

Histological Study of Aqueous Extracts Leek *Allium porrum L.* in Female Reproductive System of Laboratory White Rats

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Abstract

The aim of this study is to evaluate The effect of aqueous extracts of *Allium porrum* leafs on Reproductive system in female albino rats.

This study was conducted in laboratories of physiology and chemistry which belong to the College of Veterinary Medicine / University of kufa and laboratory of physiology of Faculty of Dentistry / University of kufa for period from 17/12/2013 to 25/11/2013 .It was used female white rats giving deferent dosages with deferent concentration (400,800) mg/kg for 20 days. It shows significantly decreased ($p \leq 0.05$) In the weights of ovaries of white female rats for both concentrations (400,800) mg/kg and the concentration 800 mg/kg is the more significant in reducing the weights of the ovaries comparing with control group . It also shows significant increase ($p \leq 0.05$) in the weights of uterus for both concentrations 400,800 mg/kg and the concentration 800 mg/kg is the more significant in increasing the weights of the uterus comparing with control group.

The histological study showed significant decrease ($p \leq 0.05$) in the number of the primary , secondary follicle and graffian follicle after dosing for both concentrations and duration , the reveals significant increase ($p \leq 0.05$) in the thickness of endothelium of the uterus in the female white rats after dosing for treatment by this was 800 mg/kg for period of 20 days compared with the control group.

In short , the aqueous of leek extract acts as anti- fertility in white female rats through its negative effects on the genitals ovaries and uterus, Effect of aqueous of leek extract for two concentration on the weight of ovaries and uterus , Also effect in the numbers of primary , secondary and graffian follicles and the effect of aqueous of leek extract in the thickness of endometrium and myometrium.

Keywords : *Allium porrum* , Reproductive system, Albino Rats.

Introduction

The leeks *Allium porrum L.* was used by the ancient Arabs as a sexual stimulant for males (Akil, 2003) . So this is the first study about using leek *Allium porrum L.* on female reproductive system and studying its effect on histological changes in Iraq and other countries according to our opinion. Leek *Allium porrum L.* belong to Alliaceceae family , were related to garlic , onion , shallots , and scallions , Leeks look like large scallions , having a very small bulb and a long white cylindrical stalk of superimposed layers that flows into green , tightly wrapped , flat leaves (Seberg,2003) Leeks contain several vitamins, the most important is Vit B6 (pyridoxal phosphate), Vit B9(folic acid), C and E. Also contain saponin and flavonoids (keampferol, querecetin), these compounds , flavonids material have been used as preventing pregnancy material (Breinholt *et al*, 2000; Iris, 2004). Leeks contain important amounts of the flavonoid(keampferol) , which has repeatedly been shown to help protect our blood vessel linings from damage , including damage by overly reactive oxygen molecules ,so it has cardiovascular support properties (Xiao *et al* 2009) . Ibn sina d.488e has mentioned that the leeks were used to cut nosebleed and the vaporizes seeds with tar for teeth in which contain worms and this kill it when draw the urine when used menstrual and stirbeh (Ibn sina, 1986).So the leeks was firstly used to see the effects on the female reproductive system for the following:

- 1- Weights of genital (ovaries and uterus)
- 2- Histological study included calculating of primary, secondary and graffian ovarian follicles and measuring the thickness of two layers of the uterus (myometrium and endometrium) .

Materials and Methods:

Preparation of Laboratory Animals

The study included 30 female white rats type *rattus ratus* age average between 8-12 week, the animals were obtained from College of Veterinary Medicine / University of Kufa . The animals were kept in animal house in the College of Veterinary Medicine / University of Kufa . The animals were put under suitable laboratory conditions, temperature is around 21-30 degree, at fixed rate of lighting system 13 hour lightness and 11 hour

darkness .It happens to get the animals increased to get the required numbers.

Preparation of warm aqueous extract for leeks plant:

Prepping the aqueous extract warm for leeks plant, taking 100 groups of powder dry leaves of plant and put it in glass beaker capacity of it 1000 ml containing 1000 ml of boiled distilled water with mixing well for a period of 10 minutes and left the flask and its contents for a period of 30 minutes, filtering the solution through a clean cloth and put the filter to a centrifuge 3000 r/min for 15 minutes, then put the filter in glass bottles and put it in oven at temperature 40C. To dry the extract , It goes through paper filter containing Millipore for sterilization, then retain it to oven to obtain dry extract of the plant sample. Then kept it in refrigerator until use.

Method of dosage with aqueous extract of leek plant:

Drinking female animals with aqueous extract of leek plant by two concentrations in the process of proestrus for the purpose of the following up the process of the follicles and the ovulation under the influence of extract, drinking the animals orally for period of 20 days.

Animals sacrifice

Sacrifice female animals after drugged textured chloroform day atheist twenty one with two concentrations of aqueous extracts dosage and duration of 20 days.

Measure the weights of the genitals:

Weights of the genitals organs (ovaries and uterus) were measured after it has been eradicated after the killing, weighted by using sensitive balance, the study conducted on the left ovary and uterine horns.

Measure the diameters of the ovaries:

It has been Used a compound microscope (ALTAY, CH3O, NOVAL) To measure the rates of the diameters of the ovaries of female rats using the extract scale of the lens kind ocular micrometer scale calibrated after careful theater stage and enlarge 4x and strength compared with control group.

Calculate the number of ovarian follicle primary, secondary and graffian ovarian follicles:

Numbers of ovarian follicle primary, secondary and graffian ovarian follicles were calculated in sequential segments and all the slide for all extract concentrations according to the rates in the ten female rats drinking the same concentration itself and compared to the control group.

Measuring the thickness of the two layers of the uterus (myometrium and endometrium)

Measured the thickness of the two layers of the uterus of female rats using the extract scale of the lens in kind after the calibrated scale of the theater and the exact magnification power 10x and compared to the control group.

Preparation of histological sections

The histological sections were prepared attended in laboratories city alsder medicine / department histological diseases and followed the method of Bancroft and Steven in 1982 by placing models of the first experiment in formation for 24 hours and then carried out the steps dehydration, clearing, infiltration, embedding, sectioning, staining and mounting.

Statistical analysis:

The results of experiments for the warm aqueous extract were analyzed for two concentrations 400, 800 mg/kg for a period of dosage 20 days after the replications ten for each concentration, using design complete randomization factorial experiments with completely randomized design, this design has been used least significant deference (L.S.D.) under standard test probability of 0.05 with standard error.

Results:

Effects the interference of different two concentrations of warm aqueous extract of leek plants in the weight of ovarian and uterus of female white rats (the weight of organ (gram))

The table (1) refers to the presence of significant decrease (in the weights of the ovaries after treatment with the aqueous extract of the leeks plant for both concentrations 400, 800 mg/kg, amounting to (0.08 ± 0.03) and (0.07 ± 0.01)g. respectively compared with the control group reaching (0.54 ± 0.14)g has expressed his focus 800 mg/kg significant decreased (p≤ 0.05) higher in weights of the ovaries.

The results showed the same table and having significant increase (in the weight of the uterus after treatment with the aqueous extract of the leeks plant for both concentrations 400, 800 mg/kg, amounting to (0.43 ± 0.04), (0.56 ± 0.20) compared with the control group reaching (0.23 ± 0.03).It has shown the concentration 800 mg/kg significant decreased (p≤ 0.05) higher in weights of the uterus.

Table (1) effect of the interference of two different concentration for the aqueous extract of the leeks plant in the weight ovaries and uterus in the female white rats (weight of organ g/100g of body weight)

Concentration Mg/kg	Weight of ovaries	Weight of uterus
	SD ±Mean	SD ±Mean
400	0.08 ± 0.03	0.43 ± 0.04
800	0.07 ± 0.01	0.56 ± 0.20
Con.	0.54 ± 0.14	0.23 ± 0.03

L.S.D = 2.25

L.S.D =5.45

Effect of the interference between concentration and period of the dosing for the aqueous extract of the leeks plant in the weight ovaries and uterus in the female white rats (weight of organ g/100g of body weight)

The results of the figure (1) shows that there is significant decrease (in the weights of the ovaries for both concentrations and duration of dosing , the concentration 800 mg/kg showed higher significant) decrease in the weights of the ovaries where as figure (2) refers to the presence of high significant) in the weight uterus in the female white rats for both concentrations , the concentration 800 mg/kg showed higher significant) increased in the weights of the uterus.

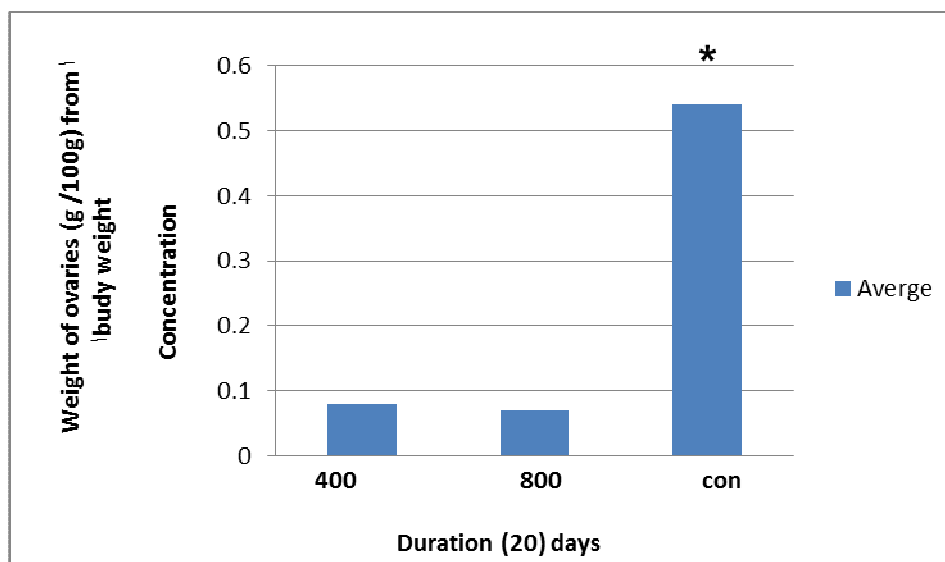


Figure (1) effect of the interference between concentrations and duration of dosing of aqueous extract of the leeks plant in the weight ovaries in the female white rats (weight of organ g/100g of body weight)

* Represents a different significant ($p \leq 0.05$) between the concentrations and control group.

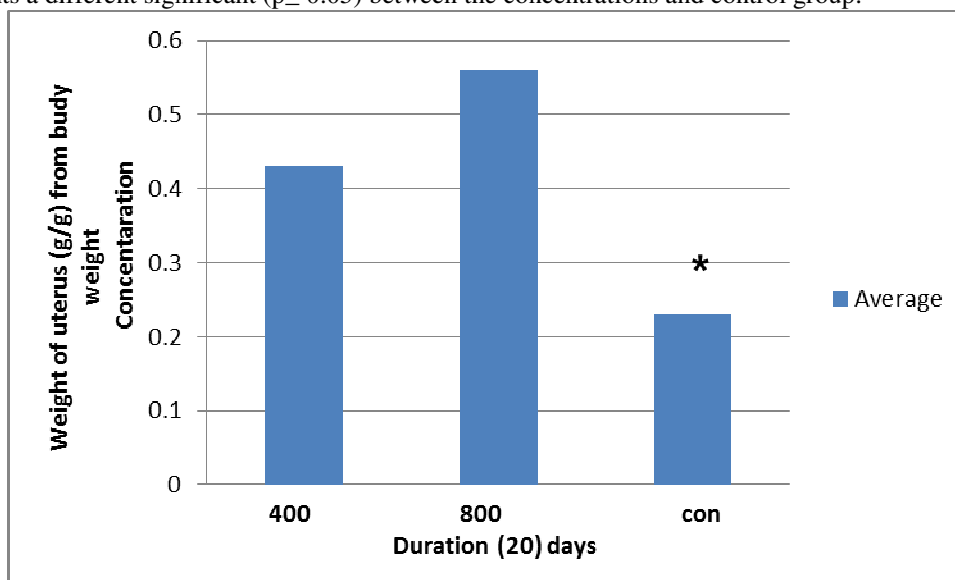


Figure (2) effect of the interference between concentrations and duration of dosing of aqueous extract of the leeks plant in the weight uterus in the female white rats (weight of organ g/100g of body weight)

*Represents a different significant ($p \leq 0.05$) between the concentrations and control group.

Histological study

The effects of different interference for two different concentrations of aqueous extract of leek plants in the number of primary , secondary and Graff follicles

The table (3) shows that there is significant decrease ($p \leq 0.05$) in the number of primary follicle for both (400 ,

800) mg / kg a mounting (2.00 ± 1.41) and (1.00 ± 0.00) respectively , compared with the control group , a mounting (4.50 ± 4.94) , and the concentration (800) mg / kg has showed significantly higher decrease in the primary ovarian follicles . the results of this table also refers to a significant decrease ($p \leq 0.05$) in the number of secondary ovarian follicles for both concentrations , as was (1.50 ± 0.70) and (0.50 ± 0.70) compared with control group reaching (2.50 ± 2.10) . The concentration (800) mg / kg has shown significantly lower decrease ($p \leq 0.05$) higher in the number of secondary ovarian follicle .

The results showed significant decrease ($p \leq 0.05$) in the number of Graff follicles for both concentrations (400 , 800) mg / kg a mounting (1.50 ± 0.70) and (0.00 ± 0.00) as compared with control group , amounting (2.00 ± 1.41) . and the concentration of (800) mg / kg has showed highly significant decreased ($p \leq 0.05$) in the number of Graff follicles .

Table (3) : The effect of different interference for two different concentrations for aqueous extract of leek plants in the number of primary , secondary and Graff follicles in the female white rats.

Concentration Mg/kg	Primary follicles	Secondary follicles	Graff follicles
	SD \pm Mean		
400	2.00 ± 1.41	1.50 ± 0.70	1.50 ± 0.70
800	1.00 ± 0.00	0.50 ± 0.70	0.00 ± 0.00
Con.	4.50 ± 4.94	2.50 ± 2.10	2.00 ± 1.41

L.S.D = 5.21
 L.S.D = 4.04
 L.S.D = 3.71

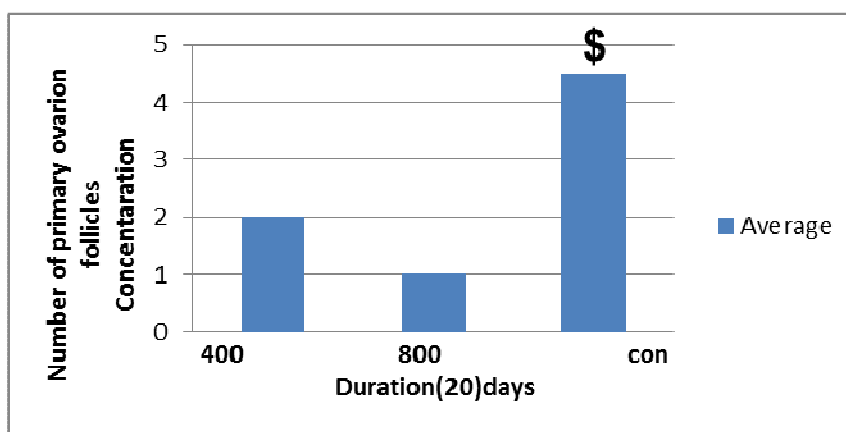


Figure (3) : The effect of interference between two concentrations and duration of dosage for aqueous extract of leek plants in the rate number of primary follicles in the female white rats .
 \$Represents a different significant ($p \leq 0.05$) between the concentrations and control group .

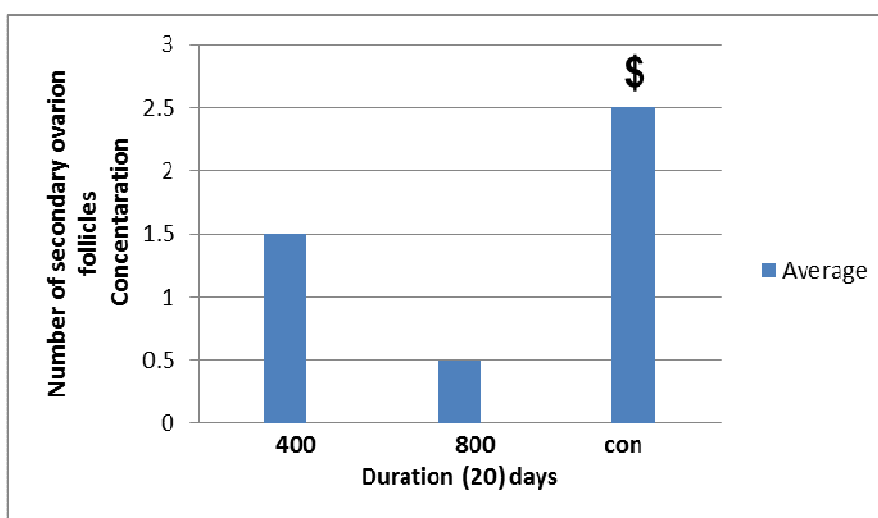


Figure (4) : The effect of interference between two concentrations and duration of dosage for aqueous extract of leek plants in the rate number of secondary follicles in the female white rats .

\$Represents a different significant ($p \leq 0.05$) between the concentrations and control group.

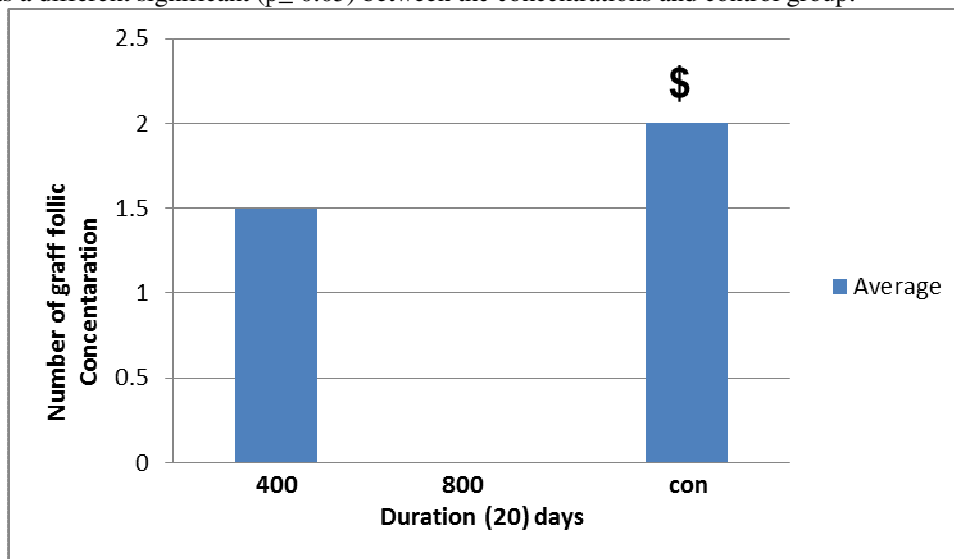


Figure (5) :The effect of interference between two concentrations and duration of dosage for aqueous extract of leek plants in the range number of Graff follicles in the female white rats .

\$Represents a different significant ($p \leq 0.05$) between the concentrations and control group.

Effects of interference of two different concentrations for aqueous extract of leek plants in the diameter range of ovaries in the female white rats.

From figure (6) the results refers to the presence significant decrease ($p \leq 0.05$) in the diameters of the ovaries after treatment for both concentrations of aqueous extract of leek plants (400, 800) mg/kg . a mounting (200.00 ± 88.38) and (153.1 ± 22.06) respectively compared with control group is a mounting (252.8 ± 67.1) . the concentration (800) mg /kg shows highly significant ($p \leq 0.05$) decrease in the ovaries diameters .

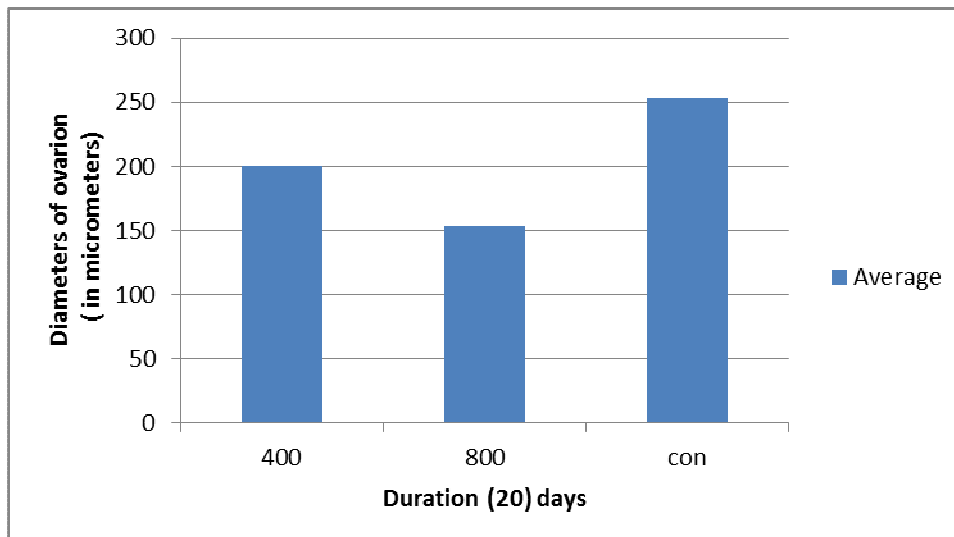


Figure (6) :The effect of interference between two concentrations and duration of dosage for aqueous extract of leek plants in the diameter range of ovaries in the female white rats .

*Represents a different significant ($p \leq 0.05$) between the concentrations and control group.

The effect influences of two different concentration of aqueous extract of leek plants in the thickness of two layers of the uterus in the female white rat (thickness of layer in micrometers).

The results of table (4) refers to the presence of high significant increase ($p \leq 0.05$) in the thickness of endometrium layer of uterus for both concentrations (400 ,800) mg/kg respectively is a mounting (81.00 ± 41.01) and (100.00 ± 7.07) compared with control group , a mounting ($77 .00 \pm 31.11$) , The results showed significant decrease ($p \leq 0.05$) in the thickness of myometrium layer of uterus for both concentrations (400 ,800) mg/kg respectively is a mounting (42.50 ± 10.06) and (35.00 ± 7.07) compared with control group , a mounting (227.22 ± 37.90).

Table (4) : The effect of interference of two concentrations of aqueous extract of leek plants in the both two thickness of uterus in the female white rats (thickness of layer in micrometers).

Concentration Mg/kg	Endometrium	Myometrium
	SD ±Mean	SD ±Mean
400	81.00 ±41.01	42.50±10.06
800	100.00±7.07	35.00±7.07
Con.	77 .00± 31.11	227.22±37.90

L.S.D = 28.7

L.S.D = 29.1

Histological changes

Ovaries

Histological changes occurred clearly in the ovaries tissues after treatment for both two different concentrations of aqueous extract of leek plants as compared with control group image (1) those influences were clear in both concentration (400, 800) mg/kg , and the most important of these histological changes : decrease in the average diameters of the ovary range and the number of ovarian follicles primary and secondary image (2)-(4) as compared with control group image (1) .

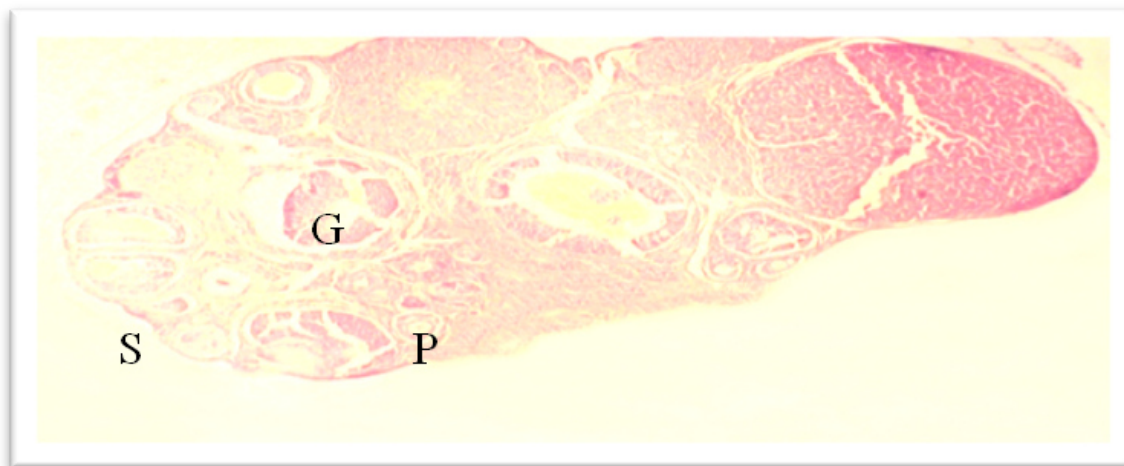


Fig.(1) :- Section of ovary for control group H&E (4x) appears of primary follicles (p) , secondary follicles (s) and Graff follicles (G).

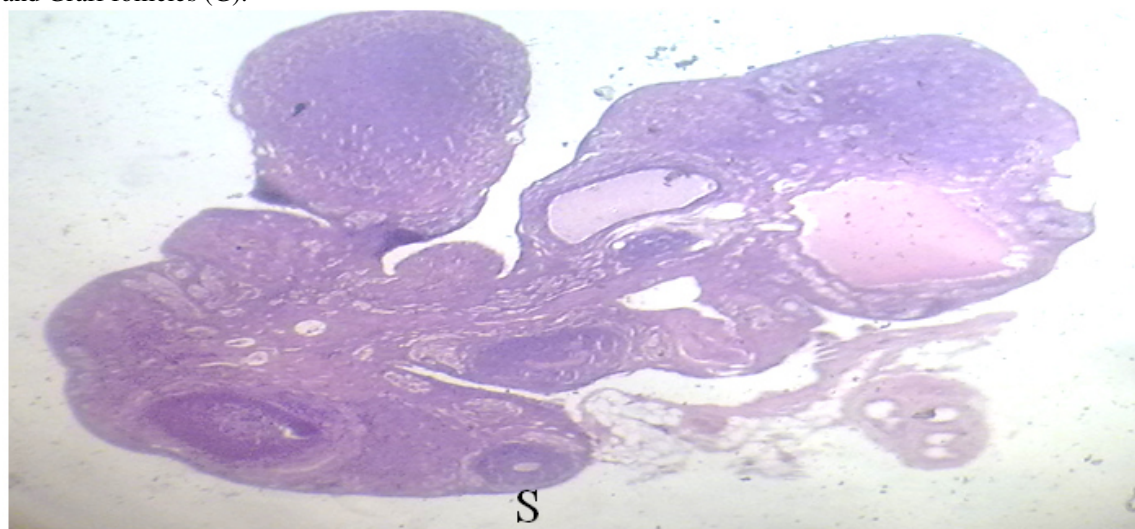


Fig.(2) :- Section of ovary after dosage with 400 mg/kg for 20 days notice of decreasing in the number of primary follicles (p) , secondary follicles (s) and absence of Graff follicles (G). H&E (4x)

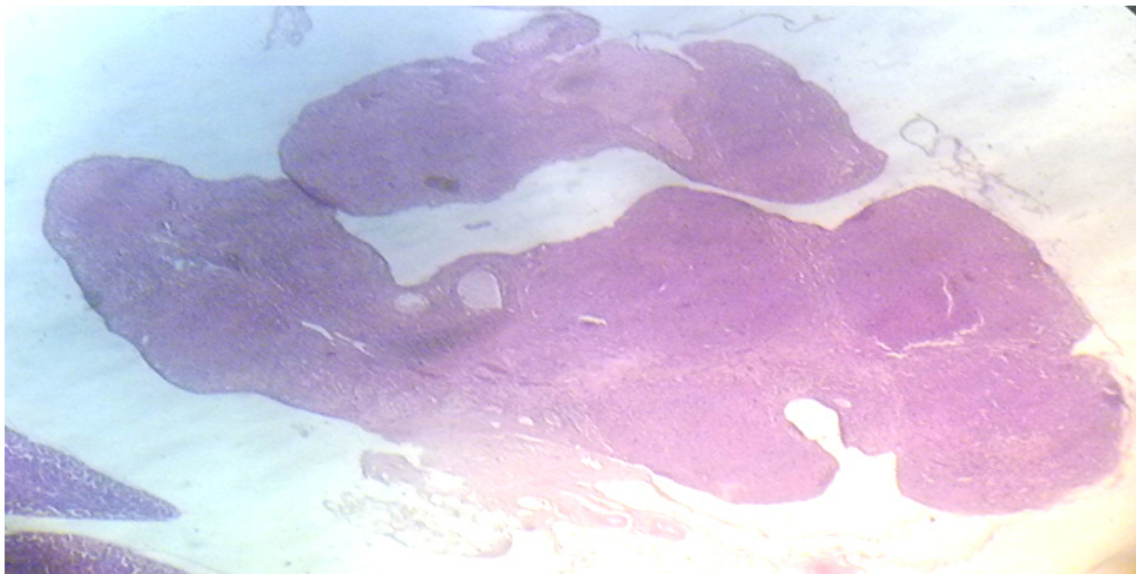


Fig.(3) :- Section of ovary after dosage with 800 mg/kg for 20 days notice the absence of primary follicles (p) , secondary follicles (s) and Graaf follicles (G). H&E (4x).

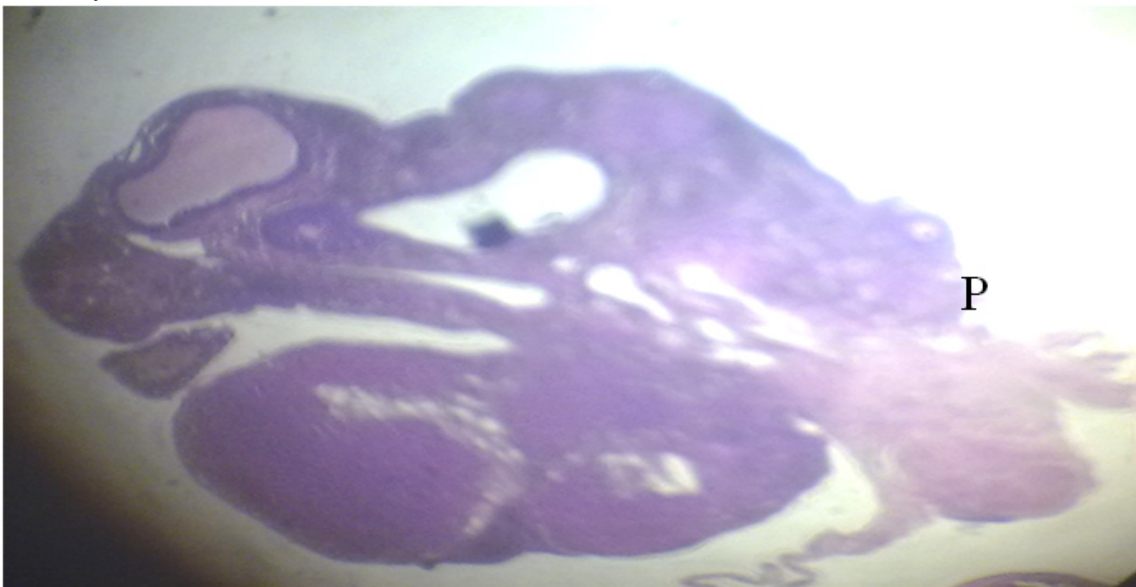


Fig.(4) :- Section of ovary after dosage with 800 mg/kg for 20 days notice of decreasing in the number of primary follicles (p) , absence secondary follicles (s) and absence of Graaf follicles (G). H&E (4x).

Uterus

Histological changes occurred clearly in the proportion of uterus that treated with two different concentrations of uterus that treatment with two different concentrations of aqueous extract of leek plants , these effects in observed the concentration of (800) mg/kg for 20 days and one of these changes , an increase in the thickness of layer (endometrium) of uterus and decrease in the thickness of myometrium , image (6)-(8) .as compared with control group image (5) .

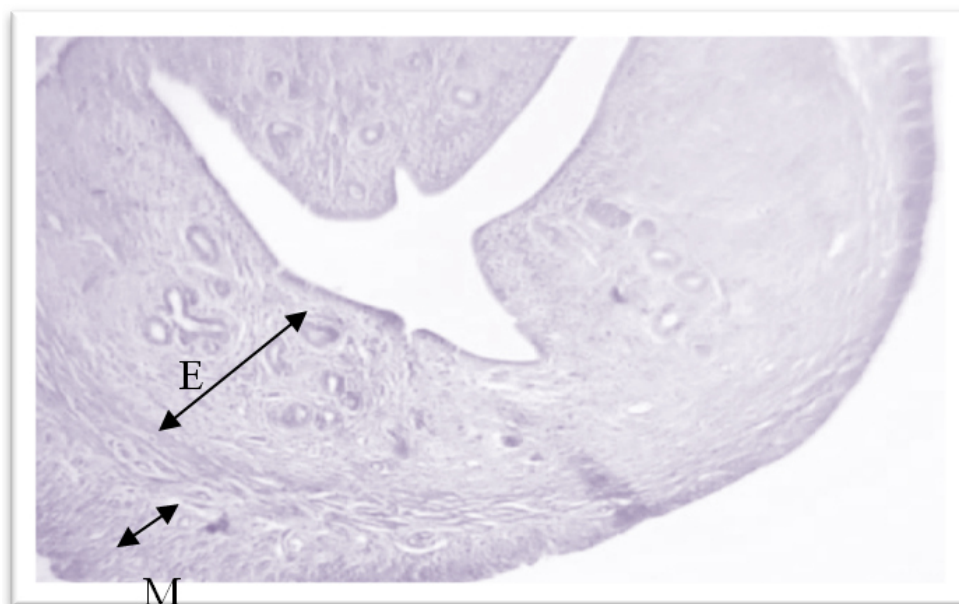


Fig.(5) :- Section of uterus for control group H&E (4x) appears of endometrium layer (E) and myometrium layers (M) with normal thickness.

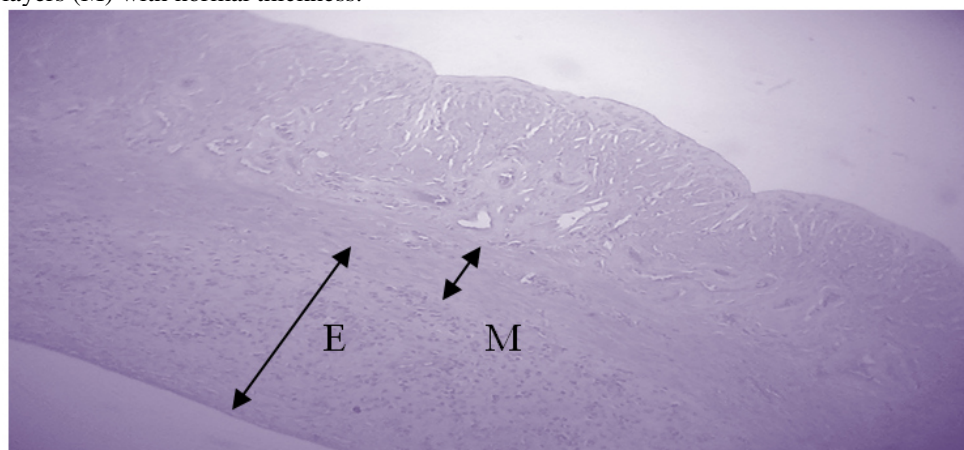


Fig.(6) :- Section of uterus after dosage with 400 mg/kg for 20 days notice of increasing in the thickness of endometrium layer.

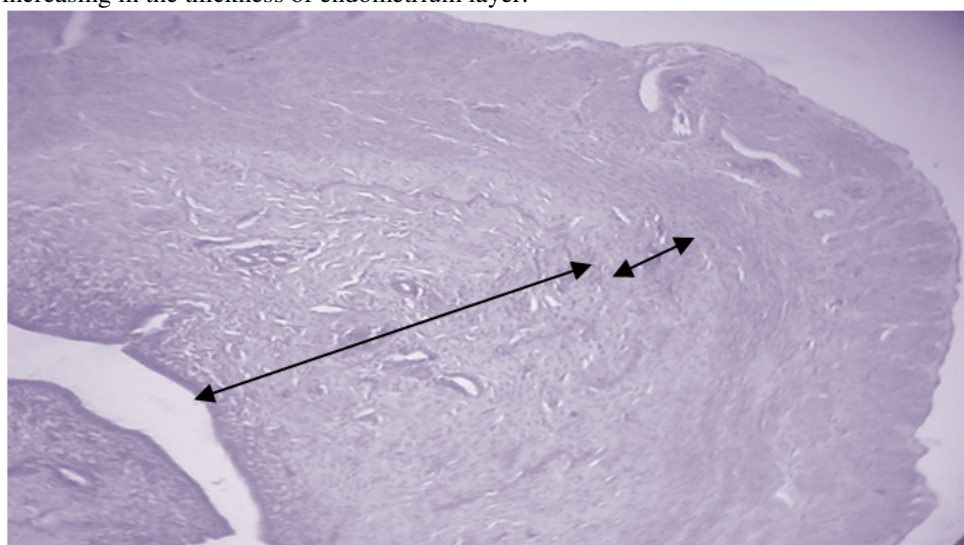


Fig.(7) :- Section of uterus after dosage with 800 mg/kg for 20 days notice of increasing in the thickness of endometrium layer.

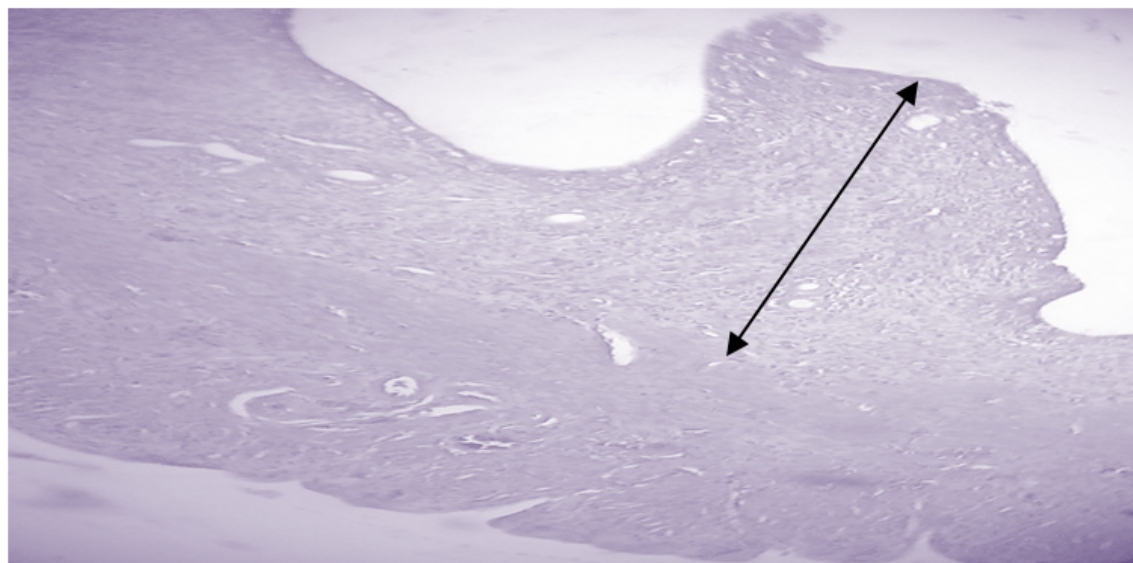


Fig.(8) :- Section of uterus after dosage with 800 mg/kg for 20 days notice of increasing in the thickness of endometrium layer.

Discussion

From the results of the current study observed the presence of a significant decrease in the weights of the ovaries after treatment with aqueous extract of leek plants for both concentrations. It may be due to a decrease in the level of follicle stimulating hormone as it is an important hormone in the development and completion of maturation of ovarian follicles . the results of the study agreed with al- shaibani (2011) , but with different plant , she used the carrot seed and noted its role in reducing the weights of the ovaries and the level of follicle stimulating hormone .

It also agreed with the results of number of researchers (Azadeh ,1999 ; Amira , 2006) , they used seeds , leaves and other effective parts of different plants and noticed the roles in reducing the weights of the ovaries and this may be due to the suppressed effect on the axis of the hypothalamus – pituitary – ovary and this due to decreasing in secretion follicle stimulating hormone and this study indicated by (Sellar & Wonda , 2001 ; Zhonghua , 2007) while the significant increase in the weights of the uterus after treatment with a queous extract of leek plants may be attributable to contain this plant on compound like (Keampferol , Quercetin) and this plant have esrogenic activity because it contains compounds and material like flavonids this compounds similar to phytoestrogenic plant , which work to increase the level of estrogens and thereby increase the weights of uterus and this results agreed with study of (Mitsutoshi et al , 2004) .the significant decrease in the number of ovarian follicles (primary , secondary and Graff) in the results of the current study are described in histological image (2 and 3) for both concentrations . that those results may be due to the role of the aqueous extract of the leek plants in reducing the level of follicle stimulating hormone (FSH) and leutinizing hormone (LH) . leeks may work to change the hormonal balance resulting in an increase in the phase of proestrus as a result of an increase of estrogen level. Which is reflected on the development of ovarian follicles and Graffian follicles as a result of negative feedback (Richard , 2001 ; Susun , 2007) and also in the current study , results of presence of a significant decrease in the rate of ovarian diameters explain this decline on the basis of reduction achieved in the preparation of ovarian follicle (primary , secondary and Graff) , ovarian diameter depends on the contents of the number of vesicles and follicles and their direct relationship correlation on the other hand a significant increase in the thickness layer of uterus and the development can be explained on the basis of what it plant contains of flavonied compounds that have estrogenic activity as well as material such as folic acid , which all operate to increase the thickness of endometrium layer of uterus , this has periously signaled a number of researcher (Al- hasnawi , 2002 ; Al- shaibany , 2005 ; Diepl *et al* 2004) in the field of plant extracts of estrogen content and using different effective material and roles of these plants in increase the thickness of endometrium layer of uterus .

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