Effect of *Nigella Sativa L.* seeds on ovaries function in adult Rats treated with Lead Acetate

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Abstract:

Background: There is an increasing interest toward medicinal plants and their active ingredients in the last years.

Aims of the Study: This study aimed to show the effect of Nagela sativa L. seeds on the functions of ovaries in adult rats treated with lead acetate.

Materials and Methods : Thirty adult female rats were randomly divided into three groups and treated orally as following for 8 weeks . Rats in the first group were received one ml of distilled water as control group . Rats in the second group were received lead acetate (10 mg / kg) as test 1 group. While rats in the third group received 100 mg/kg seeds of Nigella sativa with 10 mg /kg lead acetate as test group-2.

Fasting blood specimens were collected to poll serum 2,4,and 8 weeks for the measurement of LH, FSH

And estrogen hormone.

Results: The results revealed significant ($P<0.05$) increase in ovarian weight to body weight ratio, FSH, numbers and diameters of graffian follicles in two treated groups (T1andT2)compared with control group. Exposure of animals to lead acetate (T1) reflects a significant ($P<0.05$) increase in LH hormones with a significant decrease in estrogen compared with control group. Treatment of animals with Nigella Sativa pulse lead acetate caused significant ($P<0.05$) decrease in serum LH hormone compared with T1 group but the concentration of estrogen was significantly ($P<0.05$) increase.

Ovaries histological studies of (T1 group) indicated an increase in diameters of graffian follicles and vaculation of granulosa cells, while the ovary of the animals in (T2 group) showed decrease in number and diameter of graffian follicles in comparison with normal structure of these follicles. On conclusion it seems likely that dosage of rats with (10 mg/kg B.W) of lead acetate caused a significant redaction on ovarian function and the treatment of animals with (100 mg/kg B.W) of Nigella sativa caused a significant enhancement of its reproductive function.

Keywords : Nigella sativa , ovarian hormones, lead acetate, medicinal plants.

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http://doi.org/10.33091/AMJ.0700912011

Introduction

There is an increasing interest towards medicinal plants and their active ingredients in the last years. One of the most important motives was the synthetic drugs which show off their dangerous side effects by time, whereas medicinal plants have generally centuries-long use and little unknown side effects [1].

The plant is widely grown in different parts of the world. As an oriental spice, *Nigella sativa* has long been used as a natural medicine for the treatment of many acute as well as chronic conditions [2, 3].

Lead is considered as one of the most hazards and cumulative environmental pollutants that affect all biological systems through exposure from air, water and food sources [4] and it is one of the oldest known and most important environmental pollutions which are toxic and may affect body organ for several years even in the absence of continued exposure [5, 6,7,8, 9].

Lead exposure induces clinical pathological changes through toxicity occurred to kidney and endocrine system [10].

Lead is a heavy metal naturally found in Earth crust and also provided from anthropogenic sources in environment, feeds, and foods of vegetal and animal origin and has toxic effects on many systems of the body, particularly on the developing nervous system, the hematological and cardiovascular systems, and the kidney [11].

Materials and Methods:

1. Preparation of *Nigella sativa* Suspension:
   Black seeds were purchased from the (local market in Baghdad) and certified at the National Herbarium, in Abu Ghraib. They were cleaned and ground in a grinder. The water suspension was prepared as 1ml of suspension contains 10mg of *Nigella sativa* (10gm/litter) and each animal of treated group (T2) received 1ml/100 gm from weight of animal [12].
2. Preparation of Lead Acetate Solution:
   Lead acetate purchased from Gonane office for medical devices-Iraq, BDH Co. (England). The water solution was prepared as 1ml of solution contains 1mg of lead acetate (1gm/litter) and each animal of treated groups (T1 and T2) received 1ml/100 gm from weight of animal [13].
3. Study protocol: Thirty female rats were divided randomly into (3 groups) (10 animals per group & handled as follows:
   Control group**:** Animals of this group (10 female rats) received 1 ml/kg B.W. of ordinary tap water by oral dosage using gavage needle .
   (T1 Group): Animals of this group were received (10 mg /kg B.W.) of lead acetate solution once daily [13] .
   (T2 Group): Animals of this group were received (10 mg /kg B.W.) of lead acetate solution daily (Saied, 2005), and after three hours dosed *Nigella sativa* suspension (100 mg /kg B.W.) once daily [14].
4. Fasting blood samples:(3ml) were collected at (14, 28, 42 & 56 days) of experiment via cardiac puncture technique after general anesthesia. Blood samples were kept in to tubes and helped for not more than [4] hours before serum was collected by centrifugation (2500 rpm) 15 minutes liquidated and frozen at 20C˚until analysis taken to study parameters.
5. parameters studied:
   a. ovarian weight to body weight ratio by using the flowing equation: ovarian weight/Body weight x100.
   b. Hormonal Assay: It was conducted for the samples (Estadiol, FSH) , using kits purchased from immunothech (Marseille-France). While LH assay was done using kits obtained assay DiaSorin (Veselli-Italia).

http://doi.org/10.33091/AMJ.0700912011

Histological Study of the Ovaries:

After the end of treatment, animals were sacrificed and by cervical dislocation; ovaries were excised and cleared off the attached fat and connective tissue, Histological sections were prepared according to [15].

Statistical Analysis:

Results are expressed as mean ± SE. Statistical analysis of data was performed on the basis of Chi square (x^2), two- way analysis of variance (ANOVA II), and one- way analysis of variance. Group differences were determined using least significant difference (LSD) test at $P<0.05$ [16].

Results & Discussion:

Ovarian Weight to Body Weight Ratio (%):

The table(1) showed that Ovarian weight to body weight ratio increases significantly ($p<0.05$) in the two treated groups (lead acetate T1 and lead acetate + *Nigella sativa* T2) compared with control group. The ratio of two treated groups were (0.0352 ± 0.002, 0.0324 ± 0.003) compared with control group (0.0315±0.003). The result has also showed no significant differences between (T1 and T2) groups.

The increase of ovarian weight to body weight ratio of two treated group may be due to an increase of ovarian weight. Thus, lead acetate causes an increase of the weight of ovaries as clarified from the result of this study.

The increment of ovarian weight in lead acetate treated group (T1) may be due to an increase of gonadotropins hormones (LH and FSH) which cause some structural and histological changes of the ovaries like ovarian weight, diameters and the numbers of follicles [17].

The effect of *Nigella sativa* results in a decrease in the ovarian weight to body weight ratio (T2) in comparison with (T1) group, although these differences lack the significant ($P<0.05$) degree but they may refer to effect of *Nigella sativa* to increment of body weight and reduce the negative effects of lead acetate on ovaries and finally causes this decrease in ratio compared with lead acetate group.

Table (1): Effect of lead acetate, lead acetate and *Nigella Sativa* on the ovarian weight to body weight ratio (%), in female rats.

Groups / *Time*	*Control group*	*(T1)group*	*(T2)group*
After 56 days of treatment	**0.0315 ± 0.003** **B**	**0.0352 ± 0.002** **A**	**0.0324 ± 0.003** **A**

(T1) given lead acetate (10mg/kg B.W) and (T2) given *Nigella sativa*(100 mg/kg B.W) and lead acetate (10mg/kg B.W).

- Values are presented as means ± SE (n= 6 rats/group).
- Capital letters denote significant differences between groups ($P<0.05$).

Serum FSH Concentration (ng/ml):

Serum FSH concentration shows a significant ($P<0.05$) increase in the two treated groups compared with control group in all periods of experiment.

Also there is a significant difference in serum FSH concentration between (T1 and T2) groups (Table-2).

http://doi.org/10.33091/AMJ.0700912011

The *Nigella sativa* and lead acetate treated group (T2) causes a significant (P<0.05) decrease of FSH concentration as compared with lead acetate treated group (T1) at the eight weeks interval of experiment.

Within groups, there are significant differences in T1 at the second and fourth weeks compared with sixth and eight weeks, but there is a significant difference within T2 group at all periods except 6th week.

Serum LH Concentration (ng/ml):

The data which has referred to a significant (P<0.05) increase in LH concentration was recorded in (T1) group range values (5.55±0.68-5.88±0.50) compared to control group (3.86±0.33-4.83±0.63) in all periods of experiment except the last week (Table-3).

On the other hand, there are no differences in mean value of LH concentration between (T2) groups compared with control group except 8 week of experiments.

During the different periods of experiments there are no significant differences within (T1 and T2) groups during all experiment intervals.

The results have showed that exposure of rats to *Nigella sativa* with lead acetate causes a decrease in LH concentration (4.11±0.27 - 5.03±0.24) and the values tend to be close to control group (3.86±0.33 -4.83±0.63) but not reach the significant degree (Table-3).

Exposure to lead is associated with impairment of reproductive system organs function in both man and experimental animals specially the Gonads (ovaries) [17].

The dominant mechanism of action of the toxic effects of lead occurs at the level of the hypothalamus and /or supra-hypothalamic sites; so that the lead-treated animals responded to both GnRH stimulation in a hyperresponsive manner which lead to increased stores of pituitary gonadotropins and finally the release of these hormones will be increased in serum [18, 19]. Lead acetate causes a significant (P< 0.05) decrease in steroid hormones synthesis and release from ovary (Estrogen and Progesterone). These effects may lead to a positive feedback mechanism upon hypothalamus or pituitary gland. As a result LH and FSH hormone will increase [19, 20].

From results obtained it can be explained that *Nigella sativa* have an important role in elevation of LH and FSH level. This elevation may be due to the active constituents of *Nigella sativa* in activation of synthesis mechanisms of these hormones by stimulation of hypothalamus or pituitary glands to release and secrete Gonadotropin hormone [21].

On the other hand, the increase of LH and FSH hormones may be due to the high constituents of *Nigella sativa* from essential nutrients specially glucose and volatile fatty acid, thus these nutrients may stimulate hypothalamus and pituitary gland to increase synthesis and release of these hormones [22].

Table (2): Effect of lead acetate, lead acetate and *Nigella sativa* on serum FSH concentration (ng/ml), in female rats

http://doi.org/10.33091/AMJ.0700912011

Groups / Time	*Control group*	*(T1)Group*	*(T2)Group*
Day (14)	**6.35 ± 0.85 C a**	**8.05 ± 0.26 A a**	**7.23 ± 0.32 B a**
Day (28)	**4.95 ± 0.16 C b**	**8.51 ± 0.39 A a**	**7.13 ± 0.43 B a**
Day (42)	**6.91 ± 0.31 B a**	**7.56 ± 0.41 A b**	**5.93 ± 0.50 C c**
Day (56)	**5.41 ± 0.31 C b**	**7.46 ± 0.48 A b**	**6.86 ± 0.17 B b**

(T1) given lead acetate (10 mg/kg B.W) and (T2) given *Nigella sativa* (100 mg/kg B.W) and lead acetate (10 mg/kg B.W).
- Values are presented as means ± SE (n= 10 rats/group).
- Capital letters denote significant differences between groups (P<0.05).
- Small letters denote significant differences within groups (P<0.05).

Table (3): Effect of lead acetate, lead acetate and *Nigella Sativa* on serum LH concentration (ng/ml), in female rats.

Groups / Time	*Control group*	*(T1)Group*	*(T2)Group*
Day (14)	**4.13 ± 0.57 B a**	**5.58 ± 0.31 A b**	**4.88 ± 0.41 B b**
Day (28)	**4.83 ± 0.63 B a**	**5.88 ± 0.50 A b**	**5.02 ± 0.47 B b**
Day (42)	**3.86 ± 0.33 B a**	**5.55 ± 0.68 A b**	**4.11 ± 0.27 B b**
Day (56)	**4.73 ± 0.32 B a**	**5.61 ± 0.40 A b**	**5.03 ± 0.24 A b**

(T1) given lead acetate (10 mg/kg B.W) and (T2) given *Nigella sativa* (100 mg/kg B.W) and lead acetate (10 mg/kg B.W).
- Values are presented as means ± SE (n= 10 rats/group).
- Capital letters denote significant differences between groups (P<0.05).
- Small letters denote significant differences within groups (P<0.05).

Serum Estrogen Concentration (ng/ml):

http://doi.org/10.33091/AMJ.0700912011

The results have showed that daily treatment of animals with (10 mg/kg B.W.) of lead acetate for (8 weeks) caused significant ($P<0.05$) decrease in serum estrogen concentration in (T1) group compared with control group during all period of experiment (Table-4).

On other hand the concentration of estrogen was significantly ($P<0.05$) increase in (T2) group compared with (T1) group except at 6th week of experiment (Table-4).

Within the time there were no significant differences in mean values of serum estrogen concentration were presented in the two treated groups (T1 and T2) at all experiment period.

The elevation of steroid hormones (Estrogen) after treatment with lead acetate and *Nigella sativa* (T2) may be due to role of *Nigella sativa* components to reduce the negative effects of toxicity of lead acetate [23,24], the absent of significant differences within group (T2) at 8th week compared with 2nd week may be due to increase of lead effects or toxicity with the stages of experiment, thus lead have accumulative effect of other heavy metal so that the effect of *Nigella sativa* was decrease compared with the high toxicity of lead at the last week of experiment [25]. The increase of FSH and LH hormones in (T2) group compared with control group may be increase the follicular growth so that there were increase in the number of Graffian follicles which increase the synthesis and release estrogen hormone [26,27]. Also the increase of LH hormone in (T2) group compared with control may increase the level of estrogen because the high level of LH hormone leads to increase of its binding with the receptors on theca cells [28,29].The increase of LH hormone is considered as a principle factor to stimulate the theca interna to elevate production of pregnenolone compound which converted by granulose cells to progesterone [30].

http://doi.org/10.33091/AMJ.0700912011

Table (4): Effect of lead acetate, lead acetate and *Nigella sativa* on serum Estrogen concentration (ng/ml), in female rats.

Groups / *Time*	*Control group*	*(T1) group*	*(T2) group*
Day (14)	86.33±3.93 A a	66.01 ± 4.30 C a	76.33±2.64 B a
Day (28)	77.83 ± 2.75 A b	66.33 ± 3.80 B a	73.50 ± 3.48 A a
Day (42)	73.50 ± 2.90 A b	65.66 ± 3.69 B a	69.83 ± 1.72 B a
Day (56)	75.50 ± 2.58 A b	63.83 ± 2.14 B a	70.66 ± 2.31 A a

(T1) given lead acetate (10 mg/kg B.W) and (T2) given *Nigella sativa* (100 mg/kg B.W) and lead acetate (10 mg/kg B.W).

- Values are presented as mean ± SE (n=10 rats/group).
- Capital letters denote significant differences between groups (P<0.05).
- Small letters denote significant differences within groups (P<0.05).

Numbers and diameters of Graffian Follicles:

The data have showed that the mean numbers of ovarian follicle (Graffian follicles) increases significantly (P<0.05) in the two treated groups (T1and T2) compared with control group, (Table-5).furthermore the data showed that a high increase of graffian follicles number was clarified in lead acetate treated group (T1) compared with (T2) and control groups (8.00±2.12),(5.50±0.99) and (2.33±0.56) respectively. The data referring to the diameters of graffian follicles of control and two treated groups are shown in table (6). After the end of experiment, diameters of graffian follicles increase significantly (P<0.05) in the (T1 and T2) groups as compared with control group. Furthermore, there was a significant difference in the diameter of graffian follicle in (T1) group compared with (T2) group.

Thus, lead acetate causes a significant increase (P<0.05) in values of mean graffian follicles diameter in (T1) group compared with *Nigella sativa* treated group (T2).The result of table (5 and 6) have indicated that there is a significant (P<0.05) increase in the number of graffian follicles (Table-5) and diameters of these follicles (Table-6) with lead acetate in (T1 group) compared with control group, and this is attributed is to an increase of Gonadotropin hormones Follicular stimulating hormone and Leutinizing hormone (FSH and LH) as demonstrated in the present study (LH and FSH).These hormones are responsible of maturation and growth of ovarian follicles [30].

http://doi.org/10.33091/AMJ.0700912011

Lead acetate as discussed previously, causes a high increase in FSH and LH hormones, so that an increase in number and diameters of ovarian follicles was occurred, also lead acetate due to its specificity as a toxic materials that causes structural and functional changes of ovaries (hyperplasia) and thickening of Graffian follicles (Fig.2),

may be the cause of these alteration of diameters and numbers of ovarian follicles. *Nigella sativa* which cause an increase in the number and diameter of ovarian follicles in (T2) group compared with control group may be due to the increase of growth and maturation of ovarian follicles as a result of increase of FSH and LH hormones [21].On the other hand, the increase of diameter of follicles especially Graffian follicles in (T2) group (Fig.3 and 4) may be due to the increase of granulose cells division and high increase in follicular fluid and the numbers of theca cells [31,32].

Table (5): Effect of lead acetate, lead acetate and *Nigella Sativa* on numbers of ovarian follicles in female adult rats.

Types of follicles	***Control group***	***(T1)group***	***(T2) group***
Graffian Follicles	**2.33 ± 0.56 B**	**8.00 ± 2.12 A**	**5.50 ± 0.99 A**

(T1) given lead acetate (10 mg/kg B.W) and (T2) given *Nigella sativa* (100 mg/kg B.W) and lead acetate (10 mg/kg B.W).
- Values are presented as means ± SE (n= 6 rats/group).
- Capital letters denote significant differences between groups (P<0.05).

Table (6): Effect of lead acetate, lead acetate and *Nigella Sativa* on Graffian follicles diameter (μm) on ovarian in female rats.

Group / Times	***Control group***	***(T1)group***	***(T2)group***
After 56 days of treatment	**26.10 ± 0.98 C**	**43.13 ± 1.86 A**	**30.20 ± 0.76 B**

(T1) given lead acetate (10 mg/kg B.W) and (T2) given *Nigella sativa* (100 mg/kg B.W) and lead acetate (10 mg/kg B.W).
- Values are presented as means ± SE (n= 6 rats/group).
- Capital letters denote significant differences between groups (P<0.05).

http://doi.org/10.33091/AMJ.0700912011

The Histological Changes in Ovarian Tissue:

The histological examination of the ovary of non-treated animal (control) has showed a normal structure of the graffian follicle which consists of theca externa, theca interna, granulose cell and graffian cavity which contains oocyte (Fig. 1).

Also the histological section (Fig.2) has revealed that the ovary of animals treated with lead acetate shows an increase in the diameter of graffian follicle (43.13 ± 1.86) as compared with the diameter of graffian follicle in the normal animals (26.10 ± 0.98) (Table-6). The microscopic section has revealed vacuolation of the cytoplasm of the granulose cell the picnotic their nuclei as well as sloughing and desquamation of some granulosa cell in the fluid of graffian cavity (Fig. 2).

Also the histological section has showed an increased in the number of primary (6.66 ± 1.53), secondary (5.83 ± 0.88) and graffian follicle (8.00 ± 2.12) as compared with the normal ovary (1.16 ± 0.40, 2.00 ± 0.52, 2.33 ± 0.56) respectively. The results have explained that the ovary of the animal which treated (lead acetate and *Nigella sativa*) shows a decrease in the size of graffian follicle (30.20 ± 0.76) and a decrease in the number of primary (7.00 ± 1.60), secondary (4.16 ± 0.48) and graffian follicle (5.50 ± 0.99) as well as normal structure of the graffian follicle (Fig. 3 and Tab.5). The histological picture has showed that the primary follicles consist of single layer while secondary follicles consist of more than one layer of the cell as compared with graffian follicle (Fig.4).The histological changes in ovarian tissue may be due to hormonal changes which have occurred by lead acetate. The increase of gonadotropin hormones (FSH and LH) leads to an increase of the diameter and number of ovarian follicles, also the toxic oxidative properties of lead acetate may cause some harmful effects of histological structures of the ovaries (17).Moreover, the *Nigella sativa* component lead to enhancement of the number and the diameter of graffian follicles. This may be due to elevation of FSH hormone (33) by increasing its receptors on granulosa cells surface (34). There is significant in the number of ovarian follicles with a normal structure and an increase of follicular fluid after treatment of rats with FSH hormone at the first day before progesterone (31,21).

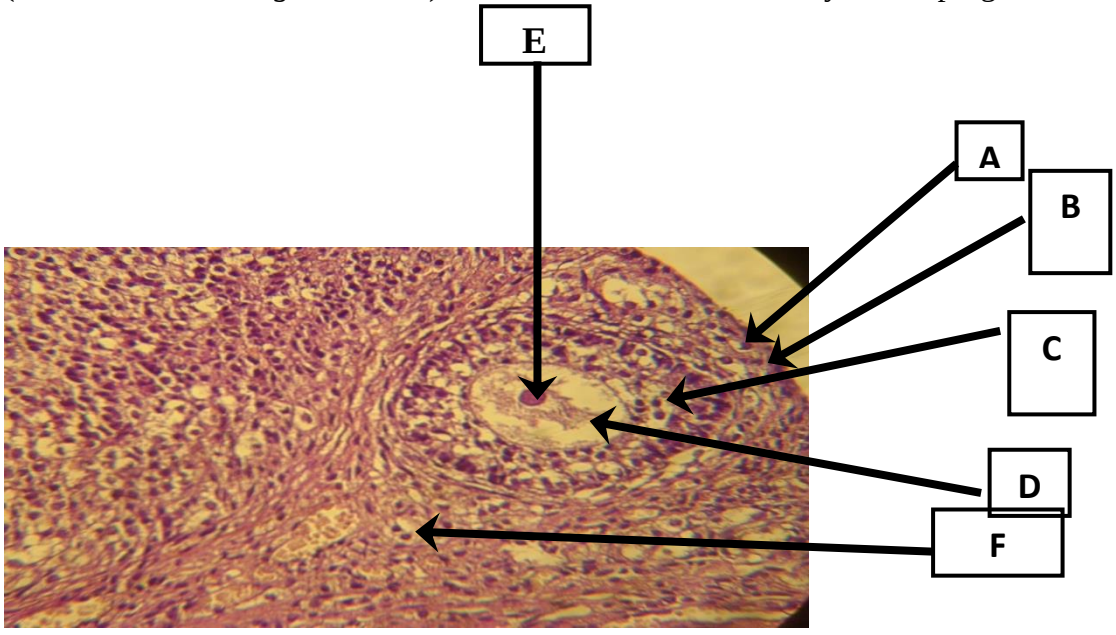

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Figure 1: Histological section in the ovary of normal animal has showed normal structure of Graffian follicles, which consist of theca externa (A), theca interna (B), granulosa cells (C), follicular cavity (D), and oocyte (E).as well as primary follicle with single cell layer(F) (H&E 40X).

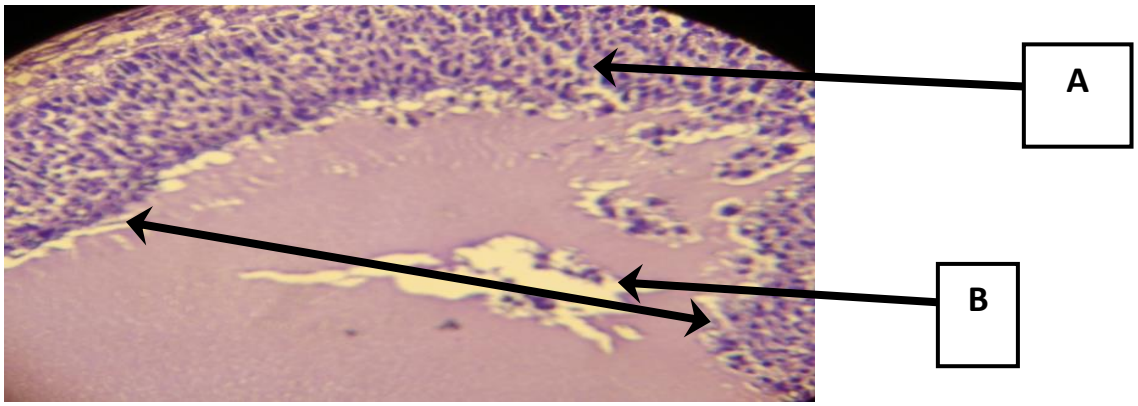

Figure 2: Histological section in graffian follicle of animal treated with lead acetate has showed vacuolar degeneration of cytoplasm of granulose cells (A), sloughing of these cells in the follicular cavity (B) and increase diameter of graffian follicles(↔ &E 40X).

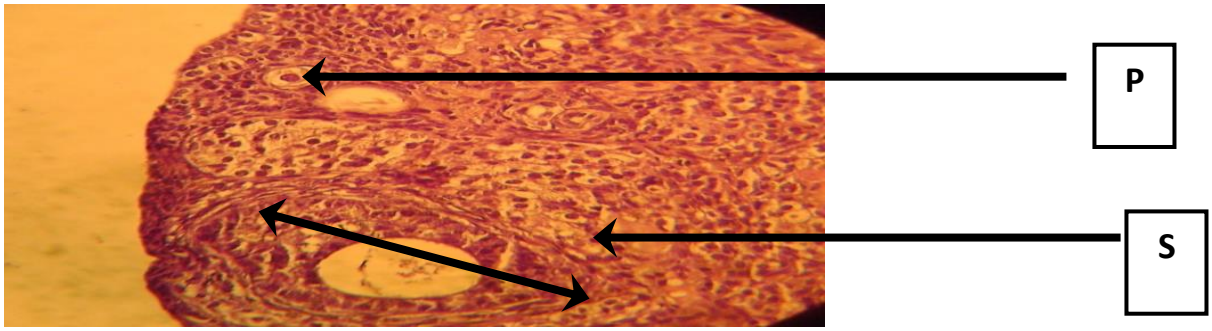

Figure 3: Histological section in the ovary of the animal administration with lead acetate and treated with *Nigella sativa* has showed a normal structure of the graffian follicle (↔) as well as a primary follicle (P) and a secondary follicle (S) (H&E 40X).

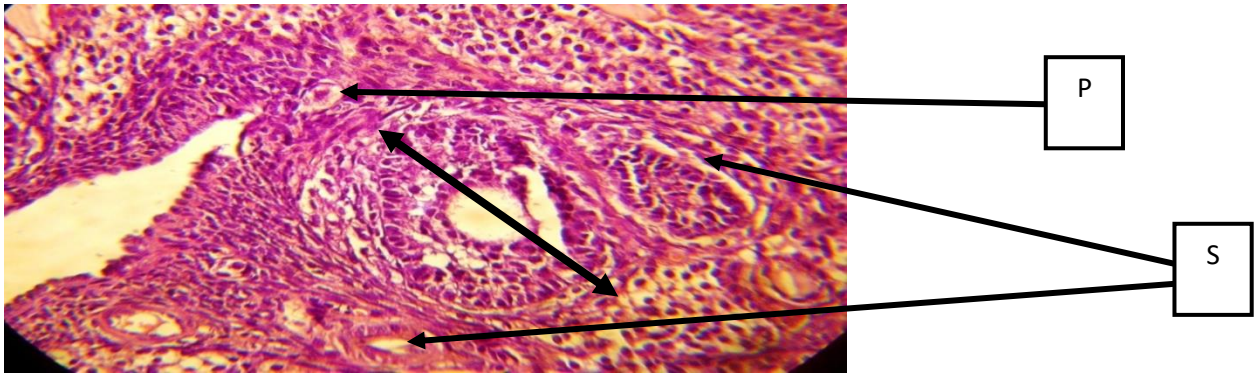

Figure 4: Histological section in the ovary of one animale treated with *nigella sativa* after administration with lead acetate has showed a normal structure of graffian follicles (↔) and a primary (P) and a secondary follicles (S) (H&E 40X).

http://doi.org/10.33091/AMJ.0700912011

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http://doi.org/10.33091/AMJ.0700912011