Anatomical Study of Cervical Vertebra in the White Albino Rat Males Treated with *Lepedium Sativium* Seeds Extract

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Abstract

The study was conducted to find out the effect of secondary compounds extracted from *Lepedium sativium* seeds on the phenotypic structure of the cervical vertebrae by measuring the weight and lengths of each cervical vertebra. The study was performed in the animal house of Department of Biology, College of Education for Girls, University of Kufa, it included the use of 40 Albino rats of the Sprague Dawley strain, one months old, the average weights range (200-250g). They were divided into 4 equal groups, the first group included the control group, which was dosed orally with distilled water only, the second group was treated with cold aqueous extract of the *Lepedium sativium* seeds 50 mg / kg, the third group was treated with cold aqueous extract of the *Lepedium sativium* seeds 100 mg / kg, and the forth group was treated with cold aqueous extract of the *Lepedium sativium* seeds 150 mg / kg of body weight. Each group was administered from the first day to experiment and until the sacrifice of animals, which was in two stages on 30 and 45 days for each group. The study included measuring the weights of the cervical vertebrae, as well as measuring the lengths (length, width, diameter) of the vertebrae. The statistical analysis of the current study results was showed significant increase in weights of the cervical vertebrae in the group treated with cold aqueous extract of *Lepedium sativium* seed at a concentration 150 mg / kg, compared to the control group. As the Average weight increases with increasing the concentration of the extract. Also, The statistical analysis of the current study results was showed significant increase in lengths of the cervical animals treated with extract of *Lepedium sativium* seed at a concentration 150 mg / kg, for periods of 30 and 45 days compared to the control group.

Key-words: *Lepedium Sativium*, Extracted.

1. Introduction

Human vertebral column it is considered the mainstay of the body, maintains its straightness and shape, and articulates with the skull from above and from below it articulates with the two hip
bones, which in turn are connected to the lower extremities [20]. It arises from somite’s that form the axial mesodermal structures located on each side of the neural tube [18]. It is considered from irregular bones where it forms with the sternum and ribs the axial skeleton and it is composed of about 2 / 5th of the height of the body and the average length in males is 71cm and in females 61cm, consists of 33 bones called vertebrae. It is divided into five regions (the cervical region, the Thoracic region, the lumber region, the sacrum region and the coccyx [3].

The cervical region of the spine consists of seven vertebrae and six intervertebral discs, extending from the base of the skull to the top of the torso [11]. Its main functions include support and promote movement of the head and neck [10]. And it is divided into two parts: the upper part of the cervical intervertebral region is made up of the atlas and the axis [12], and these two vertebrae are completely different from the rest of the cervical vertebrae[1], where the atlas vertebra is the only vertebra that lacks a vertebral body, which is a ring of bones consisting of a thick anterior arch and posterior arch is slender and two transvers process, between which there is a transvers foramen and two prominent lateral masses [14]. On each lateral mass there is an upper and lower joint. The superior articular facet is a concave renal shape and is facing up and inward. It articulates with the nuchal canals to support the base of the skull called this type of articular articulation. The alteno-occipital joint, which is responsible for 50% of the cases of head rotation, while the anterior articular facet is flat and relatively downward and inward, articulating with the upper sides of the axis and is known as the alteno-axial joint responsible for 50% of bending and stretching [15]. The second cervical vertebra is the axis, unlike the atlas, as it contains a large vertebral body from which a large, tooth-shaped bony protrusion arises. The atlas with its upper articular sides, convex upward and outward. As for the lower part of the cervical spine, it consists of the remaining five cervical vertebrae which are very similar to each other in most anatomical features, as it has a very small vertebral body whose posterior height is greater than the front part is concave on the upper side and convex from the bottom and has non-defined bony protrusions on the lateral edges. It articulates at two joints called Luschka that add to it more stability and prevent spinal slippage [4]. The cervical vertebrae have many characteristics that distinguish them from the thoracic and lumbar vertebrae, the most prominent of which is that the spinous process of the cervical vertebrae is cleft, which increases the surface area for muscle binding [17]. Another feature is that the presence of one transversal opening in each transverse protrusion surrounding the arterioles and vertebral veins with the exception of the seventh cervical vertebra [31].
The cress plant is a fast-growing, herbaceous annual plant that bears lobed, full, square or compound leaves [25]. It belongs to the bassicaceae family known scientifically as *Lepidium sativum*, it reaches a length of up to 50 cm. It grows spontaneously on the edge of rivers and lakes [9]. Its flowers are small white; its seeds are the main part of it. Triangular oval in shape and tapered at one end, soft to the touch, about 3-4 mm long and 1-2 mm wide [26]. It is a rich source of protein, dietary fiber, omega-3 fatty acids, iron and other essential nutrients and phytochemicals [28]. This plant is widely used in folk medicine to treat highly active airway disorders such as asthma [23], bronchitis and cough, and is also used as a good mediator for healing and healing fractures in the skeleton [29]., cress seed extract is an anti-bacterial diuretic, an expectorant, an antibacterial and a stimulant for the digestive system, and it can also act as an anti-rheumatic [5]. Each serving of raw cress seeds contains 32 calories, 2.3mg of protein, 4.4mg of sugar, 0.7mg of fat and 1.1mg of fiber and it does not contain cholesterol but when consuming large amounts [30], it prevents iodine from being absorbed in the thyroid gland because it contains giotrogens. Thus, it leads to hypothyroidism, and it may also cause digestive problems in some people who have sensitivity towards it [21].

2. Methodology

1. Preparing the Cold Aqueous *L.sativum* Seeds Extract

The preparation of cold aqueous *L.sativum* seeds extract was according to the method of [6].

2. Animals for the Experience

In this study, male albino rats of the Sprague dawley type were used. The number of 40 rats were purchased from the Faculty of Science / University of Kufa at the age of one month and the weight ranges between 200-250 g), then they were transferred to the animal house of the College of Education for Girls, University of Kufa and it was divided into 4 groups of each group included 10 rats for the purpose of the study The animals were placed in aluminum cages, suitable for raising animals. The floor of the cages was covered with Sawdust, to be replaced twice a week with (5) rats in each cage with the availability of appropriate laboratory conditions for them from The temperature was (25 ° C) regulated by the air conditioner and the average daily lighting of the room was 13 hours of light and 11 hours of darkness [7]. The animals were given water and animal feed intended for them (concentrated feed of wheat flour, soybeans, corn, and animal protein). Free during the study
period and the animals were left for a week for the purpose of acclimatization. Before starting the experiment, the laboratory animals were examined by a specialized veterinarian to ensure their safety from disease.

3. Experimental Groups of the Study

The first group / included 10 white males were divided into 5 males who were sacrificed after 30 days and 5 others were sacrificed after 45 days after the experiment. This group was considered the control group. The second group / included 10 males who were dosed orally with Cress extract, concentration 50, from the first day until the end of the experiment. The third group / included 10 males who were dosed orally with cress extract, concentration of 100, from the first day until the end of the experiment. The fourth group / also included 10 males who were dosed orally with cress extract, concentration of 150, from the first day until the end of the experiment.

4. Animal Sacrifice and Extraction of Vertebrae

At the beginning of the experiment, the weights of the animals were taken for all the treatments, and after the end of the 30-day period, 24 hours after the last oral dose, half of the animals (white rats) from each treatment were sacrificed (5) rats. As for the rest of the animals, they were also sacrificed after the end of the period. After the two periods, the animals were weighed and numbed with diethyl ether (Ether). The anesthetized animals were placed on a dissection plate (cork) and fixed with staples. Then the dissection process began with special dissection tools by making an incision from the dorsal side to directly access the spine, after extracting the spine. It was cut into four areas, then the cervical region was taken and placed in NAOH sodium hydroxide for 24 hours, after which it was placed in boiling water for a few minutes with monitoring in order to get the vertebrae free of muscles, ligaments and cartilage after which each vertebra was weighed and its lengths were measured (length, width and Qatar) using a digital measuring tool (Vernier).

5. The Statistical Analysis of the Study

The results of the laboratory work were subjected to microscopic examination using the ANOVA11 test in order to extract the arithmetic mean of the primary data and the standard deviation,
after which a one-way ANOVA analysis was adopted to compare the rates of calibration between groups and within each group over time and LSD was the least significant difference [32].

3. Results

The results of the current study revealed a significant increment (P< 0.01) in the weight of the Atlas, Axis and Cervical vertebrae in the treatment group with the aqueous extract of *Lepidium sativium* seeds concentration 150 in comparable to the control group and other experimental groups. Also, the results of the current study revealed a significant increment (P< 0.01) in the lengths (length, width and diameter) of the three vertebrae (Atlas, Axis and cervical vertebrae) vertebrae in the treatment group with the aqueous extract of *Lepidium sativium* seeds concentration 150 in comparable to the control group.

Table 1 - Effect of *L.sativium* Seeds Extract on the Weigh of the Cervical Vertebrae

| Groups of Study         | Atlas vertebra M ± SD | Axis vertebra M ± SD | Cervical vertebra M ± SD |
|-------------------------|-----------------------|----------------------|--------------------------|
|                         | 30 day | 45 day | 30 day | 45 day | 30 day | 45 day |
| Control group           |         |        |        |        |        |        |
| 0.265±0.004             | 0.274±0.003          | 0.244±0.003          | 0.254±0.003          | 0.21±0.002 | 0.216±0.004 |
| *L.sativium* extract group (50) mg/kg | 0.343±0.002          | 0.336±0.003          | 0.334±0.003          | 0.324±0.002 | 0.256±0.003 | 0.265±0.002 |
| *L.sativium* extract group (100) mg/kg | 0.355±0.002          | 0.342±0.004          | 0.363±0.003          | 0.334±0.003 | 0.275±0.002 | 0.284±0.002 |
| *L.sativium* extract group (150) mg/kg | *0.384±0.003         | *0.385±0.002         | *0.373±0.002         | *0.374±0.002 | *0.293±0.002 | *0.294±0.002 |
| LSD P < 0.05            | 0.108          | 0.102          | 0.111          | 0.109          | 0.062          | 0.066          |
### Table 2 - Effect of L. Sativium Seeds Extract on the Length of the Cervical Vertebrae

| Groups of Study | Atlas vertebra M ± SD | Axis vertebra M ± SD | Cervical vertebra M ± SD |
|-----------------|-----------------------|----------------------|--------------------------|
|                 | 30 day | 45 day | 30 day | 45 day | 30 day | 45 day |
| Control group   | 6.64±0.01 6 | 6.74±0.01 9 | 9.33±0.02 2 | 9.44±0.02 2 | 5.64±0.00 2 | 5.75±0.02 7 |
| L. sativium extract group (50) mg/ kg | 7.96±0.02 6 | 8.15±0.03 2 | 9.64±0.02 22 | 9.86±0.020 | 6.82±0.020 | 6.93±0.023 |
| L. sativium extract group (100) mg/ kg | 8.31±0.02 6 | 8.52±0.01 6 | 9.74±0.011 22 | 9.93±0.023 | 6.84±0.020 | 7.08±0.017 |
| L. sativium extract group (150) mg/ kg | *8.33±0.027* | *10.06±0.031* | *9.84±0.031* | *10.15±0.027* | *7.95±0.028* | *8.05±0.029* |
| LSD P < 0.05   | 1.5 | 2.7 | 0.44 | 0.67 | 1.33 | 1.22 |

### Table 3 - Effect of L. Sativium Seeds Extract on the Width of the Cervical Vertebrae

| Groups of Study | Atlas vertebra M ± SD | Axis vertebra M ± SD | Cervical vertebra M ± SD |
|-----------------|-----------------------|----------------------|--------------------------|
|                 | 30 day | 45 day | 30 day | 45 day | 30 day | 45 day |
| Control group   | 9.45±0.02 2 | 9.54±0.016 7 | 7.45±0.02 30 | 7.53±0.01 48 | 6.64±0.00 1 | 6.72±0.00 48 |
| L. sativium extract group (50) mg/ kg | 10.26±0.02 7 | 11.53±0.02 4 | 7.63±0.016 24 | 7.85±0.024 | 7.08±0.034 | 7.21±0.074 |
| L. sativium extract group (100) mg/ kg | 10.33±0.023 4 | 12.54±0.02 4 | 7.67±0.019 24 | 7.95±0.024 | 7.15±0.032 | 7.25±0.032 |
| L. sativium extract group (150) mg/ kg | *10.54±0.024* | *12.74±0.026* | *7.82±0.0016* | *8.13±0.021* | *7.25±0.024* | *7.46±0.026* |
| LSD P < 0.05   | 0.9 | 2.8 | 0.31 | 0.54 | 0.55 | 0.63 |
Table 4 - Effect of L. Sativium Seeds Extract on the Dimeter of the Cervical Vertebrae

| Groups of Study | Atlas vertebra M ± SD | Axis vertebra M ± SD | Cervical vertebra M ± SD |
|-----------------|-----------------------|----------------------|--------------------------|
|                 | 30 day | 45 day | 30 day | 45 day | 30 day | 45 day |
| Control group   | 4.64±0.01 | 4.56±0.056 | 3.25±0.02 | 3.34±0.029 | 3.55±0.03 | 3.63±0.027 |
| L. sativium extract group (50) mg/kg | 4.79±0.021 | 4.96±0.036 | 3.67±0.031 | 3.76±0.025 | 3.92±0.022 | 4.05±0.013 |
| L. sativium extract group (100) mg/kg | 4.82±0.026 | 5.26±0.027 | 3.72±0.034 | 4.12±0.024 | 3.95±0.021 | 4.20±0.011 |
| L. sativium extract group (150) mg/kg | *4.85±0.028 | *5.44±0.021 | *3.94±0.016 | *3.96±0.018 | *4.15±0.031 | *4.37±0.013 |
| LSD P < 0.05    | 0.15 | 0.77 | 0.45 | 0.55 | 0.54 | 0.63 |

Picture 1 - Shows the Atlas Vertebra in the Control Group
Picture 2 - Shows the Axis Vertebra in the Control Group

Picture 3 - Shows the Cervical Vertebra in the Control Group

Picture 4 - Shows the Atlas Vertebra Treated with *L. Sativium* Extract for 30 Day
Picture 5 - Shows the Axis Vertebra Treated with *L. Sativium* Extract for 30 Day

Picture 6 - Shows the Cervical Vertebra Treated with *L. Sativium* Extract for 30 Day

Picture 7 - Shows the Atlas Vertebra Treated with *L. Sativium* Extract for 45 Day
4. Discussion

The dosage with cold aqueous extract of *L. sativum* seeds caused a significant raise in the weight of the cervical vertebrae, this result is consistent with findings [27], which showed that there was a significant increase in bone weights when consuming *Pomegranate* peel extract at concentration of 50 mg/ kg of body weight for a period of 90 days and once a day, as it was proved that the *Pomegranate peel* extract improves the bone mass and it prevents the mineral density from being too low and also leads to reduction in osteoclasts and resorption. Also, The current study agreed with [19], which confirmed a significant increase in bone mass in mice fed with grape seed extract for a period of 6 weeks. The study also agreed with [24], who confirmed the presence of a significant increase in the weight of the bones of mice treated with *Lycium Chinese root* extract at a
concentration of 10 and 50 mg/kg for a period of 8 weeks, where it was observed that the bone mineral density was significantly higher compared to the control group. The reason is that the extract prevents loss of mineral density by promoting differentiation and proliferation of osteoblasts. The reason for the increase in bone weight and density may be due to the content of cress seeds rich in calcium known to be an important nutrient that promotes bone mineralization and prevents resorption and thus increase their density and the ability of these seeds to increase serum, alpha-linolenic acid in the liver, docosahexaenoic acid and eicosapentaenoic acid, which have been shown to have beneficial effects on Bones [8]. Or perhaps its seeds contain high amounts of vitamin C, which is known to be an essential catalyst for collagen formation, the important component in the base material of bone, as it reduces oxidative stress and resorption of the bones, increases their density and protects them from inflammation and later fractures [2]. Also, the results of the current study showed a significant increase in the dimensions (length, width, and diameter) of the cervical vertebrae in the group treated with the cold aqueous extract of cress seeds Lepidium sativum seeds extract, at a concentration of 150 mg/kg, at periods of 30 and 45 days, compared to the control group, and this is consistent with the study [16].

5. Conclusion

The eqouse extract of L.sativium seeds showed a significant effect on cervical vertebra, due to the preventive and anti-oxidative potentialities of its chemical constituents, the study recommend to use the extract of L. sativium as a dietary complement or may be a natural remedy.

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