by lessening damage and release of cysteinylleukotrienes (Cys-LTs) such as histamine and prostaglandins by exterior blood mononuclear cells, in addition, to arctigenin's of plant act as inhibitor to TNF-α formation which possible facilitated by acute inhibition the mitogen-activated protein kinases, that inhibit the activator protein-1and decrease the agglutination, the same this result reported by [22].

Agglutination % of sperms decrease in T4 group may perhaps regarded to pentoxifylline have capacity to supress the creation of pro inflammatory cytokines , also have positive consequence on sperms concentration by decrease the disruption cAMP which play vital role in sperms motion by stimulate cAMP-dependent protein Kinase (PKA), a vital modulator of spermatozoa and produces cellular glycolysis with ATP. Furthermore, the pentoxifylline have ability to inhibit free radicals , act as antioxidant and a strong immune modulatory characteristics , the similar results recorded by [23]. The enhance in agglutination percent of sperms in T2 group was recognized to gentamicin produced reduction in the sperms motility, sperms count and sperms viability and arise the abnormalities that have an important effect in surge the percent of agglutination between sperm, furthermore, the gentamicin enhanced the lipids peroxidation and free radicals production, this result agreed with results mentioned by [10].

The testosterone level in animals of (T3) and (T5) showed the best a significant increases ($p<0.05$) in mean values (3.02±0.62,3.0±0.59) respectively as compared with all others treated groups. The animals in (T2) showed a significant decreases ($p<0.05$) in testosterone levels with mean value (1.23±0.23) as compared with (T1) and animals in other groups as Table-2.

Table 2-The level of serum testosterone (Ng/ml) in different groups of rats after treatment for 30 days.

Group	T1	T2	T3	T4	T5
Testosterone level (Ng/ml)	2.01±0.51A	1.23±0.23B	3.02±0.64C	2.91±0.64A	3.0±0.59C

*** The data are expressed as mean±SE, different capital letters mean a significant differences ($p<0.05$) between groups within same column.**

Testosterone level increase in *Arctium lappa L* and gentamicin extract, possibly may be regarded to the influence of *Arctium lappa* on sexual organs by improving the action of reproductive tissue by enhancing of hypothalamus-pituitary-gonads axis guides and a arise the androgen hormone, which result from the augmentation in the GnRH-LH signaling in the testis and Luteinizing hormone (LH), which stimulate formation and secretion of testosterone hormone [24]. The a rise in testosterone hormone of group (T4) may be explained to Pentoxifylline has vital role for augmenting serums of reproductive hormones, furthermore, it caused suppress in the phosphodiesterase enzyme which is lead to reduce LH , F SH hormones and decrease the cAMP, this result agreed with result reported by

[25]. The result of of (T5) group may be attributed to the impotant constituents of *Arctium lappa* plant which act by potentiating and development the outcome of pentoxifylline, in augmentation role of sexual organs, furthermore, the extract healed many systemic adverse effect of pentoxifylline, this result agreed by result mentioned by [1]. The result of rats in (T2) group may be attributed to gentamicin was recognized to enhance the free radical production and lipids peroxidation by suppressing the antioxidant enzymes levels , furthermore, the free radical-caused oxidative degradation to sperms, Sertoli cells and leydig cells, the same results recorded by [10].

The section of rat testis in (T2) group showed several destructions in the testis(complete testicular damage) , sloughing of the germinal layer and the necrotic debris seen in the lumen of the seminiferous tubule also widening of interstitial space and dilation of seminiferous tubules also there is edema and congestion of blood vessel Figure-5, this result can be explained by inhibiting the cell dissection of gem cells and synthesis of proteins in the testis by gentamicin, in addition to increase the cell loss in the seminal vesicles, this result similar to results reported by [10]. Another researches agreed this result recorded by [9] when they said gentamicin is capable to generate superoxide reactive free radicals in addition to hydrogen peroxide radicals lead to produce oxidative and necrotic impairment.

The test is of rats in (T3) group showed mild edema in the interstitial tissue and some sections showed thickening of interstitial, in addition some somniferous tubules showed mild necrosis in the germinal layer and decrease degenerative changes of the somniferous tubules in the germinal layer Figure-6 when compared with section of testis rat in (T2) group Figure-5 and showed best improvement as compared with section of testis in (T4) group Figure-7. This result agreed with studies recorded by [26], when they said the seminiferous tubules volume density is not changed after given the *Arctium lappa* extract, also the Leydig cell remained without any variations and enhance spermatocyte , this result can be explained for plant caused augmentation of sperms formation and save the action of cells with prevent destructions. In other wise, there were researches clarified the influence of *Arctium lappa* extract by inhibiting of nuclear factors of kappa B (NF-κB), starting of antioxidant enzymes and hunting of free radical are the main mechanism of *Arctium lappa L* in preventing the inflammation, furthermore it has the ability in preventing destruction and release of cysteinyl leukotriene by external blood mononuclear cells are included inflammatory agents like histamine and prostaglandins, the block of Cys-LT is lead to suppression the inflammatory cells and edema. [27]. In addition, the *Arctium lappa L* had great abilities to scavenge the free radicals, because it included the flavonoids , in addition to steroidal saponins lead to increase testosterone formation and its physiological effect, therefore successful their manufacture and inhibiting its metabolic destruction by activating lyding cell, this result agreed with results reported by [28].

The rat testis section in (T4) group showed edema in the interstitial tissue and congestion of blood vessels, also necrosis of the interstitial tissue, in addition there is mild necrosis in the germinal layer and degenerative changes especially in the spermatocytes as in Figure-7.This results due to the pentoxifylline have anti-inflammatory and antioxidant properties by decreasing (ROS), an inhibit of lipids peroxidation associated with the plasma membranes, furthermore, it decrease of hydroxyl anions, and an TNF-α suppression lead to inhibit germs cell injury and decrease the pathological lesions severity occured by gentamicin, the same result reported by [20]. While the section of rat testis in (T5) group showed development in tissue of testis, there is regular spermatocytes, spermatozoa and spermatids, furthermore the slight variations in the testis included minor edema and congestion of blood vessels in comparison with sections of rat testis given pentoxifylline only as in Figure-8. This result may be regarded to enhance the positive influence of pentoxifylline in decreasing acute lesions induced by gentamicin when given with *Arctium lappa* extract, the numerous important constituents of extract included lignans, tannin, alkaloids, flavonoids, amino acids, phenols, minerals, sterols, vitamins, , lactone, sugars (polysaccharides), saponins, and polyacetylenes may be contributed in enhancing the influence of pentoxifylline and decrease adverse influence [29].

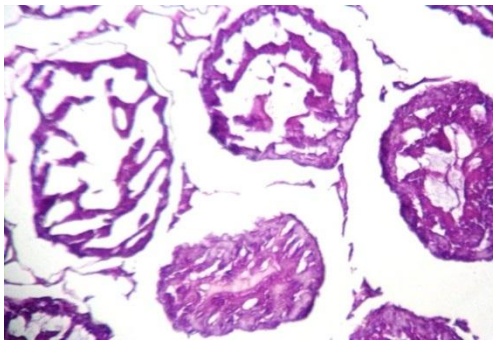

Figure 5- Tissue section in the testis of rat treated with distilled water and gentamicin showed sloughing of the germinal layer and the necrotic debris in the lumen of the semniferous tubules with odema and congestion blood vessels. H and E (x400).

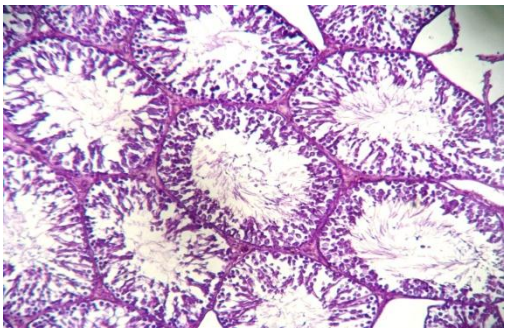

Figure 6- tissue section of rat treated with alcoholic extract of *Arctium lappa l* and gentamicin, showed normal seminiferous tubules with presence spermatocyte and spermatid H and E (x200)

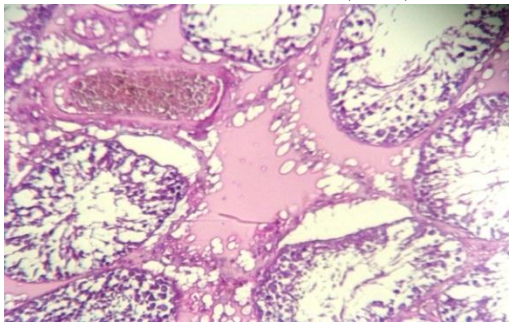

Figure 7- Tissue section in the testis of rat treated with pentoxifylline and gentamicin showed mild necrosis in the germinal layer, congestion of blood vessels and edema. H and E (x400).

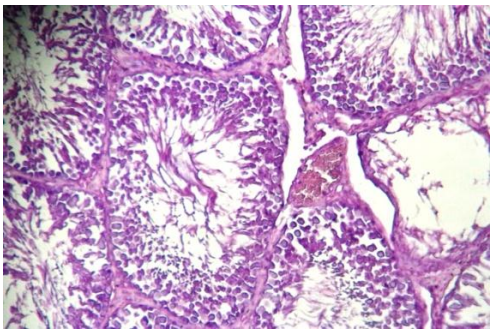

Figure 8- Tissue section in the testis of rat treated by *arctium lappa* extract with pentoxifylline and gentamicin showed normal spermatocytes, very mild changes in the testis include mild edema and congestion of blood vessels. H and E (x400).

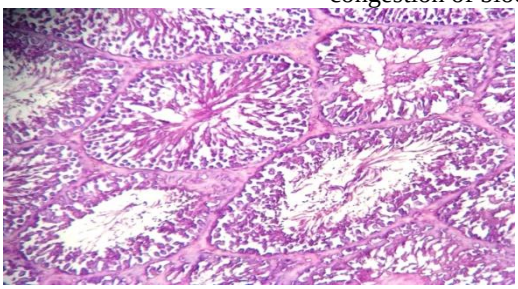

Figure 9- Tissue section in the testis of control negative group showed normal tissue H and E (x400).

Conclusion:

In this study concluded the *Arctium lappa* leaves extract and pentoxifylline have the ability to lessen the adverse action of gentamicin on DNA of sperms and *Arctium lappa* leaves caused development the action of pentoxifylline in improvement fertility.

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