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# Physiological Impacts of Cauliflower in Hyperthyroid Rabbits

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**ABSTRACT**

**This study aimed to investigate the effect of cauliflower extract as a remedy for hyperthyroid in rabbits. Twenty rabbits with mean weight 1700 ±95 g and age of 6-7 months were chosen. The animals were randomly divided into four groups (five in each group). The first group (GI) was the control and received normal diet and given tap water. The second and third groups (GII & GIII) were received 30 µg/Kg BW sodium L-Thyroxin injected subcutaneously to induce hyperthyroidism for three weeks. After that, these groups (GII & GIII) were administrated 20 & 30 mg/kg of body weight cauliflower extract for four weeks. Blood samples were collected from hyperthyroid and treated groups after two and four weeks. Serum was centrifuged at 3000 rpm for 15 minutes. The collected serum was used in the assessment of the levels of thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormones (TSH) by analytical kits which determined on ELISA method. Further, a molecular study was performed to estimate TSHR gene expression. The results of the present work showed that there was a significant ($p<0.05$) decrease in T3 and T4 hormone levels means in rabbits treated with cauliflower extract as compared with hyperthyroid. It concluded from the present study that the natural products such as phenolic compounds, carotenoids, flavonoid, and ascorbic acid from cauliflower might be used as a remedy to recover from hyperthyroid states in order to reduce the intake of chemical drugs.**

***Keywords-*** Cauliflower; hyperthyroid; TSH; T3; T4, TSHR, gene expression.

## I. INTRODUCTION

Plant-based foods such as fruit, vegetables, and whole grains, which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the risk of chronic diseases. Epidemiological evidence suggests that consumption of a diet rich in vegetables and fruits has positive implications for human health [1].

Cruciferous (brassica) vegetables are very common foods, especially in plant-based diets. They contain many healthy nutrients, including Phyto-chemicals with anticarcinogenic, antioxidative and anti-inflammatory activity. However, they also contain goitrogens such as progoitrin and thiocyanate-producing indole glucosinolates, which may interfere with thyroid hormone production or utilization [2]. The most widely used cruciferous vegetables belong to the genus Brassica and include broccoli, Brussels sprouts, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard, rutabaga and turnip. When these cruciferous are digested by intestinal bacteria, they release the goitrogens that increase the need for iodine when consumed in small amounts and can damage the thyroid gland when consumed in large amounts [3].

Thyroid is one of the principal glands in the body's endocrine system [3]. Two thyroid hormones, thyroxine (T4) and triiodothyronine (T3) are involved in the regulation of myriad of body functions including cellular metabolism, oxygen consumption, nerve conduction and reproduction [4, 5]. In addition, thyrotropes in the anterior pituitary produce Thyrotropin (Thyroid stimulating hormone (TSH)) to maintain the appropriate level of thyroid hormones (T3 and T4) [5].

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Thyroid diseases including hypothyroidism, hyperthyroidism, and thyroid nodules which in many developing countries constitute a growing health problem [4, 5, 6]. Thyroid hormones are kept within normal levels in the body via the endocrine negative feedback mechanism [5]. Alterations in the normal levels of these hormones usually lead to physiological/clinical abnormalities, such as hypothyroidism and hyperthyroidism [3]. Hyperthyroidism is present in up to 1.3% of the general population. Graves' disease is the leading cause of hyperthyroidism. Conventional therapies for the treatment of hyperthyroidism are anti-thyroid medications, radioactive iodine treatment, and thyroid surgery, each with their respective potential risks and benefits [6]. Further, these hormones have a considerable impact on oxidative stress, However, changes in their levels could alter redox environment, via causing changes in the number and activity of mitochondrial respiratory chain components resulting in increased generation of reactive oxygen species (ROS) which are often attenuated by antioxidants [5, 6]. In hyperthyroidism and hypothyroidism, disturbance of oxidant/antioxidant balance leads to cellular damage, severe metabolic dysfunctions, and damage to biological macromolecules such as proteins, lipids and DNA [3].

From above, the present work aimed to investigate the molecular and physiological effects of cauliflower extract as a remedy of hyperthyroid in rabbits.

## II. MATERIALS AND METHODS

***Preparation of Aqueous Extract of cauliflower***

The cauliflower plant was taken and washed carefully with tap water and next with distilled water. The flower was then cut into small pieces and soaked in distilled water (1:4) along the night at 6ºC. Samples were then brought into a homogenizer and the resulting homogenates which filtered with fine filter paper. The crude fluids were centrifuged at 25,000 x g for 30 min to remove any waste material. The supernatants were further filtered through two Millipore filters (0.45 and 0.22 μM). The filtrates were lyophilized and the products were stored in tight flasks at -80º C until used [7].

***Experimental Design***

This experiment was performed on 20 male rabbits weighing 1700±95 g, with an age of 6-7 months. These rabbits were obtained from Central Health Laboratories in Baghdad City, Iraq. They were kept in Animal House for one week and given a standard diet (pellets). The temperature in the Animal House was kept at 22±2°C with a relative humidity of 53±4%. Light period was about 12 hours light: 12 hours dark. The animals were randomly divided into four groups (five in each group). The first group (GI) was the control and received normal diet and given tap water. The second and third groups (GII & GIII) were received 30 μg/Kg BW Thyroxin injected subcutaneously to induce hyperthyroidism for three weeks. After that, the groups (GII & GIII) were administrated 20 &30 mg/kg, respectively of body weight cauliflower extract for four weeks [8, 7].

Blood samples were collected from hyperthyroid and treated groups after two and four weeks. Blood was divided into two parts; one for hormonal study and the other part for molecular study. Serum was centrifuged at 3000 rpm for 10 minutes. Serum was kept at -20°C and used in the determination the levels of thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormones (TSH) by chemical kits which determined on ELISA method [9,10].

***Statistical Analysis***

Results were analyzed statistically with analysis of variance test (ANOVA). Duncan test was used to determine the significantly among means with the aid the program of SPSS (ver. 17) at probability level $p < 0.05$ [11].

## III. ETHICAL CLEARANCE

This study was carried out as per ethical standards of Department of Biology, College of Science, University of Baghdad. The animal ethical clearance number for this study was 346 at 23/5/2020.

## IV. RESULTS AND DISCUSSION

The results of the present study illustrated a significant ($p<0.05$) decline in T3 and T4 levels means in rabbits treated with cauliflower extract at 20 mg/ kg body weight in comparison with hyperthyroid. Levels of T3 were 46.65 ± 2.33, 63.26 ± 2.32 and 42.54 ± 1.89 ng/dl in cauliflower extract, hyperthyroid and control rabbits, respectively. While the levels of T4 were 3.12 ± 0.23, 4.23 ± 0.36 and 1.88 ± 0.21 ng/mL in cauliflower extract, hyperthyroid and control rabbits, respectively. Concerning the levels of TSH hormone, the results demonstrated that a significant ($p<0.05$) increase in TSH hormone levels means in treated rabbits as compared with hyperthyroid. Levels of TSH hormone were 6.18 ± 0.72, 4.08 ± 0.29 and 8.02 ± 0.31 ng/mL in cauliflower extract, hyperthyroid and control rabbits, respectively. (Table 1).

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**Table 1: Thyroid hormones in hyperthyroid rabbits before and after treatment with 20 mg/ kg of cauliflower extract.**

| Groups / Hormones Levels | Control | Hyperthyroid | Cauliflower Extract (20 Mg/Kg) |
|---|---|---|---|
| T3 (ng/dl) | 42.54 ± 1.89 c | 63.26 ± 2.32 a | 46.65 ± 2.33 b |
| T4 (ng/mL) | 1.88 ± 0.21 c | 4.23 ± 0.36 a | 3.12 ± 0.23 b |
| TSH (ng/mL) | 8.02 ± 0.31 a | 4.08 ± 0.29 c | 6.18 ± 0.72 b |

*Different letters in the same row demonstrate a significant difference at $p<0.05$.

Further, the results in Table (2) demonstrated that there was a significant ($p<0.05$) decrease in T3 and T4 hormone levels means in rabbits treated with cauliflower extract at 30 mg/ kg body weight compared with hyperthyroid. In addition, there was no significant difference in T4 hormone level means in cauliflower extract as compared with control rabbits. T3 hormone levels were 45.67 ± 1.98, 65.88 ± 3.65 and 44.80 ± 1.87 ng/dl in cauliflower extract, hyperthyroid and control rabbits, respectively. While T4 hormone levels means were 2.16 ± 0.32, 3.75 ± 0.23 and 1.7 ± 0.32 ng/mL in cauliflower extract, hyperthyroid and control rabbits, respectively. On the other side, the results illustrated a significant ($p<0.05$) increase in TSH hormone levels means in rabbits treated with cauliflower extract in comparison with hyperthyroid. Further, no significant difference in TSH hormone level means in cauliflower as compared with control rabbits. The levels of TSH hormone were 7.34 ± 0.85, 6.65 ± 0.32 and 8.2 ± 0.76 ng/mL in cauliflower extract, hyperthyroid and control rabbits, respectively.

**Table 2: Thyroid hormones in hyperthyroid rabbits before and after treatment with 30 mg/ kg of cauliflower extract.**

| Groups / Hormones Levels | Control | Hyperthyroid | Cauliflower extract (30 mg/kg) |
|---|---|---|---|
| T3 (ng/dl) | 44.80 ± 1.87 b | 65.88 ± 3.65 a | 45.67 ± 1.98 b |
| T4 (ng/mL) | 1.7 ± 0.32 b | 3.75 ± 0.23 a | 2.16 ± 0.32 b |
| TSH (ng/mL) | 8.2 ± 0.76 a | 6.65 ± 0.32 b | 7.34 ± 0.85a |

*Similar letters in the same row indicate no significant difference while different letters in the same row indicate significant difference at $p<0.05$.

In hyperthyroid rabbits, the elevated levels of thyroid hormones (T4 and T3) lead to increased basal metabolic rate, elevated oxygen intake and released high quantities of reactive oxygen species which increased free radicals production and declined anti-oxidant metabolites; [11, 13, 8]. Relatively, high levels of free radicals are generated particularly in response to thyrotropin. These serve as substrate for thyroperoxidase enzymes which catalyze the synthesis of thyroid hormones, thyroxine and triiodothyronine [13].

Further, TSH was declined in the presence of increased thyroid hormone levels is related to the negative feedback effect, which regulated the secretion of TSH from the anterior pituitary gland [3].

Recent studies demonstrated that cruciferous vegetables act as an excellent source of natural antioxidants due to the high levels of carotenoids, tocopherols, and ascorbic acid, and found that these compounds may help to protect the human body against damage by free radicals. In addition to carotenoids, tocopherols, and ascorbic acid, these antioxidants which associated to plant diet intake is mainly due to the presence of phenolic compounds, which have been related with flavor and color aspects of plants [1].

There is reliable evidence that the consumption of normal dietary levels of cruciferous vegetables affects thyroid function. These crucifers release the goitrogens when digested by intestinal bacteria, and this increase the need for iodine when consumed in small amounts and can damage the thyroid gland when consumed in large amounts [13].

In adult rabbit, the epithelial cells (thyrocytes) of the thyroid follicles were flattened to low cuboidal with homogenous cytoplasm, whereas, these epithelial cells became tall cuboidal or more columnar. This may be due to increase reabsorption rate of thyroglobulin from the follicular lumen [13].

It concluded from the present work that the usage of cauliflower extract as a remedy of the hyperthyroid rabbits

may be due to the high contents of phenolic compound especially goitrogens, these compounds act to reduce the high levels of thyroid hormones and return their levels to the normal range. Further, it had been found that the higher concentration of this extract (30 mg/ kg of body weight) had a high efficacy in the recovery from hyperthyroidism than other two concentrations.

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