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PHARMACOLOGICAL ACTIVITY OF ETHANOLIC EXTRACT *LEPIDIUM SATIVUM* LINN. SEEDS ON THYROID HORMONES IN MALE RATS

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ABSTRACT: Pharmacological activities have been reported for the seed extracts of *Lepidium sativum* L.; however, the plant has not been widely studied for its effects on the thyroid gland. **Objective:** The present study was designed to investigate to possible potential pharmacological activity of ethanolic extract of *Lepidium sativum* L. seeds on regulation of thyroid hormone concentrations in male rats. **Methodology:** Each experiment was performed on Male Wistar rats, which were randomly divided into 4 groups. Group I received normal saline for 21 days and served as normal control. Group II received extract seeds of *L. sativum* at a dose of 200 mg/kg i.p. for 21 days. Groups III was treated with a 25 µg/100 g intraperitoneal (i.p.) T_3 injection for 21 days and; Group IV was treated iopanoic acid (IOP; 100 mg/kg) for 21 days. **Results:** The levels of the rats' serum Triiodothyronin (T_3), Thyroxin (T_4) and Thyroid-stimulating hormone (TSH) were analyzed. Thyroid gland specimens were processed for microscopic examination. Group II treated with extract seeds of *L. sativum* showed significant decline T_3 and T_4 levels together with significant increase in TSH concentrations as compared to control group. Histologically, this group showed congestion and dilatation of blood capillaries, cellular infiltration, follicular disintegration and vacuolar degeneration of some follicular cells. **Conclusion:** From the data, it is conformed that administration of *L. sativum* seed extract decreases the levels of the thyroid hormones indicating its thyroid inhibitory nature similar to other plants.

INTRODUCTION: Higher plants are the main source of medicine throughout the human history. Amultitude of plant species are still widely used for the traditional as well as modern systems of medicine. As per the statistic of WHO, upto 70% of population living in developing countries depend on plants for primary health care and 25% of the prescriptions in modern medicine got ingredients of plant origin.

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Garden cress (*Lepidium sativum* Linn., family - Cruciferae), a fast growing, perennial edible herb, is a native to Egypt and West Asia but widely cultivated in temperate climates throughout the world [1]. Chemically, the seeds comprise 33 - 54% of carbohydrate, 22 - 25% of protein, 14 - 27% of lipids and 8% of crude fibre [2] with good amount of calories (454 kcal/100 g).

It is a good source of thiamine, riboflavin and niacin [3]. It all so contains essential fatty acids like arachidic and linoleic acid and alpha linolenic acid, which acts as memory boosters [4]. It is a good source of calcium and magnesium which helps in normal contraction of muscle for healthy movements of limbs and heart. Iron content [3] in the seed powder often helps to cure mild anemic

conditions, especially in children. Phosphorus is needed for general healthy metabolic activities of the body. It also contains antioxidants such as tocopherols and carotenoids [5].

L. sativum is reported to exhibit antihypertensive [6], diuretic [7], anti-inflammatory, analgesic, anti-coagulant [8], antirheumatic [9], hypoglycemic [10], laxative, prokinetic [11], antidiarrheal, and anti-spasmodic [12, 13] properties. It has been shown to possess antiasthmatic [14] and bronchodilatory [15] potential in preliminary studies, but there is no report available in the literature on the pharma-cological basis for its medicinal use in endocrine disorders. However, till to date nothing is known on its thyroid regulatory ability, if any. There is no previous report of the biologically activity of the *L. sativum* extract on the thyroid gland from literature review. Therefore, it was necessary to undertake this investigation. Thyroid gland is one of the most important endocrine organs and almost all cells of the body are target sites for its hormones. The thyroid gland secretes thyroxine (T_4) and tri-iodothyronine (T_3), which are needed for proper growth and development and which are primarily responsible for determining the basal metabolic rate. The thyroid hormones are transported through the blood and act at the cellular level.

Thyroid disease is one of the commonest endocrine disorders worldwide. According to a recent projection from various studies, it has been estimated that about 6 million people in Italy suffer from thyroid diseases. About 1 to 2% of the adult population is known to suffer from thyroid disorders. The need to combat this dysfunction has risen in recent years due to its increasing prevalence. Hormone replacement has been the choice of therapy. However, alternative medicinal approaches are gaining popularity in view of their efficacy with minimal side effects. This study throws light the role of *Lepidium sativum* on thyroid and its functioning and also on the various factors associated with thyroid dysfunction.

Experimental Section:

Preparation of the Plant Extract: Dried seeds of *Lepidium sativum* Linn. were collected locally and authenticated by the Botanist Professor Carmine Guarino of University of Sannio, Department of Science and Technology and were deposited in the departmental herbarium of the same University which were allotted voucher no. RS: 321.

The collected dried seeds were washed and pulverized with electric grinder for size reduction. The powdered seeds (10 g) were extracted by Soxhlet apparatus using ethyl alcohol (100 ml) as a solvent for 8 h. The extract was filtered off using sterile filter papers (Whatman no. 1) into a clean conical flask. The filtered extract was transferred in a rotator evaporator under reduced pressure. The performance of extract was 15% w/w.

Animals: Male Wistar rats (Charles River, Lecco, Italy) having weight of 200 - 250 g (5 - 6 months old) were acclimated for 7 days prior the experiments. They were maintained at 25 ± 2 °C and relative humidity of 45 - 55% under photoperiodic condition (12 h light: 12 h dark cycle) in large and individual cages. The animals had free access to food and water *ad libitum*. The experimental was carried out in accordance to the guidelines of Institutional Animal Ethical Committee approved the protocol.

Experimental Design: The acute oral toxicity study of ethanolic extract of *Lepidium sativum* Linn. seeds was not conducted at 2000 mg/kg b.w. on Male Wistar rats because using the limit test procedure according to OECD test guidelines on acute oral toxicity test 401 (OECD-testing guideline, 401, 1981). One tenth dose of 2000 mg/kg was selected regarding toxicity study. Animals were arbitrarily divided into four groups of six animals each.

Group I received normal saline (1 ml/kg, p.o.) for 21 days and served as normal control. Group II received extract seeds of *L. sativum* at a dose of 200 mg/kg p.o for 21 days. Groups III received a 25 µg/100 g intraperitoneal (i.p.) T_3 injection for 21 days. To achieve minimum possible circulating thyroid hormone levels, iopanoic acid (IOP; 100 mg/kg) was injected for 21 days to inhibit deiodinase activity (Group IV).

All the treatments were given between 9.00 and 10.00 h of the day to avoid circadian variation. Twenty - four after the last dose, animals were sacrificed by cervical dislocation. Blood was collected and serum samples were stored at -80 °C until assayed for biochemical analysis.

Hormone Assay: Plasma levels of T_3 and T_4 were determined by radioimmunoassay (RIA). In the T_3 assay, a measured amount of sample serum and standards was added to a tube coated with anti-T_3 rabbit antibody, with a trace (4.4 (Ci) amount of radioactively labeled T_3 ([^{125}I] T_3) (Byk-Sangtec Diagnostica, Dietzenbach, Germany) and an agent-blocking Tris-buffered saline 4 mM, ANS (8-anilino-1-naphthalenesulfonic acid) 6 mM sodium salicylate with 0.2% sodium azide as a preservative (Sigma Chemical Co., St. Louis, MO) to release T_3 from serum-binding proteins. Sensitivity was 0.1 ng/mL with an accuracy of about 97%. The range of intra assay variance in 20 assays was 1.0 - 2.6%, whereas the inter assay variance ranged between 3.9% and 5.7% in 12 assays.

For T_4, a measured amount of sample serum and standards was added to a tube coated with anti-T_4 rabbit antibody, along with a trace amount of radioactively labeled T_4 ([^{125}I] T_4), 4.4 (Ci, (Byk-Sangtec Diagnostica, Dietzenbach, Germany) and a blocking agent, Tris-buffered saline 4 mM, ANS 6 mM sodium salicylate with 0.2 % sodium azide as a preservative (Sigma Chemical Co. St. Louis, MO) to release T_4 from serum - binding proteins. Sensitivity was 0.45 ng/mL, with accuracy close to 100%; the mean intra-assay and inter assay coefficients of variance were 4.6% and 4.3%, respectively. A logit - log curve fit using a %B/Bo calculation was used. T_4 and T_3 concentrations were determined by computing the % B/Bo for each sample and then finding the results on the standard curve. Cross-reactivity for T_4 in the T_3 RIA (1.3%) was not considered for data calculations, neither was that for T_3 in the T_4 RIA (0.1%).

Plasma TSH was determined by immune radio metric assay (IRMA). In the TSH procedure, sample serum and standards were added to anti ligand-coated tubes. The Tracer / Capture Reagent, a blend of ligand-tagged TSH specific antibody and ^{125}I-labeled TSH (10 µCi), was added to each tube. A cubic spline function with the zero standard as one of the standard points was used for calculations. The minimum detectable dose (MDD) was 0.01 µIU/mL, with a accuracy close to 100%, and the mean intra assay and inter assay coefficients of variance were 5.0% and 7.5%, respectively.

Histopathological Studies: Thyroid tissues were removed instantly, sliced and washed in saline. For light microscopy, each thyroid lobe was fixed in 3.7% phosphate - buffered formalin (pH = 7.2), dehydrated through an ethanol series and xylol and embedded in paraffin. For general histological analysis, as well as for stereological measurements, 5 µm thick paraffin sections stained with hema-toxylin/eosin method, taken from the anterior, medial and posterior part of the thyroid lobe (five non-serial sections per each chosen part of a lobe), were analyzed on Leica DMLB light microscope (Wetzlar, Germany). The height of the follicular cells was measured in 30 cells every 3 slides, always on the second section of normal and treated specimens using a digital system of imaging (KS 300).

Statistical Data Analysis: Results are expressed as mean ± SEM. The obtained data have been averaged prior to calculating experimental group mean and the standard error of the mean. As revealed by the X^2 test, data was not different from normal distribution. The control and experimental data of all the groups were tested together for significance using two-way ANOVA, followed by Duncan's test for multi-group comparison and Student's t-test for between group comparison using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION:

The Effect of *L. sativum* Seeds Extract on Serum Thyroid Hormone Levels: The obtained results demonstrate that *L. sativum* extract is inhibitory to thyroid function. Therefore, in *L. sativum* treated animals, both T_4 and T_3 decreased significantly in comparison to control rats ($P<0.05$) **Table 1**. The inhibition of T_3 and T_4 was about 53 and 47%, respectively, indicating that this plant extract may be very effective in the reduction of thyroid hormone concentrations which is comparable to that of a standard drug as iopanoic acid (IOP). Thyroid hormones were also decreased in PTU and increase in T_3 treated groups ($P<0.05$) **Table 1**.

Our obtained data demonstrated that the plant extract might be inhibitory the synthesis and release of T_4 directly at glandular levels and indirectly through the peripheral (hepatic)

conversion of T_4 to T_3. Therefore, these results could be attributed to two mechanisms, directly on the thyroid gland and indirectly on peripheral tissues. This inhibitory role following the administration of *L. sativum* to thyroid function is similar to that of some other plant extracts as *Agle marmelos* Linn. and *Aloe vera* L. [15]

TABLE 1: EFFECT OF LEPIDIUM ETHANOLIC SEEDS EXTRACTS ON SERUM CONCENTRATIONS OF T_3 AND T_4 (ng/ml) ALONG WITH THEIR PERCENT INCREASE (+) OR DECREASE (-) IN RELATION TO THE CONTROL VALUES. EFFECT OF IOP AND T_3 HAS ALSO BEEN PRESENTED

Groups	Dose	Thyroid Hormones			
		T_3 (ng/ml)	T_4 (ng/ml)	% changes in T_3 conc.	% changes in T_4 conc.
Control		1.32±0.15	46.02±2.1		
L. sativum	200 mg/kg	0.61±0.05	24.23±1.5	-53.78	- 47.34
T_3	25 µg/g	2.78±0.12	123.01±9.32	+110.60	+167.29
IOP	100 mg/kg	0.56±0.03	19.21±2.1	-57.57	-58.25

Each value is the mean ± SEM (n = 10)

Interestingly, on the other hand, the level of TSH of same rats showed a highly significant increase ($p<0.05$) as compared with control rats **Table 2**. This finding might result from acute effects of *L. sativum* extract. Serum levels of T_3, T_4, and TSH were generally used as reliable indicators of the thyroid function in both humans and experimental animals. All reactions necessary for the formation of T_3 and T_4 are influenced and controlled by TSH level. However, our data suggested that elevated serum level of TSH occurred due to active feedback mechanism. When the serum levels of T_3 and T_4 were decreased due to the *L. sativum* extract effects on the thyroid gland, the anterior pituitary gland released more TSH in the serum to stimulate the thyroid gland to produce more T_3 and T_4.

TABLE 2: EFFECT OF LEPIDIUM ETHANOLIC SEEDS EXTRACTS ON SERUM CONCENTRATIONS OF PITUITARY HORMONE TSH (µIU/mL) ALONG WITH ITS PERCENT INCREASE (+) OR DECREASE (-) IN RELATION TO THE CONTROL VALUES. EFFECT OF IOP AND T_3 HAVE ALSO BEEN PRESENTED

Groups	Dose	TSH (µIU/mL)	% changes in TSH conc.
Control		2.6±0.02	
L. sativum	200 mg/kg	5.2±0.05	+100
T_3	25 µg/g	1.1±0.04	- 57.69
IOP	100 mg/kg	5.4±0.07	+107.69

The Effect of *Lepidium Sativum* Seeds Extract on Histology of the Thyroid Glands:

Group I: The thyroid follicles were covered by a cuboidal epithelium, whose cells showed round or ovoid nuclei with sparsely distributed chromatin and evident nucleolus. The cytoplasm showed some vacuolization and intense acidophilia. The thyroid follicles were filled with colloid showing a characteristic acidophilia. Groups of inter follicular cells were observed. The stroma showed some fibroblasts, few collagen fibers and a great number of blood vessels. Additionally, para follicular cells (C-cells) were observed in some follicles resting on the basement membrane and had large pale nuclei. Vascular connective tissue septa were seen between the follicles **Fig. 1a**.

Group II: The gland in the group treated with seeds extract of *L. sativum* showed disintegration and disorganization of thyroid follicles. Some follicles appeared with interrupted follicular wall. Congested blood vessels were seen in C.T septa **Fig. 1b**. Many collagen fibers were detected in the connective tissue septa. Additionally, some follicular cells had darkly stained nuclei and inter follicular cellular infilteration. Desquamated epithelial follicular cells in the lumen are also seen.

Group III: In T_4-treated groups **Fig. 1c**, thyroid parenchyma appeared less well ordered. In both treated groups, thyroid follicles were enlarged and seemed distended due to colloid accumulation. Follicles were lined mostly with flattened thyrocytes containing oval nuclei with increased chromatin density.

Large follicles of irregular shape suggesting disruption of follicular walls and fusion of neighboring follicles, as well as an inter follicular barrier consisting of a single row of thyrocytes, were also apparent. Inside the lumen of some irregular follicles desquamated thyrocytes were noticed. The amount of inter follicular connective tissue was reduced, and capillaries were collapsed.

Group IV: In the animals treated with propyl-thyouracil (PTU), most of the follicles were covered by a columnar epithelium whose cells had round or eliptic nuclei. The chromatin was sparsely distributed and the nucleoli were evident. The cytoplasm was sometimes vacuolized and showed a slight acidophilia. The number of follicles was increased, showing an intensive hyperplasia of the epithelial lining. There was a decrease in the follicles diameter, and in some of them it was not possible to see their lumen. The colloid was absent or decreased but lightly basophilic when it was present. Mitotic figures were present in the epithelial lining of the follicles. The stroma was reduced and few fibroblasts, together with a great number of congested blood vessels were seen **Fig. 1d**. Data about the height of follicular epithelium after treatments are shown in **Table 3**.

TABLE 3: EFFECT OF ETHANOLIC SEEDS EXTRACTS OF *L. SATIVUM* ON EPITHELIUM HEIGHT OF THE FOLLICULAR CELLS OF THE THYROID GLAND ALONG WITH THEIR PERCENT INCREASE (+) OR DECREASE (-) IN RELATION TO THE CONTROL VALUES. EFFECT OF IOP AND T_3 HAVE ALSO BEEN PRESENTED

Groups	Dose	Height of follicular epithelium (µm)	% changes in Height of follicular epithelium (µm)
Control		8.3±0.15	
L. sativum	200 mg/kg	1.61±0.05	-80.60
T_3	25 µg/g	12.8±0.12	+54.21
IOP	100 mg/kg	1.56±0.03	-81.20

Each value is the mean ± SEM (n=10)

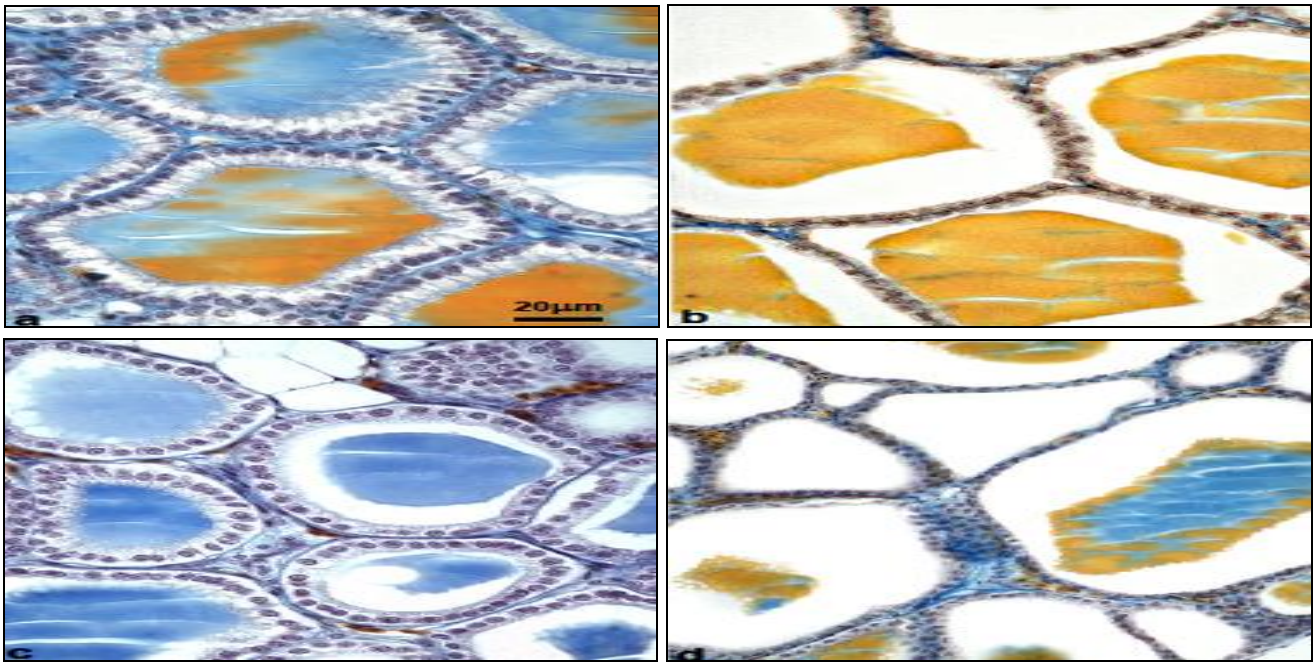

FIG. 1: THYROID GLAND CONTROL GROUP (I): 1A: H AND E STAINED SECTION SHOWING VARIABLE SIZED FOLLICLES. CENTRAL FOLLICLES ARE LINED BY SINGLE LAYER OF CUBOIDAL CELLS WITH ROUNDED NUCLEI WHILE THE PERIPHERAL FOLLICLES HAVE FLATTENED OR LOW CUBOIDAL CELLS. THEIR LUMINA ARE FILLED WITH HOMOGENOUS ACIDOPHILIC STRUCTURELESS COLLOID. IT SHOWS CONSPICUOUS PERIPHERAL VACUOLES. IN EXPOSED GROUP OF SEEDS EXTRACTS (II) 1B: H AND E STRAINED SECTIONS SHOWING DISINTEGRATION AND DISORGANIZATION OF THYROID FOLLICLES WITH INTERRUPTED FOLLICULAR WALL. T_3 - TREATED GROUP (III) 1C: H AND R STRAINED SECTION REVEAL MANY RECOVERED THYROID FOLLICLES. THESE FOLLICLES ARE LINED BY CUBOIDAL CELLS WITH ROUNDED NUCLEI. NOTE, RETURN OF PERIPHERAL VACUOLATION IN THE COLLOID. IOP-TREATED GROUP (IV) 1D: H AND R STRAINED SECTIONS SHOWING SOME FOLLICLES APPEAR DISTENDED WITH COLLOID AND LINED BY FLAT CELLS (SCALE BAR = 20 µm)

CONCLUSION: From the results, it is demonstrated that administration of *L. sativum* seed extract decreases the levels of both the thyroid hormones indicating its thyroid inhibitory nature similar to other plants [16]. In the present work, alterations in thyroid gland function in the exposed group were further confirmed by the histological examination of the thyroid follicles which showed apparent light microscopic and structural changes. Many of these follicles underwent disintegration and disorganization. Some follicles appeared involuted and the others had interrupted follicular

wall. The follicular cells had vacuolated cytoplasm and others had shedding of their epithelial lining in the lumina. Additionally, some follicular cells had darkly stained nuclei and cellular infiltration in interfollicular septa.

The histological alterations of thyroid follicles could be explained by deficiency of stimulatory effects of TSH on the gland. The alterations in epithelial height were confirmed by morpho-metrical results. In conclusion, the thyroid gland is sensitive to *L. sativum* exposure. This exposure induced morphological changes with drop off in serum T_4 and T_3. These changes remained to the end of the experiment indicating that a longer period of time is required. However, this extract may a better choice for mild hyperthyroidism cases, as it does not exhibit any toxic effect, rather is hepato-protective in nature. Therefore, further studies are recommended before human therapy.

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