



# HISTOLOGICAL STUDY OF CELERY AQUEOUS SEEDS EXTRACT (*APIUM GRAVEOLENS* L.) ON OVARIES IN ADULT FEMALE RATS

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## Abstract

Most recent studies have focused on the use of herbal extracts in medicine and treatment of many diseases especially in the side of fertility because the containment of active compounds that play an important role in stimulating hormones responsible for fertility, one of the examples of these herbs is celery. For this reason, this study was designed to determine the importance of the aqueous extract of celery seeds in the increase of fertility of adult female rats through its reflection on the diameter of both ovary and uterus also the number of ovarian follicles.

Thirty healthy adult female albino rats (2-3 month) aged with (130-170 gm) weight randomly divided into three groups: control group and two experimental groups, 10 rats for each group. The control group was given an equal amount of orally distilled water daily for 28 days while experimental group animals were given a single dose orally (0.057 g/kg per weight) and double dose (0.114 g/kg per weight) from celery seed aqueous extract also for 28 days. Five females randomly selected from each group for anesthesia and histology. After the sacrifice, the ovaries were removed from the body to be weighed and then prepared for the routine histological preparation stages to obtain colored microchips with a haematoxylin-eosin thickness of 5-7 micrometers ready for imaging and microscopic measurements.

Results showed increased in the ovaries and uterus weights of female experimental groups that received celery extract as compared with control group. Microscopically sections of ovary were indicated noticeable increasing in the thickness of ovary and uterus muscle layer also increasing in the numbers of primary, secondary, Graafian Follicles and Corpora Lutea of both experimental rats that received single and double dose of celery extract as compared with control rats but not depended on the dose.

**Key words:** *Apium graveolens* L., uterus muscle layer, herbal extracts, fertility.

## Introduction

In the past, medical plants were considered as the only form of health care, by the time the interest towards medicinal plants was increasing through their importance in traditional medicine (WHO, 2000). Herbal was applied in treating of numerous diseases, such as experimental wound healing, hypertension, diabetes, as well as reproductive performance (Modaresi and Gholchobian., 2012). In recent years, many developed countries have paid great attention to studying the effects of different plants on the fertility through the huge experiment on laboratory animals (Tasdighi *et al.*, 2012).

For a long time, human being used many kinds of

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plants in medicine and try to find their role in fertility by used them as sex stimulants some of these plants are: *Salvia officinalis*, *Tribulus terrestris* and *Nigella sativa* (Craig., 1999).

WHO gave a great attention to use medical herbs in scientific research's in order to improve the human health in the world and especially in third world countries (Chen., 1977).

Reproduction is one of the special character of living organisms to keep continues and rise organism's species by heredity the life from generation to another, so a lot of medical plants was used to increase the fertility and reproduction of animals, one of that plants was *Apium graveolens* (Atta, 1998; Takagi *et al.*, 2007).

Celery, *Apium graveolens* L., is a plant with high taste, dietetic, and medicinal values (WHO, 2000). It is a spice plant prized for its content of volatile odorous compounds terpenes and phthalides (Jarvenpaaw *et al.*, 1997). It had a great biological importance because of having active chemical substance that play important role as antimicrobial against bacteria, fungi and also parasites (Zheng *et al.*, 1993). Antimicrobial substances that found in Celery for instance, sedanolide active against nematode, mosquito larvae and fungi (Momin and Nair., 2001), furanocoumarins inhibited *Listeria monocytogenes*, and *Micrococcus luteus* (Ulate-Rodrigues *et al.*, 1997).

Ovaries of the rat are grape-like structures that vary in size and gross appearance, depending on the stage of the oestrous cycle. Ovarian surface covered by a single layer of modified peritoneal mesothelium, the ovarian surface epithelium (OSE), which is continuous with the broad ligament (mesovarium) that supports the ovary (Gbotolorun *et al.*, 2001). The OSE of a single ovary can range from squamous to cuboidal, columnar or pseudostratified columnar in type; this regional variation in OSE morphology accompanies the cyclical changes that occur within the underlying ovarian parenchyma during the oestrous cycle (Gbotolorun *et al.*, 2011).

In sexually mature rats the cortex contains numerous follicles at various stages of development. Five stages of follicular maturation (folliculogenesis) were described: Primordial follicle, Primary, Secondary, Tertiary and Preovulatory (Graafian) follicle (Gbotolorun *et al.*, 2001; Radwan., 2010).

## Materials and Methods

### Plant extract preparation

Celery plant seeds was purchased from the local market in the province of Wasit, Kut district and diagnostic in the College of Agriculture, University of Sumer. To prepare extract, Mix 10 g of celery seed powder with 150 ml distilled water with continuous stirring for 24 hours then filtration by filtration leaves what man No.1. Concentrate the whole leachate into a heavy leach and keep in the refrigerator until use (Khuon., 2012).

### Experimental animals, Treatment and dosing

Thirty healthy virgin female albino strain rats, weighting (130-170 g) aged (2-3 months) were used for present work. Rats were maintained in clean special room in cages in the animal house of Wasit university, College of Science, fed with standard pellet diet and tap water and libitum, left period before the experiment.

Rats were randomly divided into three groups: Control and two experimental (Ten female rats in each group).

Rats in control were administered with distilled water orally for 28 days while those in experimental groups received orally celery extract at single dose 0.057 g/kg B.W and double doses/daily (0.114 g/kg B.W.) of celery extract by gavage for 28 days.

### Histology

Five female rats from each group were chosen for histology. Under deep ether anesthesia animals were sacrificed, The ovaries were dissected, isolated clean from the surrounding adipose tissues then fixed in 10% neutral buffered formalin and Boun's solution for 24-48 h. Specimens were processed and embedded in paraffin. Serial sections 5-7  $\mu\text{m}$  thick were routine histology section, stained routinely in Hematoxylin and Eosin (H&E) (Bancroft and Steven., 1982). Stained tissue sections of uterus and ovary were examined by light microscope with canon digital camera.

Measuring the Ovaries Diameters Thickness and calculate the number of the different follicles and corpora lutea female ovaries diameter, uterus endometrial thickness and numbers of the different follicles and corpora lutea were calculated by using ocular micrometer after calibrated with the stage micrometer and 40 X ocular lenses to examination and comparison with control group.

### Statistical Analysis

The data of work were analyzed by ANOVA. Tukey HSD used for comparisons between the experimental and control groups.  $P < 0.05$  was considered statistically significant. All results were expressed as mean  $\pm$  standard error of the mean (M  $\pm$  S.E.M).

## Results

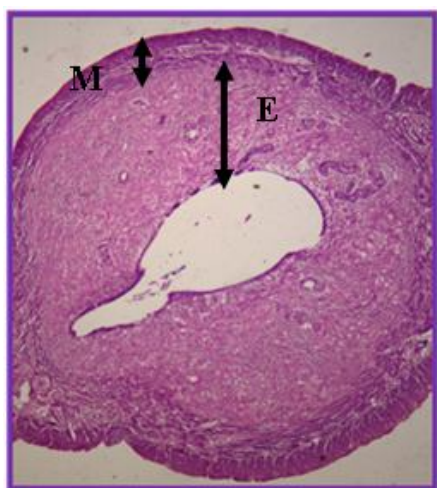
### Ovary and Uterus in Adult Female Rat

In relation to the ovaries diameters, the microscopic sections of ovaries in both single and double doses group of celery extract showed that there was a considerably significant increase ( $p < 0.05$ ) in the ovary diameter reached to highly value (3794.17  $\pm$  2.63  $\mu\text{m}$ ) for double dose and (3790.33  $\pm$  1.05  $\mu\text{m}$ ) for single dose as compared with the ovary diameter of control female rats (3519.33  $\pm$  1.22  $\mu\text{m}$ ). But this increase in ovarian diameters were not different significantly between single and double dose groups (table 1). As for the thickness of Endometrial and Myometrial, Depending on the apparent results listed and indicated in (table 1) and (figs. 1, 2) that showing noticeable increase in endometrial and myometrial thickness of uterus female rats which drinking celery extract in single and double doses reached to (503.66  $\pm$  1.18 and 232  $\pm$  0.85  $\mu\text{m}$ ) for single group and (505.5  $\pm$  1.87 and 235.33  $\pm$  1.39  $\mu\text{m}$ ) for double dose

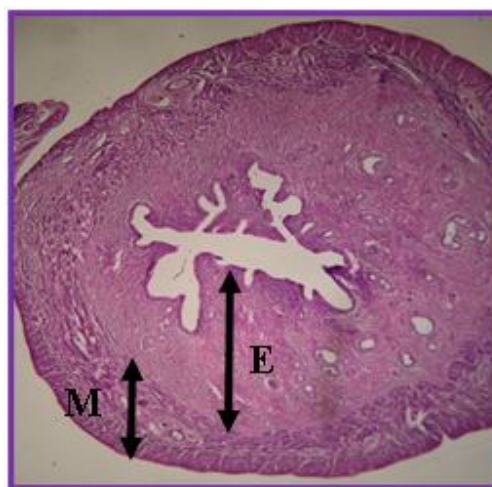
respectively comparing with control that drinking only distilled water ( $469.5 \pm 0.68$  and  $225 \pm 0.58 \mu\text{m}$ ). In spite of this significant increase in endometrial and myometrial thickness between control animals and drinking extract animals, no significant increase was observed between the animals of drinking celery extract depended on the dose (single and double) which noticed in (table 1).

**Table 1:** Effect of orally administration of celery extract (*A. graveolens L.*) on the diameter and uterus thickness of ovary female rats after 28 days

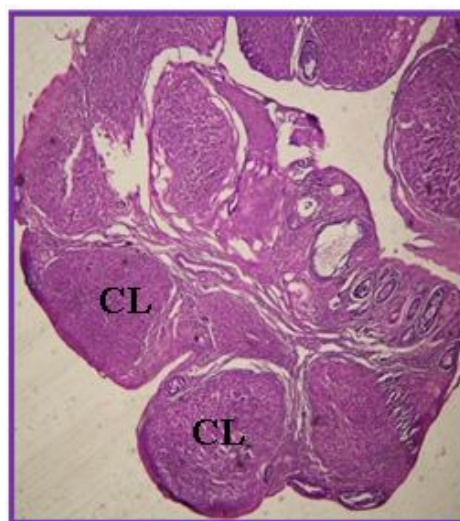
Parameters Group	Ovary diameter ( $\mu\text{m}$ )	Endometrial thickness ( $\mu\text{m}$ )	Myometrial thickness ( $\mu\text{m}$ )
Control	<sup>a</sup> 3519.33 $\pm$ 1.22	<sup>a</sup> 469.5 $\pm$ 0.68	<sup>a</sup> 225 $\pm$ 0.58
Group A	<sup>b</sup> 3790.33 $\pm$ 1.05	<sup>b</sup> 503.66 $\pm$ 1.18	<sup>b</sup> 232 $\pm$ 0.85
Group B	<sup>b</sup> 3794.17 $\pm$ 2.63	<sup>b</sup> 505.5 $\pm$ 1.87	<sup>b</sup> 235.33 $\pm$ 1.39



**Fig. 1:** Section of uterus in rat administrated orally distilled water shows myometrial (M) and endometrial thickness (E) (H and E  $\times$  40)



**Fig. 2:** Section of uterus in rat administrated single of celery extract shows an increase in myometrial (M) and endometrial thickness (E) (H and E  $\times$  40)



**Fig.3:** Section of the ovary in rat administrated orally distilled water shows Corpora Lutea (CL) (H and E $\times$ 40).

**Table 2:** Effect of orally administration of celery extract (*A. graveolens L.*) on the diameters of primary, secondary, Graafians follicles and Corpora Lutea in female rats after 28 days.

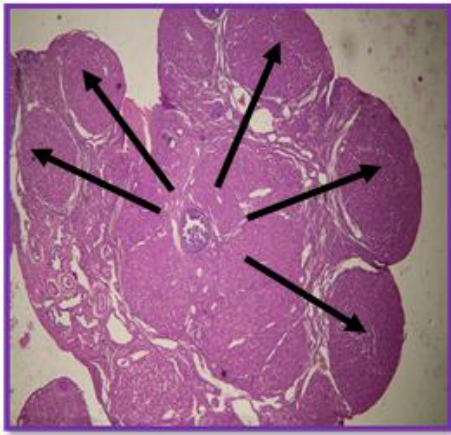
Group	Primary follicles Diameters ( $\mu\text{m}$ )	Secondary follicles Diameters ( $\mu\text{m}$ )	Graafians follicles Diameters ( $\mu\text{m}$ )	Corpora Lutea Diameters ( $\mu\text{m}$ )
Control	a51.0 $\pm$ 0.51	a141.5 $\pm$ 0.57	a236.0 $\pm$ 0.37	a661.17 $\pm$ 0.40
Group A	b58.67 $\pm$ 1.77	b150.3 $\pm$ 2.06	b253.17 $\pm$ 0.47	b687.33 $\pm$ 0.33
Group B	b59.0 $\pm$ 1.03	b156.7 $\pm$ 1.67	b253.33 $\pm$ 0.76	b687.83 $\pm$ 1.60

**Table 3:** Effect of orally administration of celery extract (*A. graveolens L.*) on the numbers of primary, secondary, Graafians follicles and Corpora Lutea in female rats after 28 days.

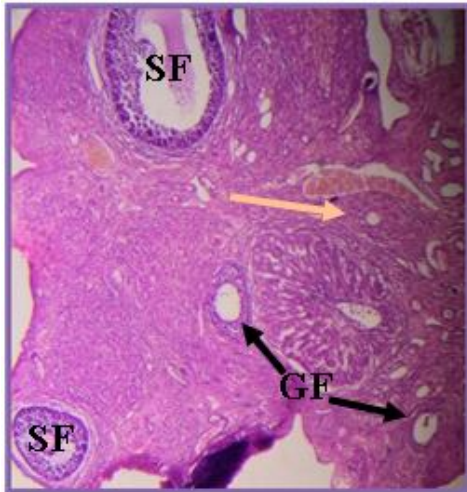
Group	Primary follicles Diameters ( $\mu\text{m}$ )	Secondary follicles Diameters ( $\mu\text{m}$ )	Graafians follicles Diameters ( $\mu\text{m}$ )	Corpora Lutea Diameters ( $\mu\text{m}$ )
Control	a7.0 $\pm$ 0.37	a5.1 $\pm$ 0.30	a3.7 $\pm$ 0.21	a5.33 $\pm$ 0.4
Group A	b8.8 $\pm$ 0.30	b0.33 $\pm$ 7.3	b5.3 $\pm$ 0.21	b7.83 $\pm$ 0.30
Group B	b8.5 $\pm$ 0.22	b0.51 $\pm$ 8.0	b6.0 $\pm$ 0.36	b8.5 $\pm$ 0.22

Results of microscopic measurements as shown in (Tables 2, 3) also figures from (4) to (7) refers to a significant difference ( $p < 0.05$ ) of both numbers and diameters of primary, secondary, Graafian's follicles in addition to corpus lutea in single and double dose as compared with control, which means that noticeable increase in celery drinking animals showed after treated with extract as compared with control animals. However, there was no noticeable difference in both numbers and diameters observed depended on dose of the celery extract treatment.





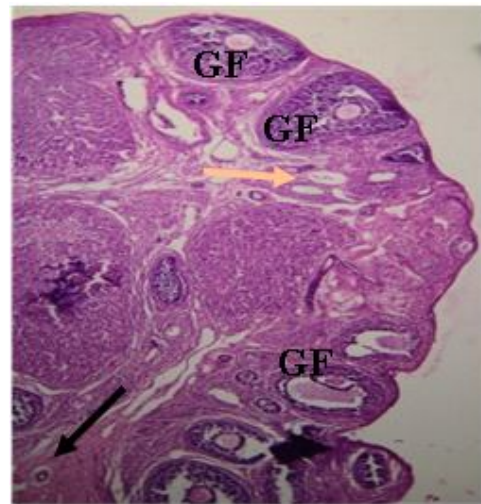
**Fig. 4:**Section of ovary in rat administrated double dose of celery extract tea shows an increase in Corpora Lutea (→) (H and E×40).



**Fig. 5:**Section of ovary in rat administrated orally distilled water shows primary follicle (→), secondary follicle (SF) and Graafian follicle (GF) (H and E × 100)



**Fig. 6:**Section of ovary in rat administrated single dose of celery extract shows an increase in primary follicle (→), secondary follicle (→) and Graafian follicle (→) (H and E×100)



**Fig. 7:**Section of ovary in rat administrated double dose of celery extract shows an increase in primary follicle (→) secondary (→) and Graafian follicle (GF) (H and E×100)

## Discussion

Several studies have shown the importance of herbal products as extracts in the treatment of fertility such as celery as an alternative to synthetic drugs because of its effective compounds that impact on the fertility rate (Al-Obaidi *et al.*, 2011; Sakran *et al.*, 2016). The result of this study was concerning with knowing the effect of different doses of celery seeds extract on mature female ovary rat's, by histological measurements.

Microscopically hematoxylin and eosin stained sections of control rat's ovary groups, were similar to those of the natural description of the ovaries in the rest of the research related to reproductive system of rats (Radwan, 2010; Tomma, 2011).

The increase in the diameters of ovaries of celery extract treated group (single and double) which are indicated by microscopic measurements may contribute to the effect of extract on the content of ovarian follicles during 28 days. It's well known that the ovarian diameter depends on its content of follicles that reflexes on the size and diameter of ovary (Biennial, 2000; Greydanus *et al.*, 2001; Fraser and Kovacs, 2003) by increase the numbers of primary, secondary, and Graafian follicles and corpora lutea. Another possible for diameters increasing might be retunes to the role of Follicle stimulating hormone (FSH) on ovary growth the high concentration of the FSH hormone which stimulate the growth of follicle in the ovary reflects on the numbers of follicles that reach maturity (Auchi, 2004).

A lot of research refers to the effect of celery on the fertility of rats (male and female), results of (Mahdi, 2015) shows increase in the fertility of male rats reflects

in number of spermatids and diameters of seminiferous tubules, also the results refers by (Kooti *et al.*, 2016) on the study of celery (*Apium graveolens*) on reproductive parameters in male wistar rat reflecting the ability of celery in the increase of fertility.

Furthermore, the present results are similar to the findings of (Al-Gharbawi, 2011) who reported that orally administrations of alcoholic and aqueous extracts of celery leaves on female mice showed a significant increase of the numbers and diameters of the primary, secondary follicles and corpus lutea. Also, (Noor, 2008) who reported that there is an increase in the numbers and diameters of the primary, secondary and Graafian's follicles in female rat after treatment with Alcoholic extract of *Nigella Sativa* for 40 days. (Marzouni *et al.*, 2016) also agreed with histological result in their study of aqueous extract of leafs celery on rats.

### Conclusions

Elevated in the weights of the ovaries and uterus as well as increased in the thickness of the muscle layer and numbers of the primary, secondary, Graafian Follicles and Corpora Lutea of these organs in lab animals associated with received of the celery extract.

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