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A COMPARATIVE STUDY BETWEEN THE EFFECTS OF *ARCTIUM LAPPA L.* LEAVES EXTRACT AND PENTOXIFYLLINE ON FERTILITY OF MALE RATS TREATED WITH GENTAMICIN

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ABSTRACT
The objectives of this study was to compare the effect between *Arctium lappa L.* alcoholic extract and Pentoxifylline drug on the enhancement of fertility in rats. Leaves of *Arctium lappa* was extracted by making alcoholic extract 70%. Thirty five male rats were divided into five groups and the period of treatment was 30 days: the first group (T1) was treated with daily dose of (600) mg/kg of *Arctium lappa L.* and gentamicin (5) mg/kg, the second group (T2) was treated with a daily dose (100mg/kg) of pentoxifylline and gentamicin (5mg/kg), the third group (T3) was treated with dose of (300mg/kg) of *Arctium lappa* extract with dose (50mg/kg) of pentoxifylline and gentamicin, the fourth group (T4) was treated with distilled water and gentamicin. The fifth group (T5) was left without any treatment (negative control group). The results of motility% and life sperm% in T3 group showed the best significant increase and best reduction in abnormalities % as compared with T2, T4 and T5 groups. Body weight and testis weight in T3 group showed a significant increase as compared with T1, T2, T4 and T5. In other hand the gonadosomatic index in T3, T4 and T5 groups showed a significant increase as compared with T1 and T2 groups. In this it could be concluded that the alcoholic extract of *Arctium lappa* leaves and pentoxifylline have a significant influence to reduce the side effect of gentamicin in fertility. On other hand, the extract of *Arctium lappa* leaves lead to improve the effect of pentoxifylline in enhancement fertility in rats.

KEYWORDS: *Arctium lappa*, pentoxiphylline, fertility, gentamicin.

INTRODUCTION

Products with Natural source have been used in the dealing and treating of various chronic pathological conditions of animals because they are mostly antioxidants rich[1]. Therefore, the common uses of medical herbal plants are incorporate with different medicinal drugs potentiate drug effect and minimize adverse effects[2]. This plant is well-heeled in anti-oxidative agents, such as tannin, quercetin, gallic acid, and caffeoylquinic acid. Some trials show that this plant has used to treat the infertility without or less side effect. The leaves of this plant have also been traditionally used for treatment of impotence and sterility[3]. Pentoxifylline is a methyxanthin derived in the same pharmacologic group as caffeine that constrains the breakdown of cyclin adenosine monophosphate (cAMP). This causes cellular glycolysis and endogenous adenosine triphosphate (ATP) production that stimuli the sperm motion characteristics[4,5]. In overall, Pentoxifylline has been reportedly active in protective sperm motility in vitro, also when administrated orally to the animal[6]. Antibiotic drugs used to treat many diseases such as infections have many side effects and may lead to decrease fertility[29]. This study was performed to study the following aims, to compare the effect between *Arctium lappa L.* alcoholic extract and Pentoxifylline drug on the enhancement of fertility in rats and treat the adverse effect of gentamicin on DNA of sperm by using *Arctium lappa L.* alcoholic extract and Pentoxifylline.

MATERIALS & METHODS

Extraction leaves of *Arctium lappa L.*

The powder of *Arctium lappa L.* leaves was extracted with 70% ethyl alcohol 150 grams of the leaves powder were placed in 1 liter flask with 700 ml (70% ethyl alcohol). A Teflon magnet was covered the orifice of flask, then the flask was placed on hot plate magnetic stirrer. The temperature of extraction was 40-45°C. The solution was left stirring for 72 hours and then sieved by using sterile gauze to get rid of coarse particulars. The solution then filtered through Whitmann filter. The filtrate was poured in clean and sterile petridishes and kept in incubator at temperature of 45°C until dryness after that kept in deep freeze [7].

Experimental Animals

Thirty five albino Swiss male rats weighting 250 gram were used in the present study. Rats were housed in plastic cage 60x20x20 cm placing in the room until the beginning of experiments. Standard rodent diet (commercial feed pellets) and Tap *ad. lib*. Water was freely available. Housing conditions were maintained at 28±2°C and light /dark cycle (14/10 hours). The litter of cages was changed every 7 days with taken care to avoid unnecessary stress.

Experimental designs

Thirty five male rats were distributed into five sets (7 rats in each one):

Group (1):

The first group (T1) was left without any treatment (negative control group).

Group (2):

The second group (T2) was treated with distilled water given orally by stomach tube and gentamicin (5mg/kg) I/P for 30days (positive control group).

Group (3):

The third group (T3) was treated by daily dose of (600) mg/kg body weight of *Arctium lappa L* given orally by stomach tube and gentamicin *(*5) mg/kg body weight given I/P for 30 days.

Group (4):

The fourth group (T4) was treated with a daily dose (100mg/kg) of pentoxifylline given orally by stomach tube and gentamicin (5) I/P for 30days.

Group (5):

The fifth group (T5) was treated with a daily dose orally of (300) mg/kg body weight of *Arctium lappa* extract with a daily dose orally (50mg/kg) of pentoxifylline and gentamicin (5) I/P for 30days.

Semen Collection

The thirty five groups were killed by merciful way for get sperms, the testis were detached laterally with left epididymis. The caudally epididymis were detached from the testis, marked with filter paper and place in watch glass with 1ml of warm sodium citrate 2.9% then incise and cut about 200 sections by micro scissor [30].

Progressive sperm motility

This parameter is complete directly afterward the assortment of semen. Semen was pressed from the epididymis caudal part on a pre-warmed (27°C) optical microscope slide; the slide was at that point protected with a warm accommodation slip-up and inspected in the light optical microscope using (400x) magnification. Ten fields of the optical microscope were arbitrarily chosen and the spermatozoa motility of ten spermatozoa was measured on each field. So, the motility of 100 spermatozoa was measured arbitrarily. Spermatozoa were considered as sluggish, motile, or immotile. The measurement of motile sperms was well-defined as the motile sperms number divided by the entire number of calculated spermatozoa (*i.e*. 100) [8].

Spermatozoa viability (Life/dead ratio)

The measurement of life and dead sperms was done according to method recorded by[9]. This procedure was complete by addition 2 droplets of Nigrosin / Eosin color wash (stain) to the seminal fluid on a slide that pre warmed , a constant smear was then completed and dehydrated out by air; the color slide was directly inspected under the optical microscope using x400 magnification. The living spermatozoa cells were stainless while the dead spermatozoa cells absorbed the stain. The sperm that unstained were counted and the percentage was calculated as in the following equation:

Percentage of dead sperms = (No. of live sperms / Total sperm No.) X100.

Abnormal Sperm morphology

The abnormalities were measured according to method recorded by[9]. This was done by addition two droplets of warm Walls and E was stain to the semen on a pre-warmed slide, a unchanging smear was then finished and air-dried; the pigmented slide was directly inspected under the light microscope using (The morphologically abnormal sperms were estimated depending on sperms abnormality *e.g.* tapered head, tailless head, coiled tail, bifurcate tail and broken tail.

Abnormal sperms morphology was calculated according to the following equation:

Percentage of abnormal sperms= ((No. of morphologically abnormal sperms / Total sperm No.) X100.

Sperm Concentration

Sperm count was done according to[10 & 11], by using Hemocytometer (Neubauer Type). The Hemocytometer slides were filled with 50 µl of a sperm suspension by micropipate and covered by cover slide, the sperms were counted in twenty-five small squares of the chamber in the squares of red blood cell by using light microscope.

Estimation of sperm was made according to the following formula:

Sperm concentration= Number of sperms x 1000 x10x400/80.

Gonadosomatic index (%)

After the end of treating period, the rats were weighed and the testis organ weight measured after scarified animals, testis were removed and balanced by sensitive balance after being cleaned from the accessory connective and adipose tissues, in order to know the effect alcoholic extract and pentoxiphylline on body weight.

The percentage of Gonadosomatic index was calculated as in the next equation according to [12 & 13].

$$\text{Gonadosomatic index} = \frac{\text{wieght of testis(gm)}}{\text{body wieght of rat(gm)}} \text{ X } 100$$

RESULTS

Sperms function test

The results of sperm function test in present study was demonstrated in table (1) and figure (1) in all groups, the percentage of motility in rats treated with *Arctium lappa* extract and gentamicin showed the best significant increase ($p<0.05$) in mean value (72.57±1.95) as compared positive control treated group, pentoxifylline and gentamicin treated group and *Arctium lappa* extract with pentoxifylline treated

group there is no significant difference ($p<0.05$) as compared with negative control group. While the results of motility percentage of sperm in positive control treated group appeared significant decrease ($p< 0.05$) in mean value (36.28±3.25) as compared with negative control group and other treated groups. In addition the results of rats treated with *Arctium lappa* extract with pentoxifylline and gentamicin showed improvement in motility percentage in mean value (61.43±3.89) as compared with pentoxifylline and gentamicin treated group (54.85±4.49). In current study the mean values of the sperms count of rats treated with *Arctium lappa* extract and gentamicin, pentoxifylline and gentamicin, pentoxifylline with *Arctium lappa* extract showed significant increase ($p< 0.05$) in mean values (78.57±1.51), (69.71±3.19) and (75.71±3.24) respectively as compared with positive control treated group (51.14±4.22) and no significant ($p<0.05$) as compared with negative control group. The life sperm percentage of group treated with *Arctium lappa* extract and gentamicin showed important increase ($p< 0.05$) in mean value (80.43±2.43) when compared with, pentoxifylline and gentamicin treated group and *Arctium lappa* extract with pentoxifylline treated group in means values (63.57±4.60) and (70.75±2.28) respectively. In otherwise the results of life sperms percentage in positive control treated group appeared significant depletion ($p<0.05$) in mean value (29.71±3.60) as compared with negative control and other treated groups. The table and figure also showed attend of abnormalities to decrease significantly ($p<0.05$) in group treated with *Arctium lappa* extract and gentamicin, pentoxifylline and gentamicin treated group and *Arctium lappa* extract with pentoxifylline in means values (20.25±2.22, 58.00±3.41 and 41.28±2.81) respectively comparing with positive control group (75.85±3.46), which revealed enhancement of abnormalities significantly ($p<0.05$) as compared with negative control group. On the other hand animals receiving extract of *Arctium lappa* and gentamicin was the best recorded in the lack percentage of abnormalities as compared with other treated groups, in addition to improvement of abnormalities significantly ($p<0.05$) in animals receiving extract of *Arctium lappa* with pentoxifylline together when compared with animals receiving pentoxifylline and gentamicin only.

TABLE 1: Effect of *Arctium lappa* extract , pentoxifylline , *Arctium lappa* L extract with pentoxifylline , distilled water and gentamicin on motility%, abnormality%, Life sperm% and Concentration ($x10^6$ /ml) after 30 days of the treatment.

Param. / Group	Motility	Count of sperm	Life sperms	Abnormalities
T 1	76.57±1.46A	81.71±2.53A	84.57±2.13A	15.00±1.72A
T 2	36.28±3.25B	51.14±4.22B	29.71±3.60B	75.85±3.46B
T 3	72.57±1.95A	78.57±1.51AD	80.43±2.43C	20.25±2.22C
T 4	54.85±4.49D	69.71±3.19D	63.57±4.60D	58.00±3.41D
T 5	61.43±3.89D	75.71±3.24AD	70.75±2.28D	41.28±2.81E

T1 control group (negative control).
T2 treated with distilled water and Gentamicin (positive control).
T3 treated with extract of *arctium lappa* L. and gentamicin
T4 treated with pentoxifylline drug and gentamicin.
T5 treated with extract of *arctium lappa* L with pentoxifylline .
*mean±SE, different capital letters mean significant difference ($p<0.05$) among columns.

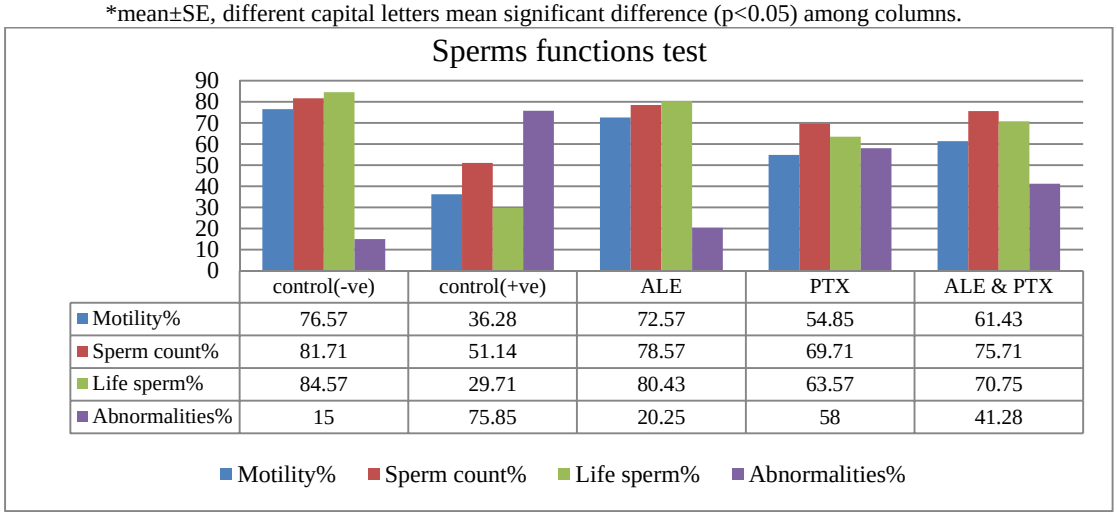

FIGURE 1: Sperms functions test after 30 days of treatment.

⋆*Arctium lappa* extract (ALE).
⋆ Pentoxifylline (PTX).

Body weight, testis weight and gonadosomatic index percentage

The results of body weight, testis weight and gonadosomatic index, parameters are shown in the groups treated with *Arctium lappa* extract and gentamicin, pentoxifylline and gentamicin, *Arctium lappa* extract with pentoxifylline and gentamicin showed significant increase ($p<0.05$) in body weight as compared with positive control treated group, whereas the best significant increase ($p<0.05$) in body weight was observed in group treated with *Arctium lappa L.* extract and gentamicin in mean value (238.28±2.32) as compared with that pentoxifylline and gentamicin, *Arctium lappa* extract with pentoxifylline and positive control treated groups in mean values (226.71±2.90, 226.85±3.38, 205.42±1.53,) respectively. In current study the mean values of testis weight of *Arctium lappa L.* extract and gentamicin treated group (1.79±0.09) showed significant increase ($p<0.05$) as compared with pentoxifylline and gentamicin, *Arctium lappa* extract with pentoxifylline and positive control treated groups in mean values (1.61±0.17, 1.71±0.09, 1.42±0.15) respectively, with no significant difference as compared with negative control group. The gonadosomatic index percentage of group treated with *Arctium lappa L.* extract and gentamicin, pentoxifylline and gentamicin, *Arctium lappa* extract with pentoxifylline and gentamicin , showed significant increase ($p<0.05$) in mean values (0.75±0.09, 0.71±0.17, 0.75±0.09) respectively as compared with positive control and negative control treated groups (0.61±0.15, 0.69±0.08) respectively, in addition the positive control treated group revealed a significant reduction($p<0.05$)as compared with all other treated groups.

DISCUSSION

Sperm function test: After 30 days of daily oral treatment with *Arctium lappa L.* crude extract and gentamicin injection the percentage of motility, life sperms and count sperms was increased in addition the percentage of abnormalities were decreased when compared with other treated group, the commentary for this result may be due to the extract enhance the activity of reproductive system through the stimulating of hypothalamic-pituitary-gonadal axis, in addition to many active ingredients of extract as probable bioactive agents in charge for enhancement male sexual behaviour, these contain steroids and steroidal saponins, which may work as mediators in the steroidal pathway of androgen creation, thus increasing their production. Furthermore, flavonoids have been concerned preventing testosterone metabolic degradation[14]. In the other hand there were many bioactive compounds of plant extracts also give aphrodisiac actions by acting directly on the central nervous system to change the action of neurotransmitters and gonadal tissue in males, which can also modify and enhancement sexual behaviour. Alkaloids of extract enhance the vasodilation in the sexual organs and it have antioxidant effect, this results supported by [15]. The antioxidant properties of *Arctium lappa L.* extract preserved the spermatozoal membrane against lipid peroxidation because the increase of lipid peroxidation in spermatozoal membrane will decrease the natural motility of sperms, increase the viability and decrease the morphologically abnormal sperms, this results agreed with results reported by[16,17]. Therefore, *Arctium lappa L.* caused certain improving effects on the cell membrane by the antioxidants character of *Arctium lappa L.* that can inhibit the oxidation of 3,4 dihydroxyphenylalanine[18]. The increases in of motility %, life sperms % and count sperms of pentoxifylline and gentamicin treated group may be attributed to pentoxifylline act as alike to other methylated xanthine derivatives, in addition pentoxifylline is a determined as nonselective phosphodiesterase inhibitor, this result agreed with results reported by [19], in another study recorded by[20] they mentioned that the pentoxifylline reduces the break of cyclin adenosine monophosphate (cAMP) Intracellular cAMP level which are recognized to play important role in sperm motility by activate cAMP-dependent protein Kinase (PKA)which itself induce sperms tails protein phosphorylation with successive increase in sperm motion and a vital modulator of sperms and creation cellular glycolysis and adenosine triphosphate (ATP) that important for energy generation necessary for sperms motion, in addition to Pentoxifylline has important role for increasing serum levels of sexual hormones and augments acrosomal reactions, leading to increase sperm motility, count and depletion in abnormalities. The *Arctium lappa* extract with pentoxifylline and gentamicin treated group showed improvement in motility%, life%, count and morphological percentage when compared with pentoxifylline treated group only are possibly due to the crude extract of *Arctium lappa* have many active ingredients which may potentiated and improvement the effect of pentoxifylline, in addition to may reduced adverse systemic effect of pentoxifylline that may considered as stress factors, this result agreement with another study recorded by [21]. The decreases in motility%, life sperms % and count sperms with enhancement the abnormalities of distilled water and gentamicin treated group when compared with all treated group may be regarded to Gentamycin increase the free radical formation and lipid peroxidation, and by decreasing antioxidant enzyme that reduce fertility, this result agreed with results recorded by[22,23].

Gonadosomatic index

The increase in body weight, testis weight and gonadosomatic index of *Arctium lappa L* extract and gentamicin treated group when compared with gentamicin treated group perhaps is due to the ability of *Arctium lappa* extract to recover the weight depletion in rats, via improved glucose utilization to produce energy, in addition to many active ingredients of extract as probable bioactive agents, these contain steroids and steroidal mediators such as saponins, flavonoids and alkaloids which may work as mediators that keep and balance the normal weights from depletion, as well as its ability in enhancement of androgen, the same result reported by[24,25]. The Pentoxifylline and gentamicin treated group showed increase in body weight, testis weight and gonadosomatic index when compared with gentamicin treated group, and this result may be regarded to

that Pentoxifylline had the ability in increasing serum levels of sexual hormones and improve albumin concentration, in addition to inhibit cytokines that suppress food intake and inhibit gastric emptying, this result supported by results reported by[20,26].

The decrease in body weight, testis weight and gonadosomatic index of Distilled water and gentamicin treated group can be attributed to increase catabolism and anorexia are responsible for decreased food intake and causes body weight loss [27]. Further, subsequent loss of the tubular cells, involved in renal water reabsorption leads to dehydration and decreases body weight, the same result reported by[28].

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