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Histological effect of *Lepidium sativum* extract on the pituitary glands in female rabbits

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***Abstract*—The pituitary gland plays a central role in regulating endocrine functions, including growth, reproduction, lactation, and metabolic balance. Objective: Study of the effect of *Lepidium sativum* plant extract on the histological structure of the pituitary gland. Materials and methods: A total of 20 sexually mature female domestic rabbits were randomly allocated into 4 categories, each one consisting of 5 pets, and were selected based on 2.5 to 3 kg weights, divided into two main groups. Group 1: Non-pregnant, non-lactating rabbits, and included Section A1 (control): were given sterile water. Section B1 was treated with *L. sativum* extract powder at a dose of 200 mg/kg body weight. Group 2: Lactating rabbits, Section A2 (control), were treated with sterile water. Section B2 was treated with *L. sativum* extract at a dose of 200 mg/kg body weight. The experiment lasted for three weeks. a histological examination of the pituitary gland. Histological results showed that administration of Lepidium sativum extract resulted in significant changes in the anterior lobe of the pituitary gland. The treated groups, particularly during lactation, exhibited hypertrophy and hyperplasia of lactotroph cells, with prominent nuclei and cytoplasmic activity, indicating a stimulatory effect of the extract on prolactin-secreting cells compared to the normal control group.**

Keywords_ *Lepidium sativum,* pituitary glands, Prolactin, Histological study

INTRODUCTION

The natural plant extracts' potential therapeutic benefits Nand bioactive ingredients have sparked a lot of interest in their application in biological and medical research (1). There are over 350,699 plant species in the world, 75,000 of which are utilized as food, and many of which have medicinal properties (2). This highlights the importance of dynamic studies in understanding the use of plants for food and medicine. Garden cress, or Lepidium sativum, is an annual vegetarian plant with several traditional therapeutic uses. This plant is distinguished by its small top leaves and lobed lower leaves, as well as by its potent, fragrant scent. Its bioactive ingredients, which are utilized in many cultures to treat illnesses and advance general health, provide it therapeutic significance. its potential effects on endocrine functions, specifically those of the pituitary and breast glands. Its bioactive ingredients might affect the function and structural integrity of the mammary and pituitary glands (3).

The pituitary gland comprises three important parts (anterior, posterior, and intermediate lobes). The anterior lobe was the major and largest lobe and was greater than the posterior lobe. The intermediate lobe and the cleft split the posterior lobe from the anterior lobe. The intermediate cleft divided the gland into two separate and different parts, the anterior lobe and the posterior lobe (4). The pituitary gland is situated within the hypophyseal fossa, a fibro-osseous compartment near the center of the cranial base. In that location, the gland has proximity to several important cranial nerves and vascular structures at the skull base (5).

MATERIALS AND MTHODS

Collection of Lepidium sativum

Collecting wild cress (Lepidium sativum) from the Samawah desert in the winter season of the poison 2025 and after the rains, Washed the plant and cleaned it well. Cut the plant into small parts. Dried the herb in the shade and at room temperature (6). Ground the plant using a home herb grinder. Soaked the dry plant for 28 hours with ethanol alcohol at a concentration of 70% and a liter per 100 grams of plant. Used the rotary shaker device for 18 hours. Nomination of the first: extract with medical gauze to remove large impurities. Filtration of the second extract using filter paper, which is known as the cold extraction process, Dried the extract in the oven at a temperature of 50 ° to obtain powder, Saved the extracted material at a refrigerator temperature of 4°. I repeated this process several times to get the sufficient amount of extract (7).

Preparation of ethanolic extract of Lepidium sativum

The work was conducted in the laboratories of Al-Ameed University using the dried plant material of wild arugula (Eruca sativa). The alcoholic extract was prepared by weighing 50 grams of the dried plant and grinding it using a household herb grinder to obtain a fine powder. The powder was then placed in a glass flask, and 500 mL of ethanol at 70% concentration was added. The mixture was left at room temperature for a specified period with intermittent stirring, and then it was filtered to obtain the extract. The yield of the dried extract was 3.3%, equivalent to 3.3 grams of extract per 50 grams of dried plant material.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

Sample preparation

Extracts were prepared using 0.4 g of the sample dissolved in 10 ml of pure methanol at 60 °C for GC–MS. The suspension was filtered through a 0.45 µm filter before being sent to the GC–MS for analysis.

Method

Thermal decomposition products of the samples were characterized by an Agilent (7820A) GC Mass Spectrometer, Analytical Column: Agilent HP-5ms Ultra inlet (30m length x 250µm diameter x 0.25 µminside diameter) Injection volume1µl Pressure11.933 psi GC Inlet Line Temperature250˚C Aux heaters Temperature310˚C Carrier GasHe99.9% Injector Temperature250˚C Scan Range:m/z50~500_Injection TypeSplitless Oven Program Temperature (Table 3-3)/Scan Program Temperature (Table1)

Table 1. Oven program temperature of GC-MS

RAMP	TEMP./TIME
Ramp 1	55 ˚C hold to 2˚C/min
Ramp 2	55 ˚C to 180 ˚C 7˚C/min
Ramp 3	180˚C to 280 ˚C 8˚ C/min
Ramp 4	280˚C hold to 2˚ C/min

Time amounted to about 34 minutes. The chemical constituents of clove bud extract were identified by comparing the results of the chromatogram and reference retention time using the Wiley mass spectra library (Wiley W9N11) [8].

Histochemical Study:

1- Hematoxylin and Eosin stains: for general histological examination according to [9]**.** 2- The Periodic Acid-Schiff (PAS) stain is a histochemical technique used in microscopy to highlight specific carbohydrates in tissues [10]**.** 3- Mallory trichrome stain is a valuable histological technique used in the examination of collagen, muscle fibers, and cytoplasm, allowing for a clearer assessment of the tissue architecture [11]**.**

Animals Experimental

This study was conducted for the period from the beginning of one month in 2025 to the end of the second month in 2025, with 20 adult females who were randomly allocated into four categories, each one consisting of 5 pets whose weights ranged from 2.5 to 3 kg and ages ranged from 9 to 13 months. And were housed and maintained in the animal house/college of veterinary medicine/university of Kerbala. Animals were placed in special cages with lighting 12 hours a day and good ventilation; the floor was furnished with soft sawdust, and care was taken care of the cleanliness of the cages and change the floor constantly and sterilized with disinfectants as well as continuous care for the cleanliness of irrigation bottles and the shelter room, and provide animals with water and a standard diet freely throughout the duration of breeding and research. Rabbits were left to acclimatize for three weeks before the start of the experiment**.**

Experimental Design

The animals were randomly divided into two main groups, each consisting of 10 adult female rabbits, as follows:

1-First Group (G1): Non-pregnant, Non-lactating Mature Females This group was subdivided into two subgroups (5 animals per subgroup):

A- Control Group (A1): Received only standard feed and clean drinking water,

B- Treatment Group (B1): Received L.S. extract orally, once daily for three consecutive weeks, at a dose of 200 mg/kg body weight [12]**.**

2- Second Group (G2): Lactating Females After natural mating and parturition, this group of females entered the lactation phase. On the first day of lactation, the group was divided into two subgroups (5 animals per subgroup):

A-Control Group (A2): Received standard diet and water only during the lactation period.

B- Treatment Group (B2): Received L.S. extract orally, once daily for three consecutive weeks during lactation, at the same dose used in the first group.

Histological study of pituitary gland Histomorphology examinations for cell shape comparison of all samples, cell diameter, and nucleus size of pituitary cells (acidophilic cells).

Statistical analysis

The Statistical Packages of Social Sciences (SPSS) program (2019) was used to detect the effect of different groups in study parameters. The t-test was used to compare significant differences between means in this study.

STATISTICAL ANALYSIS

The Statistical Packages of Social Sciences (SPSS) program (2019) was used to detect the effect of different groups in study parameters. The t-test was used to compare significant differences between means in this study.

RESULT AND DISCUSSION

Pituitary gland diameter

Table (2) showed the pituitary gland measurement diameter of acidophil cells in the different rabbit groups. The results indicate statistically significant differences between the control and treated sections. In non-lactating rabbits (Group 1), the pituitary gland diameter increased in section B (treated with *L. sativum* extract), recording 14.36 ± 0.23 µm, compared to section A (control), which recorded 9.94 ± 0.32 µm. Similarly, in lactating rabbits (Group 2), section B (treated) showed a higher diameter of 28.16 ± 0.75 µm, compared to section A (control), which recorded 25.33 ± 0.64 µm.

Table 3. Comparison between groups 1 and 2 in Pituitary gland measurement diameter of acidophil difference sections in rabbits

Section	Mean ± SD of Pituitary gland measurement diameter (micron)		T-test (P-value)
	Group 1	Group m2	
Section A	9.94 ±0.32	25.33 ±0.64	5.164 ** (0.0001)
Section B	14.36 ±0.23	28.16 ±0.75	5.702 ** (0.0001)
T-test (P-value)	1.263 ** (0.0079)	2.307 * (0.0388)	---
(P<0.05)		(P<0.01).	

The findings suggest that *L. sativum* extract plays a role in stimulating the activity and growth of acidophil cells in the pituitary gland, with a more pronounced effect observed in lactating rabbits. The statistical analysis confirmed these differences, showing a highly significant increase (P<0.01) within groups and a significant difference between non-lactating and lactating groups (*P<0.05* to P<0.01). This indicates that physiological status (lactating vs. non-lactating) and treatment with *L. sativum* both influence pituitary gland activity. Biologically, this increase in the size of pituitary eosinophils in treated human subjects can be explained primarily by two factors. The fact that mammalian eosinophils belong to the lactotroph family of cells contributing to prolactin (PRL) (13). Any increase in the stimulation of prolactin secretion, whether it occurs by direct cell stimulation or as a result of hypophysiotropic release from the hypothalamic-pituitary axis, results in hypertrophy (enlargement) of these cells since they become more active and produce larger amounts of protein. Secondly, a set of plant-diverse compounds in the *L. sativum* extract could modulate these two pathways via a variety of paths (14).

Chemically, *L. sativum* is rich in bioactive compounds (flavonoids, saponins, sterols, alkaloids, and phenolic compounds) with reported hormone-like and nutraceutical activities (15). These types of compounds could have positive effects on prolactin secretion via two principal mechanisms: (a) estrogen-like activity (phytoestrogenic/sterol) potentiating lactotrophs and increasing expression of genes for prolactin, and (b) modulation of cerebrovascular signals influencing dopaminergic mediation to inhibit prolactin. Some phytoextracts may minimize activation of aura centers and be low-grade D2 blockers at the receptor level, such that they diminish suppression to lactotrophs in the anterior pituitary and increase PRL release (16). The common modes of action for galactagogues propose inhibition of dopamine as the optimum approach to increase prolactin (17).

There is direct experimental support for the idea that consuming *L. sativum* seeds/extract enhances lactation function and milk production. Animal and human studies have reported increased milk production and improved reproductive performance indicators with this substance, which is consistent with the observed elevations in prolactin and lactotroph hyperplasia in our lactating and treated groups (18). Additionally, studies in rabbits have shown that feeding garden cress seeds to females improved lactation yields and physiological indicators associated with lactation (19), which is consistent with the results of the current study.

Histological Results of pituitary gland

G1 (section A1): The results of histological examination in the healthy rabbit showed cells small and elongated to oval in shape with darkly stained nuclei. Most of them are pituicytes (supporting glial cells), which have small, round to oval nuclei and very little visible cytoplasm (20). Occasional larger cells with more cytoplasm can be seen, representing different supportive or vascular-associated cells. Cells are relatively sparse and scattered throughout the tissue as compared to the dense arrangement in the anterior pituitary. The majority of the slide is filled with nerve fibers and capillaries, and only moderate numbers of nuclei are present between them. The cytoplasm is generally indistinct under light microscopy due to the predominance of eosinophilic (pink-stained) background from nerve fibers. Most pituicytes appear to have scant, poorly demarcated cytoplasm, making the nuclei more prominent than the cell bodies. The pituitary gland (posterior lobe) shows scattered small glial-like cells (pituicytes) with round to oval basophilic nuclei and inconspicuous cytoplasm, embedded in a dense eosinophilic neuropil composed of unmyelinated nerve fibers and rich networks of capillaries (21). **Figure 1**

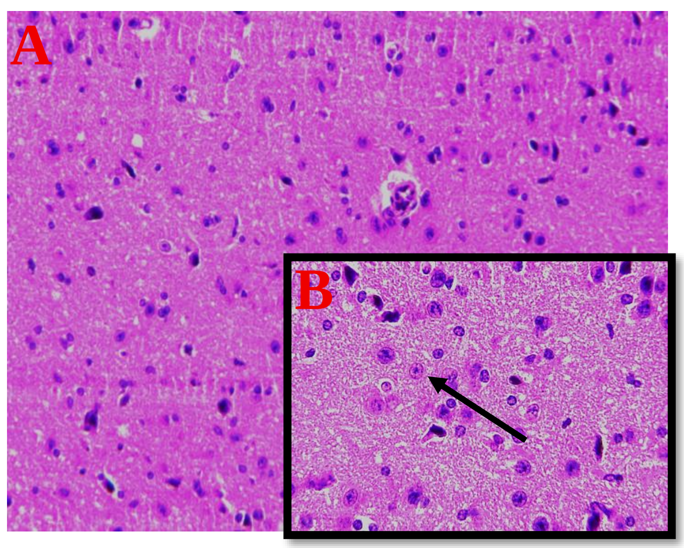

Figure 1. G1 (section A1), histological section pituitary gland, acidophile cell (black arrow), H&E stain, A. 100X B. 400X,

G1 (section B1): treated with the *Lepidium sativum* extract showed moderately enlarged pituitary cells compared to normal cells, with a more prominent cytoplasm (22). The density of cells is markedly increased; the field is packed with closely aggregated cells, leaving very little intercellular space. The cytoplasm is more conspicuous and eosinophilic, with many cells showing abundant granular cytoplasm, indicating active secretory function. Some cells display clearer cytoplasmic boundaries compared to normal tissue. The nuclei are round to oval, deeply basophilic, and relatively large in proportion to the cell, with increased nuclear crowding evident throughout the section. The anterior pituitary in this slide shows increased cell population, larger and more active secretory cells with abundant cytoplasm, and crowded nuclei (23). These features are consistent with stimulatory or trophic effects of garden cress extract on pituitary cells, possibly enhancing their activity and secretory function (24). **Figure 2**

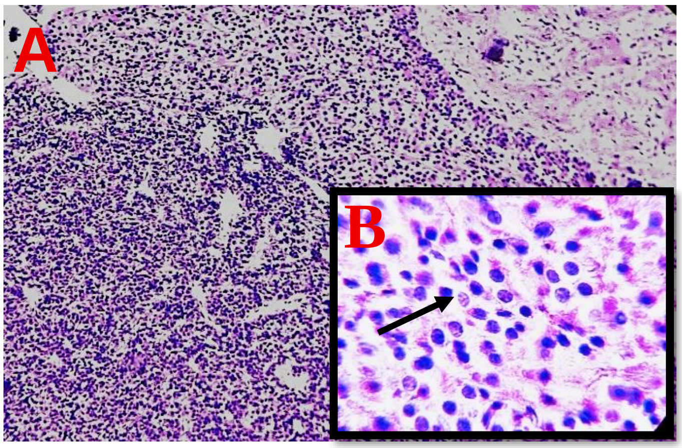

Figure 2. Histological section in G1 (section B1) pituitary gland، acidophile cell, H&E stain, A.100X, B.400X.

G2 (section A2): This group shows the anterior pituitary gland (adenohypophysis) during lactation, when lactotroph (prolactin-secreting) cells are maximally active. The cells are enlarged and hypertrophic, considerably bigger than in the non-lactating state (25). This increase in size reflects the high synthetic and secretory activity of the gland. Most cells are round to polygonal, with well-defined borders. Some cells appear irregular due to crowding and tight packing. The cytoplasm is abundant, eosinophilic, and granular, corresponding to the presence of secretory granules, mainly prolactin [26]. In many cells, the cytoplasm fills a large portion of the cell body, giving them a swollen appearance. The nuclei are large, round to oval, centrally located, and basophilic. Many nuclei are prominent, with open chromatin, indicating high metabolic and transcriptional activity. Cells are densely clustered and arranged in cords and groups, with minimal intercellular space. They are separated by fine connective tissue strands and a rich capillary network, facilitating rapid hormone release into the bloodstream. In lactation, the anterior pituitary gland is characterized by large, polygonal secretory cells with abundant granular eosinophilic cytoplasm, prominent basophilic nuclei, and crowded arrangements within a vascular stroma. These features represent the intense activity of lactotrophs producing prolactin to sustain milk secretion. **Figure 3**

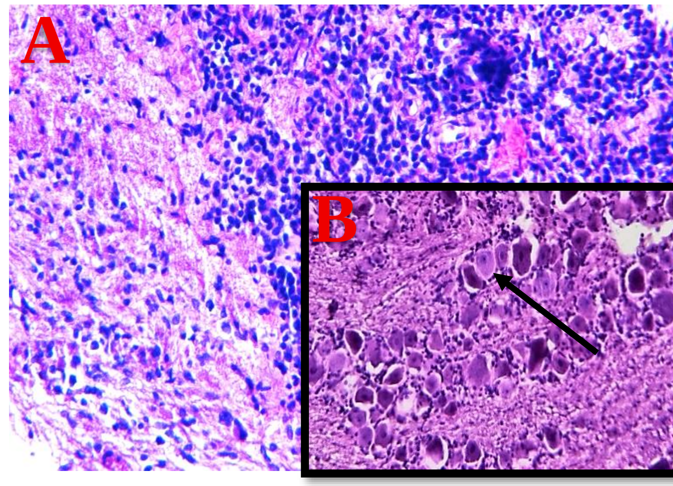

Figure 3. G2 (section A2), histological section pituitary gland, acidophile enlarged and increased in size and nuclei large, round to oval, predominantly location (black arrow), H&E stain, A. 100X, B. 400X

G2 (section B2): this group shows the pituitary gland section after administration of wild cress (*Lepidium sativum*) extract during the lactation period. The pituitary gland section exhibits distinct morphological alterations consistent with a stimulatory effect of wild cress extract during lactation. The endocrine cells, predominantly lactotrophs, appear enlarged and hypertrophied, with rounded to polygonal shapes. The nuclei are mostly central, euchromatic, and relatively large, with prominent nucleoli, reflecting an active transcriptional state. Occasional nuclei display irregular contours, correlating with intense metabolic and synthetic activity. The cytoplasm demonstrates strong acidophilic to basophilic staining, depending on the secretory granule content. Many cells show dense granularity, indicating accumulation of hormone-containing vesicles, most notably prolactin in lactotrophs. The cytoplasm is expanded and filled, with reduced vacuolation compared to quiescent phases, supporting the notion of active protein synthesis and hormone release. The cytoplasmic characteristics—granularity, hyperchromatic staining, and relative homogeneity—are clear markers of exaggerated secretory function. Histological examination of the pituitary gland under wild cress extract during lactation shows enlarged, hypertrophied endocrine cells with large euchromatic nuclei and prominent nucleoli. The cytoplasm is intensely granular and highly stained, reflecting increased synthesis and release of hormones, particularly prolactin, indicating a strong secretory state. **Figure 4**

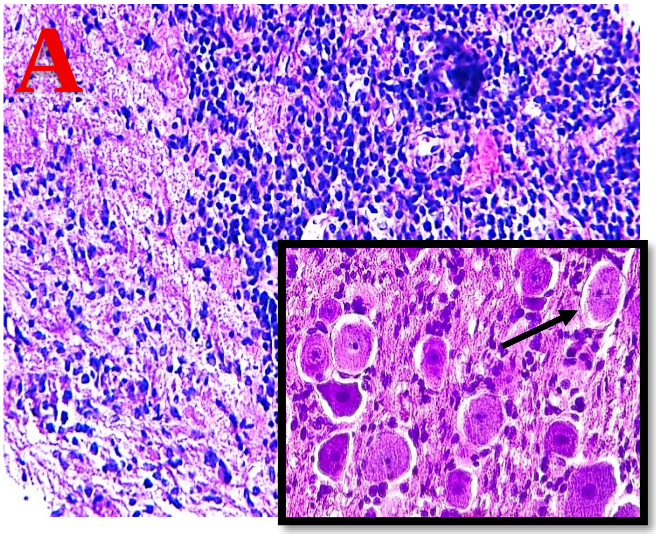

Figure 4 G2 (section B2), histological section pituitary gland, acidophile enlarged and hypertrophied, rounded to polygonal shapes and nuclei round to oval, centrally located (black arrow), H&E stain, A. 100X, B. 400X

Conclusion

The GC/MS analysis of *Lepidium sativum* extract revealed the presence of several bioactive compounds, including flavonoids and phenolic derivatives, which are known for their anti-inflammatory and hormone-modulating properties. These phytochemicals have been reported to enhance cellular activity and influence endocrine regulation. Such active constituents may explain the observed histological changes in the pituitary gland, where lactotroph cells appeared enlarged and metabolically active. This suggests that the extract exerts a stimulatory effect on prolactin secretion, supporting its potential role in promoting lactation and pituitary function.

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