Evaluation of the regulatory effects of broccoli and Brazil nuts on experimentally induced thyroid disorders in female rats

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Abstract

This study evaluates the protective and therapeutic role of broccoli and Brazil nuts in in vitro-induced thyroid disorders in female albino rats. The experiment was conducted in the laboratories of the College of Veterinary Medicine at Tikrit University. The study aimed to investigate the effect of these dietary components on physiological variables associated with hyperthyroidism and hypothyroidism. These variables included changes in body weight, blood glucose levels, and hormonal changes in thyroid function (T3, T4, and TSH). The methodology involved inducing hypothyroidism and hyperthyroidism in experimental groups using thyroxine and carbimazole, while incorporating broccoli and Brazil nut powder into their diets at specific ratios for 28 days. The significance of this research lies in exploring dietary alternatives rich in bioactive compounds such as sulforaphane and selenium, and their potential to restore hormonal and metabolic balance in the face of thyroid dysfunction. This contributes to providing scientific insight into the safety and efficacy of these foods in therapeutic diets.

Keywords: Broccoli, Brazil nuts, Hyperthyroidism, Hypothyroidism.

Introduction

Thyroid disorders are a diverse group of diseases resulting from abnormalities in the structure or function of the thyroid gland. They include hypothyroidism, hyperthyroidism, structural abnormalities, and thyroid cancer [1][2]. The increasing life expectancy of the world's population has led to a rise in the incidence of thyroid disorders, particularly among the elderly. It is estimated that thyroid disorders such as hypothyroidism affect 5% of the global population [3]. Hyperthyroidism affects approximately 1.3% and 0.8% of the populations in the United States and Europe, respectively [4]. The global incidence of thyroid cancer is 10.1 per 100,000 women and 3.1 per 100,000 men [5].

Broccoli (Brassica oleracea) is a biennial plant belonging to the Brassicaceae family. It is one of the most important vegetable crops worldwide, experiencing increasing market demand due to its high nutritional value. It is widely cultivated in temperate regions from late summer to late spring [6]. Broccoli has recently gained significant attention thanks to its excellent nutritional composition and numerous health benefits. It is a rich source of several vitamins (C, K, and significant amounts of B and E vitamins). It also contains many active compounds such as folic acid and Omega-3 fatty acids. In addition to its content of minerals such as selenium, calcium, iron, chromium, magnesium, phosphorus and zinc, it is also a good source of plant proteins. Broccoli contains phytochemicals with antioxidant properties, such as polyphenols, including

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kaempferol, glucosides, quercetin, isoramnitin, and others [8][7]. Despite the alleged potential negative effects of broccoli sprouts on thyroid function due to their high content of glucosinolates and their derivatives, most notably sulforaphane, the limited studies conducted on humans and animals have not clearly confirmed this effect [9]. On the contrary, some studies have shown that broccoli extract has a very clear protective effect in safeguarding thyroid tissue and improving its function. This effect is attributed to its high antioxidant content [10]. Furthermore, one study indicated that consuming broccoli sprout syrup rich in sulforaphane is safe for the thyroid gland and its functions in the long term [11].

Brazil nuts (Bertholletia excelsa) belong to the Lecythidaceae family and grow on the giant Brazil nut tree, which typically reaches a height of 60 meters. These trees grow in groves containing between 50 and 100 trees [12]. Brazil nut cultivation is widespread throughout Brazil, and it is also produced in several other countries along the Amazon basin. The Brazil nut is native to the Amazon rainforest in South America [13][14]. Brazil nuts are considered a promising food source due to their rich nutritional content and numerous health benefits. They contain many bioactive phytochemicals, most notably a high selenium content, as well as other minerals. They possess significant biological and medicinal benefits, playing a role in the prevention and treatment of various diseases. They can also be used as a dietary supplement and a sustainable functional food [15]. Brazil nuts are the richest known food source of selenium; a single nut often meets the recommended daily intake of selenium. Selenium supplements, even those taken as capsules, and even those consumed as part of the Brazil nut diet, show health benefits. Additional advantages have been observed when consuming Brazil nuts compared to taking selenium supplements [16]. Selenium, along with other active compounds in Brazil nuts such as phytosterol polyphenols and flavonoids, is known to have potent antioxidant properties [17].

Material and Methods

Preparing broccoli feed:

Broccoli was purchased from markets in Fallujah. The broccoli florets were washed with distilled water to remove impurities and then cut into small pieces. These pieces were spread out in the shade and left to dry, being stirred continuously. After complete drying, which took more than two weeks, they were ground into a powder using an electric grinder. The powder was then placed in airtight glass containers and stored at room temperature until use. It was then added to the basic diet (the standard ration for the animal house of the College of Veterinary Medicine). The broccoli powder was added to the basic diet at a rate of 10%, according to the method of Tahoon (2022). [18].

Preparation of the Brazil nut sample:

Brazilian hazelnuts were obtained from Baghdad Governorate. After ensuring their cleanliness and freedom from dirt and dust, the kernels were ground, mixed, and added at a rate of 10% to the standard feed ration.

laboratory animals:

This study used 20 female albino rats, aged 2-3 months and weighing 190-200 grams. The rats were obtained from the University of Tikrit, College of Veterinary Medicine, Animal House. They were examined by a veterinarian at the Animal House to ensure their health and well-being. They were then left for five days to acclimate to their environment, during which time they were given water and the standard diet for rats in the Animal House.

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Experiment design:

The laboratory animals were distributed among five individual plastic cages with metal mesh lids. The cages were sterilized and cleaned, with each cage containing four rats. Sufficient water and food were provided throughout the 28-day experiment. The groups were divided as follows:

Group 1: The healthy control (Co): This group consisted of four rats fed only the center's standard diet.

Group 2: Thyroxin (Th): The rats in this group were treated with thyroxine at a concentration of 10 micrograms/kg of body weight, as described in NovoPro, n.d. [19].

Group 3: Carbimazole (Ca): The rats in this group were treated with carbimazole at a concentration of 1.35 mg/kg of body weight, as described in NovoPro, n.d. Group 4: Broccoli + Hyperactivity (ThB): This group consisted of four rats with hyperactivity who were treated with broccoli.

Group 5: Broccoli + Reduced Activity (CaB): This group consisted of four rats with reduced activity who were treated with broccoli.

Group 6: Brazil Nut + Hyperactivity (ThN): This group consisted of four rats with hyperactivity who were treated with Brazil Nut.

Group 7: Brazil Nut + Reduced Activity (CaN): This group consisted of four rats with reduced activity who were treated with Brazil Nut.

After the 28-day experiment, the laboratory rats were fasted for 12 hours and weighed using an electronic scale. They were then anesthetized with chloroform, and blood samples were drawn using the cardiac puncture method. The samples were placed directly into gel tubes and centrifuged at 3000 rpm for 15 minutes to isolate the serum. Subsequent tests, including thyroid hormone and blood glucose levels, were performed according to the instructions provided by the manufacturers of the testing equipment.

Results and Discussion

Body weight

The results of the current study showed that the hyperthyroid group fed the standard (Th) diet exhibited a significant decrease in body weight compared to the healthy control group. This may be due to the strong association between hyperthyroidism and weight loss [20]. Some studies have indicated that hyperthyroidism increases the basal metabolic rate and energy expenditure, leading to weight loss. Hyperthyroidism can sometimes cause loss of appetite due to the appetite-suppressing effect of the T3 hormone [21].

The results showed an increase in body weight in the reduced-activity group on a standard diet compared to the healthy control group. This increase was also observed in the hyperactive group. This may be due to hypothyroidism causing a decrease in metabolic rate, leading to weight gain [21]. It may also be due to the slowed intestinal motility experienced by hypothyroid patients, which causes chronic constipation resulting in weight gain [22].

The group receiving reduced activity and broccoli showed a decrease in weight compared to the hyperactive group. This is likely due to broccoli containing active flavonoid compounds such as kaempferol, which has a weight-reducing effect [23]. Meanwhile, the hyperactive group receiving broccoli showed an improvement in weight compared to the hyperactive group, due to broccoli containing active

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compounds that contribute to weight improvement, such as glucopanin [24].

Table 1. The effect of different factors on body weight rate

Sample	Initial weight/ g	Final weight/ g	weight difference/ g
Co	193	216	23
Th	196	210	14
ThB	193	213	20
ThN	196	215	22
Ca	195	230	35
CaB	196	225	30
CaN	196	227	31

The results showed an increase in body weight in the hyperactive rat group fed Brazil nuts. Some studies have indicated a body weight-enhancing effect of Brazil nuts [25]. Conversely, the hypoactive Brazil nut group showed a decrease in body weight compared to the untreated group. Other studies have suggested that the selenium content of Brazil nuts reduces body fat loss and increases lean muscle mass, thus leading to a decrease in body weight [26]. Therefore, it may be preferable to study the effect of Brazil nuts on body weight independently of underlying medical conditions.

Blood sugar level:

The current study showed a significant increase ($P<0.05$) in blood glucose levels in the hyperthyroid (Th) group compared to the healthy control (Co) group. This may be due to hyperthyroidism causing blood glucose disturbances, accelerating insulin breakdown, and reducing insulin half-life [27][28]. Our results also showed a significant increase in blood glucose levels in the hypothyroid (Ca) group compared to the healthy control group and even the hyperthyroid group. Hypothyroidism leads to impaired glucose absorption from the gastrointestinal tract and the development of peripheral insulin resistance [29]. Furthermore, the results of the current study showed that the hyperthyroid and hypothyroid groups treated with broccoli achieved a significant decrease in blood glucose levels compared to the untreated hypothyroid and hyperthyroid groups. These levels were comparable to those recorded in the healthy control group. This may be attributed to broccoli's sulforaphane content, which has the ability to lower blood glucose levels and reduce insulin resistance. These effects are linked to the activation of Nrf2 and PI3K/Akt pathways, and the inhibition of NF-κB, GSK3β, α-glucosidase, and DPP-IV [30]. Some studies have shown that broccoli is able to lower blood sugar levels and reduce insulin resistance [31].

Table 2. Effect of different treatments on blood sugar levels

Sample	Glucose
Co	111 ± 10.31 e
Th	154 ± 11.25 b
ThB	135 ± 11.42 c
ThN	115 ± 11.49 de
Ca	167 ± 14.23 a
CaB	140 ± 10.83 c
CaN	120 ± 10.73 d

The numbers represent the mean ± standard error. Letters similar to the control group indicate no significant differences; letters different from the control group indicate significant differences.

Our current results also showed a significant decrease in blood glucose levels in the two groups treated with Brazil nuts compared to the hyperactivity and hypoactivity control groups. This is likely due to Brazil nuts' ability to improve serum glucose levels. Studies have shown that Brazil nuts are rich in many active compounds, most notably selenium, which has demonstrated numerous health benefits in improving insulin and glucose response, and thus the ability to lower blood glucose levels [32]. Studies also indicate that nuts in general, including Brazil nuts, are a healthy alternative for blood glucose control [33].

The effect of different treatments on thyroid hormone levels:

The results showed a significant increase in T3 and T4 levels, along with a decrease in TSH concentration, in the rats treated with thyroxine. The decrease in TSH levels resulting from thyroxine treatment is attributed to a negative feedback mechanism. An increase in T4 levels stimulates thyrotrophs in the anterior pituitary gland to lower TSH levels [34][35]. Similarly, carbimazole treatment causes a decrease in T4 levels and a significant increase in TSH levels [36]. The results for the hyperactive broccoli-sprouted group (ThB) showed a significant decrease in thyroid hormone levels, reaching levels comparable to those of the healthy control group. The ThB group recorded values similar to or lower than those of the Ca group, without any statistically significant difference. This may be because adding broccoli sprouts to the diet has a protective effect against thyroid damage. Broccoli has the potential to reduce hyperthyroidism through the action of bioactive compounds such as brassicoside, 24-methylene-25-methylcholesterol, and 5-dehydroavenasterol. These compounds have the ability to inhibit the overproduction of thyroid hormones [37].

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Table 3. Effect of different treatments on thyroid hormone levels

Sample	TSH/ Mlu/ml	T4/ Mlu/ml	T3/ Mlu/ml
Co	1.24 ± 0.221 ^{c}	81.21 ± 7.19 ^{b}	87.14 ± 7.66 ^{b}
Th	0.812 ± 0.131 ^{d}	113.31 ± 11.22 ^{a}	121.12 ± 10.24 ^{a}
ThB	0.914 ± 0.212 ^{cd}	80.06 ± 7.53 ^{bc}	85.34 ± 6.49 ^{bc}
ThN	1.18 ± 0.346 ^{c}	110.24 ± 10.33 ^{a}	118.67 ± 9.93 ^{a}
Ca	2.83 ± 0.630 ^{a}	65.32 ± 8.19 ^{d}	65.45 ± 6.88 ^{d}
CaB	2.05 ± 0.542 ^{b}	62.13 ± 6.74 ^{d}	63.23 ± 7.14 ^{d}
CaN	2.34 ± 0.711 ^{ab}	75.41 ± 5.94 ^{c}	80.56 ± 8.03 ^{c}

The numbers represent the mean ± standard error. Letters similar to the control group indicate no significant differences; letters different from the control group indicate significant differences.

This underscores the need for empirical evidence to support claims regarding the negative effects of broccoli sprouts on thyroid function due to their high glucosinolate content. On the contrary, broccoli sprouts have been found to contain compounds that play a protective role against sulfadimethoxine-induced thyroid damage. In a study by Paśko et al. (2018), a significant increase in thyroid hormone receptor (TR) activity was observed in hypothyroid animals after consuming broccoli sprouts. Furthermore, a positive effect on cellular antioxidant levels in the thyroid gland was also noted. It was also observed that broccoli reduced IL-6 levels [38]. The group receiving reduced activity with broccoli showed a significant decrease in T3 and T4 levels, while TSH levels remained elevated compared to the healthy control group. No improvement in thyroid function was observed compared to the groups treated for hypothyroidism with respect to T3 and T4 levels, although TSH levels did improve. The CaB group showed a significant decrease in this hormone compared to the untreated group. This indicates that broccoli did not significantly improve the condition of hypothyroidism.

The hyperthyroid group fed Brazil nuts showed no significant differences in T3 and T4 thyroid hormone levels compared to the untreated hyperthyroid control group (Th). However, there was a significant decrease in TSH levels compared to the same group. This suggests that Brazil nut diet does not have a clear therapeutic effect on hyperthyroidism. Further studies are needed to investigate the mechanism by which Brazil nuts affect thyroid function, especially given our findings of a contraindication in the treatment of hypothyroidism. Stefani et al. (2020) noted that the role of selenium supplementation, to which most of the effect of Brazil nuts on thyroid activity is attributed, in thyroid diseases has shown conflicting results [39]. Selenium acts as a buffer regulator and therefore does not prevent hyperthyroidism itself, especially if the hyperthyroidism is induced by external factors. However, its effective role lies in protecting thyroid tissue from damage due to its antioxidant activity and its ability to ensure the efficient conversion of T4 to T3 [40][41]. The current results for the hyperthyroidism group treated with Brazil nut showed a significant improvement in T3 and T4 thyroid hormone levels, with no significant differences in TSH levels compared to the untreated hypothyroid group. This may be because consuming Brazil nut as part of a regular daily diet improves antioxidant levels by increasing selenium levels, as previously mentioned, and enhances glutathione peroxidase activity [42]. The results of the current trial, examining hyperthyroidism and hypothyroidism and the therapeutic effect of Brazil nut on both conditions, clearly indicate that its selenium content does not have a regulatory role but rather stimulates the thyroid and its systems,

thus increasing the release of thyroid hormones, not decreasing it. This is further supported by the results showing that Brazil nut did not lower the elevated thyroid hormone levels in the hyperthyroid group treated with Brazil nut (ThN).

Conclusion

The study concluded that broccoli and Brazil nuts have a significant and varied effect on managing thyroid disorders and associated metabolic disturbances. Broccoli demonstrated its ability to improve hyperthyroidism, contributing to lowering elevated hormone levels and restoring normal body weight. It also played a crucial role in reducing high blood sugar levels and insulin resistance thanks to its sulforaphane content. The study further concluded that Brazil nuts are a potent stimulant for thyroid function, particularly in cases of hypothyroidism. Their high selenium content contributed to raising thyroid hormone levels. However, they did not appear to be effective as a regulator in hyperthyroidism, indicating that their effect is stimulatory rather than inhibitory. The study's results confirmed the safety of these nutrients when consumed in appropriate proportions.

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