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Bunium incrassatum Bois. Batt. Trab. (Talghouda) in the improvement of thyroid tissue damages in female rats

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Abstract

This study aims to determine the nutritional and therapeutic effects of dried *Bunium incrassatum* Bois.Batt.Trab. Tubers powder on rats with hyper and hypothyroidism. Thirty female Wistar rats were divided into 6 groups. G1 (C) received a normal diet. G2 (CT) received a normal diet with dried Talghouda tubers powder dissolved in water. G3 (Hpo) was treated with Carbimazole® (5 mg/day) for 6 weeks. G4 (HpoT) was treated with Carbimazole® (5 mg/day) for 6 weeks and Talghouda for 7 weeks after induced hypothyroidism. G5 (Hpy) was treated with Levothyroxine® (600µg/Kg body weight/day) for 6 weeks. G6 (HpyT) had hyperthyroidism induced by Levothyroxine® (600µg/Kg body weight/day) for 6 weeks and then treated with Talghouda for 7 weeks. A dose of Talghouda was chosen at 1.03g/Kg body weight/day, and all treatments were given by gavage. Phytochemical analysis was carried out for Talghouda, and the thyroid glands of each group were then recovered for histological study. Talghouda treatment showed a highly significant ($p < 0.01$) increase in body weight in groups G2, G4 and G6 with gland repair and reactivation of thyroid follicles in hyper and hypothyroid rats compared to G1, G3 and G5. The powder of dried Talghouda tubers, used as traditional medicine in Algeria, improved the damages caused by hyper and hypothyroidism.

Keywords: *Bunium incrassatum*; Histology; Hyperthyroidism; Hypothyroidism; Talghouda; Thyroid

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INTRODUCTION

By its strategic position and its richness in floral diversity, «Mediterranean, Saharan and Paleo tropical flora», Algeria constitutes a phylogenetic reservoir of more than 3000 species, 15% are endemic.[1] The Eastern and Saharan regions are known for their traditional medicine and artisanal preparation of herbs rich in bioactive substances, used for their nutritional and therapeutical virtues. Thyroid dysfunction is among the metabolic diseases that constitute a real public health problem. In 1990, the World Health Organization recognized iodine deficiency as the principal cause of mental retardation globally. It is considered an important public health problem in 129 countries, in which 1.5 billion people (29%

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of the world's population) live at risk of iodine deficiency.[2] In human and clinical nutrition, the use of medicinal and nutritive plants, their mode of preparation and administration, and their effects on certain diseases constitute the interest of new research and projects, but some of them remain unidentified. Among these species, which cover a large part of the flora of the Tell regions, *Bunium incrassatum* Boiss. Batt. Trab is commonly called "Talghouda". It was the only food source for Algerians during the colonial period when animal and plant resources were completely exhausted. This tuber used as flour in bread and cakes, or as a vegetable in culinary dishes, has nutritional and therapeutical virtues, which have contributed, on the one hand, to maintain a balanced status of health during the war, and on the other, to cure certain diseases such as cystitis, pyelonephritis, intestinal worms and wounds, by stimulating the immune system and playing a role of nervous relaxation.[3] Moreover, in Eastern Algeria, it is known for its therapeutic effect on thyroid dysfunction (hypo/hyperthyroidism and thyroid nodules). Noteworthy, many plant constituents such as polyphenols and alkaloids can interfere with thyroid hormones production or metabolism and may have antiproliferative effects on thyroid cancer cells.[4] Recent studies have reported that Talghouda has a good phytochemical composition.[5] To the author's knowledge, there is no published work describing the use of *Bunium incrassatum* Boiss. Batt. Trab. for the treatment of thyroid disorders.[6] This study aims to evaluate the nutritional and therapeutic effects of dried Talghouda tuber powder on rats suffering from hyper and hypothyroidism and determine the nutritional and phytochemical composition.

METHOD

Reagents

All reagents were purchased from Sigma (Sigma-Aldrich, MO, USA) while Carbimazole® (ATHYROZOL 5mg, Genericlab), Levothyroxine® sodium 100 µg Tab were from MERCK.

The material used came from our research laboratory of Food Technology and human nutrition for physicochemical and phytochemical analysis and Cypress DIAGNOSTICS for blood analysis (Hematology automat Model CYANHemato). Leica DM750 microscope was coupled to the Leica ICC50 camera for histological study.

Plant material

The dried tubers were of commercial origin (herbalist) from Setif (Algeria), collected in cereal fields in 2020. Plants were taken for identification in the herbarium of the National Higher School of Agronomy, El-Harrach.

Research Process

Nutritional composition

The evaluation of the nutritional composition of the dried tuber powder of *B. incrassatum* was made according to the following reference methods: Moisture and dry matter (ISO712:2009), protein content by Kjeldahl method (ISO20483:2013), lipid content by Soxhlet method (AFNOR NF-V03-908), fatty acids profile by GCP(ISO5509:2000), and ash content by incineration (ISO2171:2007). Starch and congeners content was determined according to the following formula: Starch and congeners (%) =100% - (Protein% + Lipid% + Ash% + Moisture%) Mineral content (Sodium, Potassium, Calcium) by flame photometry.[7]

Phytochemical study and antioxidant activity

The phytochemical study was carried out after methanolic extraction of dried Talghouda tuber powder. Preliminary tests were made to reveal the presence of some secondary metabolites. The determination of total polyphenol content by Folin-Ciocalteu method,[8] flavonoid content by Islam et al.[9] alkaloid content by Spectrophotometry[10] and Titrimetry[11] and coumarin content by spectrophotometry.[12]

The antioxidant capacity was evaluated by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method[13] and by the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging method[14]. Reducing power (RP) was measured[15] by comparing Butylated hydroxytoluene (BHT) and ascorbic acid.

Animal studies

Toxicity: A preliminary toxicity study was conducted using the "acute oral toxicity class" method of OECD line 423. Female mouse Balb-c were divided into 5 groups of 6 animals. They fasted for 4 hr prior to and 2 hr after the experiment. The control group was treated with distilled water, and the other 4 groups were orally administered with a single and increasing dose of Talghouda dried tuber powder dissolved in water (85, 250, 500, 1000 mg/Kg BW). After dosing, each animal was observed in the first 2 hr for behavioral signs of toxicity (changes in skin, hair, eyes, autonomic and central nervous systems, motor activity, convulsion, tremors, diarrhea), and they were monitored for 14 days for the long-term possible lethal outcome.

Animals

Thirty Wistar female rats of SPF sanitary status (Specific Pathogen Free) weighing 170-200 g were acquired from the Pasteur Institute of Algeria. They were housed in 6 cages, 5 rats each, for 20 days. The cages were made from polypropylene with a stainless-steel lid and a bottom lined with bedding, renewed 3 times a week. The animals were kept in a constant temperature (22 ± 2 °C), with a relative humidity of (62 ± 7%), 12 h light/12 h dark cycle and fed with a standard diet and drinking water ad libitum. The experimental procedures were approved by the Algerian Institutional Animal Care Committee, which belonged to the National Administration of Algerian Higher Education and Scientific Research (Algiers). Thyroid dysfunction was induced following the protocol of Zhou et al.[16] for hypothyroidism and Azharuddin et al.[17] for hyperthyroidism. G1 served as control (C), G2 control with Talghouda (CT), G3 Hypothyroidism without treatment (Hpo), G4 Hypothyroidism treated with Talghouda (HpoT), G5 Hyperthyroidism without treatment (Hpy) and G6 Hyperthyroidism treated with Talghouda (HpyT). The experimental protocol is represented in Figure 1.

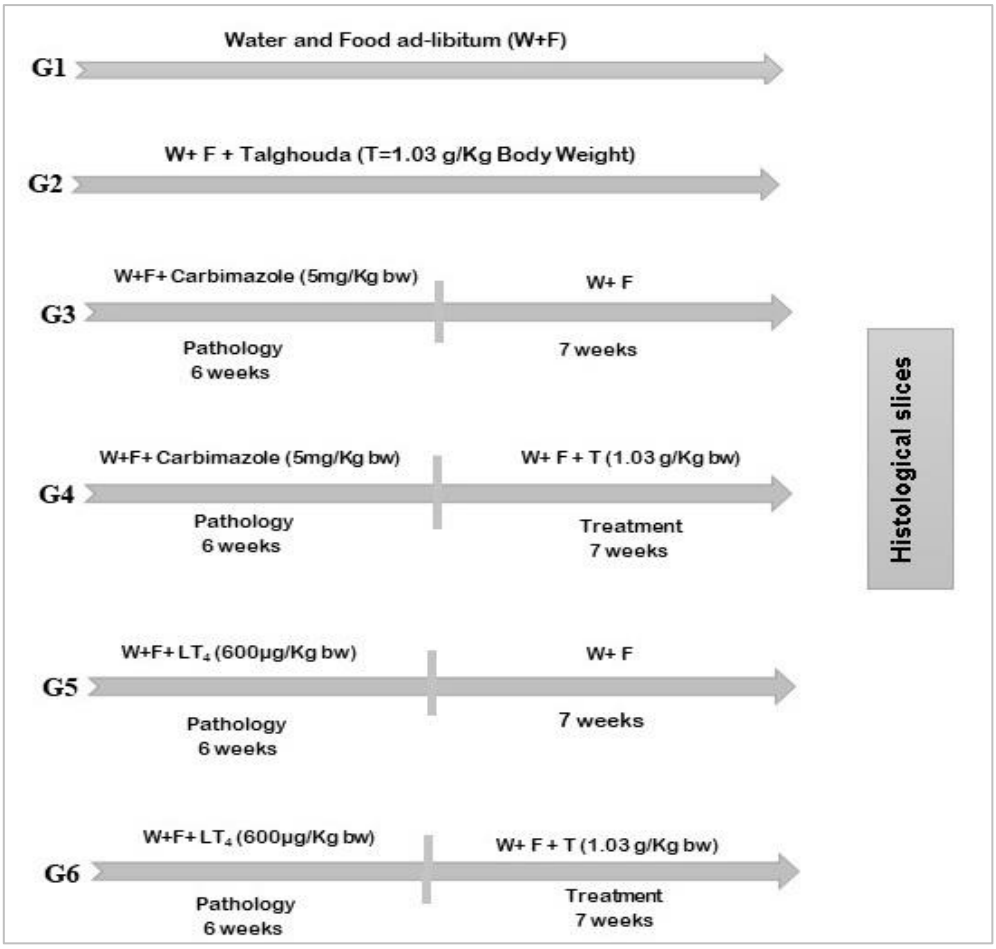

Figure 1. Experimental Protocol

G1: Control (C)
G2: Control with Talghouda (CT)
G3: Hypothyroidism without treatment (Hpo)
G4: Hypothyroidism treated by Talghouda (HpoT)
G5: Hyperthyroidism without treatment (Hpy)
G6: Hyperthyroidism treated by Talghouda (HpyT)

The dose of Talghouda powder was chosen due to the HED (Human Equivalent Dose).[18] The weight of the rats was evaluated at each step of the experiment (pathology and treatment phases). Blood samples were obtained from the retro-orbital sinus to evaluate the hematologic profile.

At the end of the experiment, the rats were sacrificed, and the organs were weighed (liver, kidneys, heart, and thyroid). Thyroid glands, obtained by excision, were fixed in Bouin Holland liquid for histological study.

Histology

The histological study was conducted in the Histopathology Laboratory located at the Anapathology department of the Higher National Veterinary School of Algiers. Two strains were used: Hematoxylin-Eosin (*H&E*) (routine method for disease diagnosis) and Masson's Trichrome (*MT*) to visualize connective tissues and cytoplasmic elements. Histological slices were observed by Leica DM750 microscope, and the Leica LAS Microsystems software carried out the image processing.

Statistical study

Analysis of Variance (ANOVA) and the results obtained (expressed in Mean ± Standard Error) was analyzed by using STATISTICA software (StatSoft, Inc. (2007), version 8.0) and EXCEL (Microsoft office professional plus, Excel 2019 MSO). The significance levels were expressed as follows: Not significant ($^{NS}p > 0.05$), Significant ($^{a}p < 0.05$), Highly significant ($^{b}p < 0.01$), very highly Significant ($^{c}p < 0.001$).

RESULTS AND DISCUSSION

The identification of the plant revealed a robust stem from 10 to 50 cm height, an umbel with long and robust peduncles, from 8 to 12 spread out rays. The white "umbellate" flowers had an involucre and an involucel with five leaves at the base. A calyx constituted each flower with triangular lobes, well-marked and rigid teeth, and a conical stylopodium with persistent styles (Figure 2.1).

The roots were tuberous, coming out of a voluminous tuber (2 - 5 cm), rough and characterized by a white color inside and a black-brown scaly bark outside (Figure 2.2).

Figure 2. *Bunium incrassatum* Bois. Batt. Trab (Original Photos)

Nutritional composition

The evaluation of the nutritional composition of the dried tuber powder of *B. incrassatum* is shown in Table 1.

Table 1. Nutritional composition of *Bunium incrassatum*

Composition	Powder of Talghouda (%)
Moisture	12.41 ± 0.19
Dry matter	87.59 ± 0.19
Proteins	6.87 ± 0.28
Lipids	1.59 ± 0.09
Starch and congeners	75.79 ± 0.26
Ash	3.34 ± 0.14
Mineral (mg/100g)	
Sodium	26.126
Potassium	289.17
Calcium	449.6

Phytochemical composition and antioxidant activity

Total polyphenols, flavonoids, alkaloids and coumarins are presented in Table 2.

Table 2. Phytochemical composition of Talghouda

Composition	Content
Polyphenols	37.37 ± 0.46 mg GAE/g extract
Flavonoids	2.36 ± 0.06 mg QE/g extract
Alkaloids	0.82 ± 0.02 mg AE/g extract 0.214 g of total alkaloids
Coumarins	17.94 ± 5.25 mg CE/g extract

GAE: gallic acid equivalent; QE: quercetin equivalent; AE: atropine equivalent; CE: coumarin equivalent.

Reduction of free radicals DPPH, ABTS, and ferric ions are expressed as IC_{50} (half maximal inhibitory concentration) (Table 3).

Table 3. IC_{50} Values of methanolic extract of *Bunium incrassatum*

	Half maximal Inhibitory concentration (IC_{50})
DPPH	1.602 ± 0.002 mg/mL
ABTS	0.744 ± 0.0001 mg/mL
FRAP	2.85 ± 0.003 µg/mL

The results obtained showed an increase in antioxidant activity when increasing the concentration of *B. incrassatum* extracts. The reducing power of extract compared to BHT and ascorbic acid is shown in Figure 3. The plant extract showed a concentration-dependent reducing power, with a reduction close to vitamin C at 3 and 4 mg/mL concentrations, respectively.

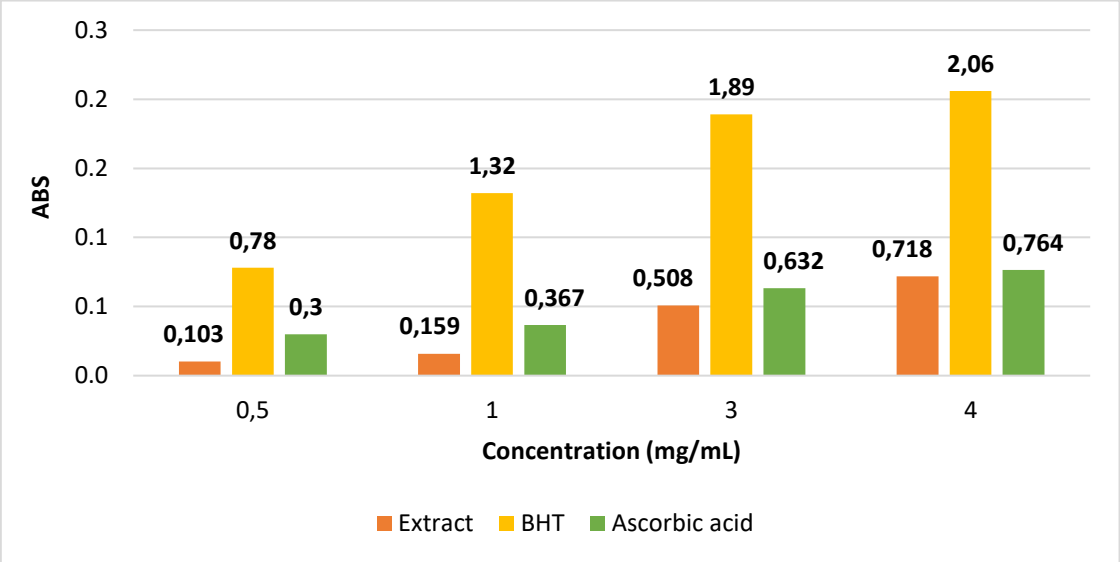

Figure 3. Reducing power of *B. incrassatum* extract, BHT and ascorbic acid

The phytochemical composition of Talghouda powder (Table 2) revealed 37.37 ± 0.46 mg EGA/g extract of polyphenols content and 2.36 ± 0.06 mg EQ/g extract of flavonoids. An analysis made by Dehimi et al.[5] on polyphenols and flavonoids content in *B. incrassatum* suggested a polyphenols level of 13.00 ± 0.09 mg EGA/g extract and flavonoids of 16.32 ± 0.05 mg EQ/g extract. This result indicated that *B. incrassatum* used in our study had the highest polyphenols content, with fewer flavonoids. This difference was due to edaphic, physiological and genetic factors.

Experience

Toxicity: In the acute toxicity study, a single oral dose (85, 250, 500, 1000 mg/Kg BW) of Talghouda powder showed no mortality or signs of toxicity during the 14-day observation period. No significant changes in body weight gains were detected, but hyperactivity was noted after 30mn of administration, with increasing dose, followed by a sedative effect confirmed by light and noise stimulus. The stimulating, sedative and sleep-inducing effect of the Talghouda plant was due to the presence of alkaloids among its active principles. Alkaloids presented a wide range of pharmacological and psychotropic activities, with stimulating or frenemy action of the sympathetic and parasympathetic systems, mainly the atropine alkaloids (atropine, hyoscyamine, scopolamine)[19].

Bodyweight

Pathological phase

The control group (C) did not have a significant weight gain (p>0.05), while the hypothyroid and hyperthyroid groups had a very highly significant weight difference (p< 0.0001) (Figure 4).

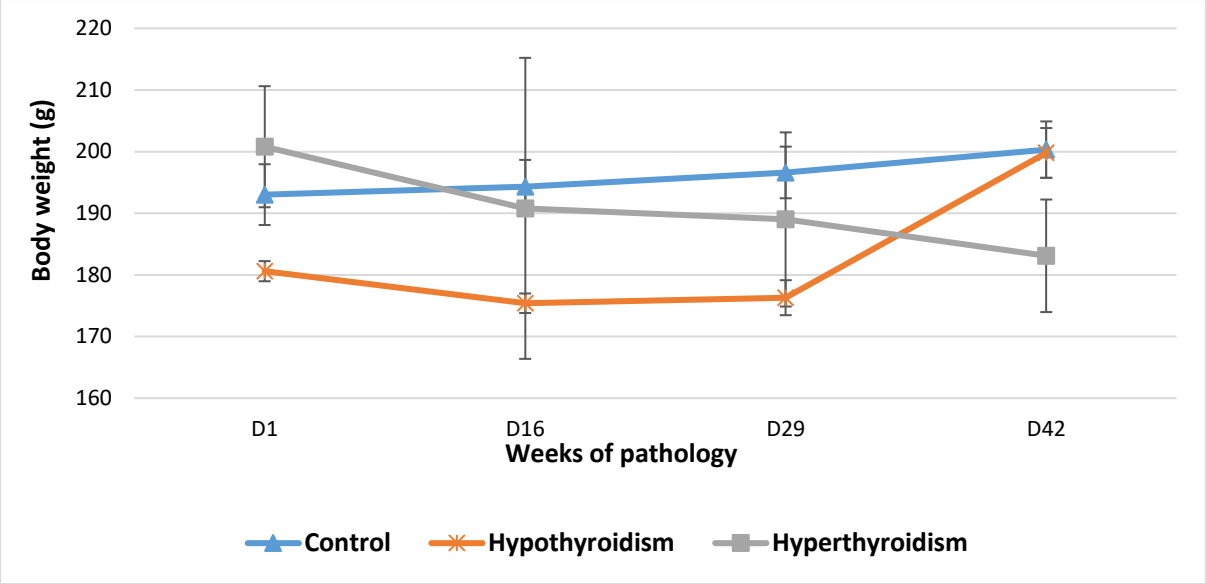

Figure 4. Evolution of Body Weight During Pathological Phase in Groups (T, Hypo and Hyper)

Treatment phase

The evolution of the weight and weight gain are shown in Figure 5. There was no significant difference in body weight in CT and Hpy groups.

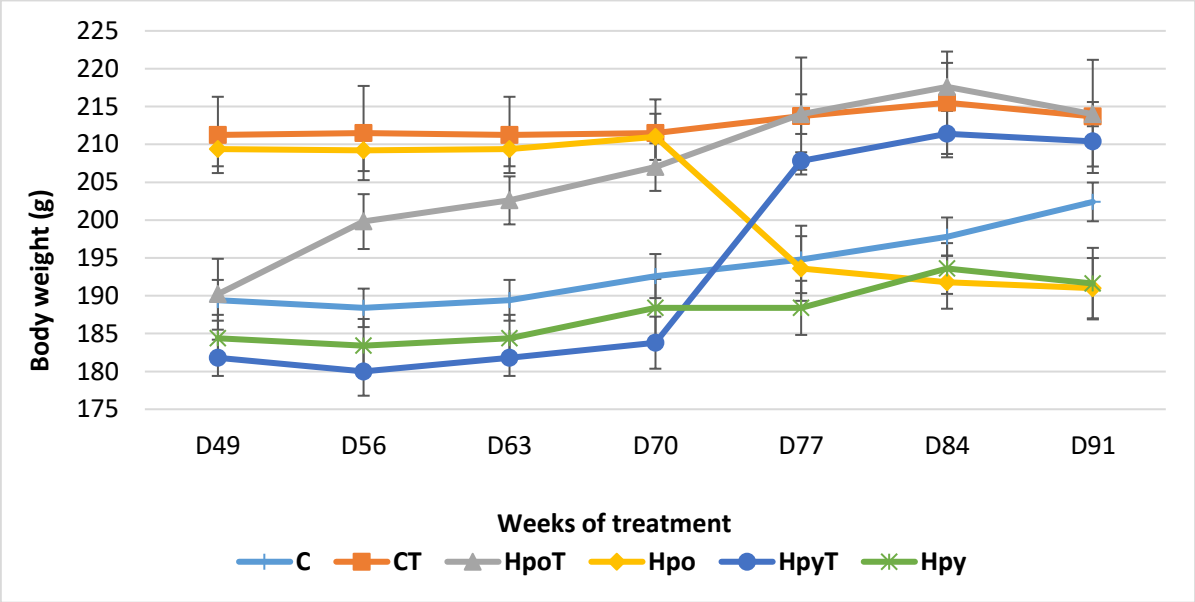

Figure 5. Evolution of body weight during the treatment phase

The Control group showed a highly significant increase. The HpoT significantly increased body weight (p<0.05) after treatment with Talghouda powder. At the same time, the weight continued to decrease in the Hpo group. The treatment of the HpyT had a very highly significant bodyweight increase.

Hematological profile

Pathological phase

Hematological analysis before (To) and after (Tf) induction of pathology are presented in Table 4.

Table 4. Hematological Profile of Rates After Pathological Phase

Groups		Control	Hypothyroidism	Hyperthyroidism
RBC (1012/L)	To	8.74 ± 0.15	8.52 ± 0.11	8.67 ± 0.25
	Tf	8.08 ± 0.23a	8.32 ± 0.46NS	8.68 ± 0.19NS
Hb (g/dL)	To	15.40 ± 0.17	15.40 ± 0.13	15.27 ± 0.29
	Tf	13.45 ± 0.43b	11.57 ± 1.06b	14.04 ± 0.35a
HT %	To	51.24 ± 0.77	50 ± 0.50	49.02 ± 1.44
	Tf	41.44 ± 1.16c	50.17 ± 11.45NS	42.19 ± 2.93NS
MCV (fL)	To	58.5 ± 0.67	58.67 ± 1.12	56.5 ± 0.22
	Tf	52.86 ± 0.34c	50.71 ± 1.87b	53.29 ± 1.02b
MCH (pg)	To	17.65 ± 0.26	18.05 ± 0.25	17.62 ± 0.21
	Tf	17.11 ± 0.13 NS	14.24 ± 2.12 NS	15.67 ± 0.4c
MCHC (g/dL)	To	30.07 ± 0.31	30.78 ± 0.19	31.15 ± 0.35
	Tf	32.41 ± 0.24c	27.41 ± 3.83NS	29.5 ± 1.04 NS
WBC (109/L)	To	6.60 ± 1.13	7.38 ± 0.34	10.38 ± 2.59
	Tf	3.41 ± 0.65a	3.04 ± 0.68c	4.35 ± 0.67b
LYM %	To	48.65 ± 17.9	70.15 ± 3.01	62.1 ± 5.3
	Tf	55.61 ± 3.13 NS	55.93 ± 3.75a	58.26 ± 4.12 NS
PLT (109/L)	To	694.68 ± 44.65	685.5 ± 26.41	743.67 ± 50.53
	Tf	639.14 ± 31.2 NS	827 ± 43.6a	696.29 ± 80.30 NS
RBC (1012/L)	To	8.74 ± 0.15	8.52 ± 0.11	8.67 ± 0.25
	Tf	8.08 ± 0.23a	8.32 ± 0.46NS	8.68 ± 0.19NS
Hb (g/dL)	To	15.40 ± 0.17	15.40 ± 0.13	15.27 ± 0.29
	Tf	13.45 ± 0.43b	11.57 ± 1.06b	14.04 ± 0.35a

RBC (Red Blood Cells), Hb (Hemoglobin), HT (Hematocrits), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), WBC (White Blood Cells), LYM (Lymphocyte), PLT (Platelets); Values are expressed as Mean ± Standard Error; Not Significant ($^{NS}p > 0.05$), Significant ($^{a}p < 0.05$), Highly Significant ($^{b}p < 0.01$), Very Highly Significant ($^{c}p < 0.001$); each group (n=10).

All hematological parameters decreased significantly in each group. No significant changes were observed in RBC for the Hypothyroidism group, while lymphocytes and platelets were for Control and Hyperthyroidism groups.

Treatment phase

The hematological results in Table 5 showed an increase in Hb and WBC in groups treated with Talghouda, but it was statistically insignificant. There was a significant increase in RBC and platelets in the CT group. The C, Hpo and Hpy groups did not have a significant difference in their hematological profile, but it was noted that the levels of MCHC and WBC in the sick groups decreased.

Table 5. Hematological Profile of Rates After treatment Phase

Groups		C	CT	Hpo	HpoT	Hpy	HpyT
RBC (10^{12}/L)	To	8.17 ± 0.12	7.79 ± 0.5	7.18 ± 0.2	9.24 ± 0.57	8.39 ± 0.14	8.93 ± 0.38
	Tf	8.02 ± 0,16^{NS}	10.31 ± 0.34^{b}	8.27 ± 0.35^{NS}	10.77 ± 0.57^{NS}	8.43 ± 0.65^{NS}	10.03 ± 0.3^{NS}
Hb (g/dL)	To	14.03 ± 0.14	13.4 ± 0.76	12.63 ± 0.52	10.2 ± 2.45	13.43 ± 0.3	14.57 ± 0.67
	Tf	13.53 ± 0.45^{NS}	14.08 ± 0.21^{NS}	13.1 ± 0.13^{NS}	14.4 ±0.46^{NS}	12.33 ± 0.43^{NS}	15.1 ± 0.2^{NS}
HT %	To	42.81 ± 0.57	41.59 ± 2.17	40.36 ± 2.08	63.6 ± 27.48	44.93 ± 0.63	37.99 ± 6.64
	Tf	42.23 ± 0.57^{NS}	53.59 ± 2.56^{b}	46.27 ± 1.01^{a}	53.7 ± 3.17^{NS}	46.99 ± 1.01^{NS}	49.46 ± 1.34^{NS}
MCV (fL)	To	52.67 ± 0.33	53,33 ± 0.67	52.67 ± 0.88	47 ± 3.21	53.67 ± 1.67	53 ± 2.08
	Tf	52.66 ± 0.65^{NS}	51.91 ± 1.02^{NS}	56.17 ± 1.81^{NS}	49.84 ± 0.98^{NS}	56.9 ± 5.21^{NS}	49.41 ± 1.54^{NS}
MCH (pg)	To	17.2 ± 0.21	17.2 ± 0.17	15.4 ± 0.44	10.83 ± 4.55	16.03 ± 0.37	15.13 ± 0.84
	Tf	16.84 ± 0.39^{NS}	13.69 ± 0.45^{c}	15.92 ± 0.54^{NS}	13.43 ± 0.45^{NS}	14.79 ± 0.78^{NS}	15.1 ± 0.5^{NS}
MCHC (g/dL)	To	32.83 ± 0.47	32.2 ± 0.12	31.37 ± 0.35	21.97 ± 8.75	29.9 ± 0.68	28.7 ± 2.55
	Tf	32.01 ± 0.83^{NS}	28.47 ± 3.17^{NS}	28.35 ± 0.52^{b}	26.98 ± 1.06^{NS}	26.26 ± 1.01^{a}	30.59 ± 0.79^{NS}
WBC (10^{9}/L)	To	3.98 ± 1.23	3.56 ± 0.73	4.38 ± 0.49	2.28 ± 1.15	5.38 ± 0.36	3.29 ± 1.38
	Tf	3.55 ± 0.83^{NS}	6.01 ± 0.32^{b}	3.23 ± 0.18^{NS}	5.17 ± 0.8^{NS}	3.8 ± 0.48^{NS}	5.57 ± 0.58^{NS}
LYM %	To	60.78 ± 4.28	55.35 ± 0.95	64 ± 1.1	50.4 ± 4	54.49 ± 2.59	61 ± 9.4
	Tf	60.93 ± 3.53^{NS}	47.45 ± 10.38^{NS}	45.43 ± 11.2^{NS}	42.28 ± 9.64^{NS}	61.85 ± 5.44^{NS}	62.83 ± 4.2^{NS}
PLT (10^{9}/L)	To	711.33 ± 35.58	575.33 ± 28.29	810.33± 72.47	874 ± 73.71	781 ± 38.44	568.33 ± 170.64
	Tf	735.5 ± 30.03^{NS}	758.5 ± 39.41^{b}	816.75± 55.62^{NS}	931.25 ± 55^{NS}	824 ± 35.34^{NS}	766.75± 99.54^{NS}

Values are expressed as Mean ± Standard Error. Not significant (^{NS}p> 0.05), Significant (^{a}p< 0.05), Highly significant (^{b}p< 0.01), Very Highly Significant (^{c}p < 0.001); each group (n=5)

Organ weight

Organ weights are shown in (Table 6).

Table 6. Organ weights of rats

Groups	Liver (g)	HSI (g)	Thyroid Gland (mg)	Heart (g)	Right kidney (g)	Left kidney (g)
C	9.23 ± 0.7	4.58 ± 0.39	56.96 ± 4.27	0.73 ± 0.04	0.75± 0.03	0.69 ± 0.04
CT	9.99 ± 0.17	4.69 ± 0.13	41 ± 5.27	0.82 ± 0.04	0.79± 0.04	0.73 ± 0.04
Hpo	7.7 ± 0.6	3.84 ± 0.33	20.38 ± 4.75	0.71 ± 0.05	0.69 ± 0.04	0.62 ± 0.04
HpoT	8.08 ± 0.4	3.82 ± 0.24	53.94 ± 3.38	0.72 ± 0.04	0.75± 0.04	0.68 ± 0.03
Hpy	8.3 ± 0.31	4.33 ± 0.16	75.22 ± 6.0	0.91 ± 0.1	0.79 ± 0.05	0.73± 0.04
HpyT	8.19 ± 0.26	3.89 ± 0.11	45.38 ± 1.72	0.87 ± 0.08	0.76± 0.03	0.72± 0.04
***p* value**	*p*>0.05	*p*>0.05	***p*<0.001**	*p*>0.05	*p*>0.05	*p*>0.05

Liver, hepatosomatic index (HSI), heart and kidney weights showed no significant difference. However, a highly significant difference (p<0.001) was noted in the thyroid gland weight: decreased in the Hpo group and increased in the Hpy group.

Histological study

Histological slices of the thyroid glands of rats are illustrated in Figure 6.

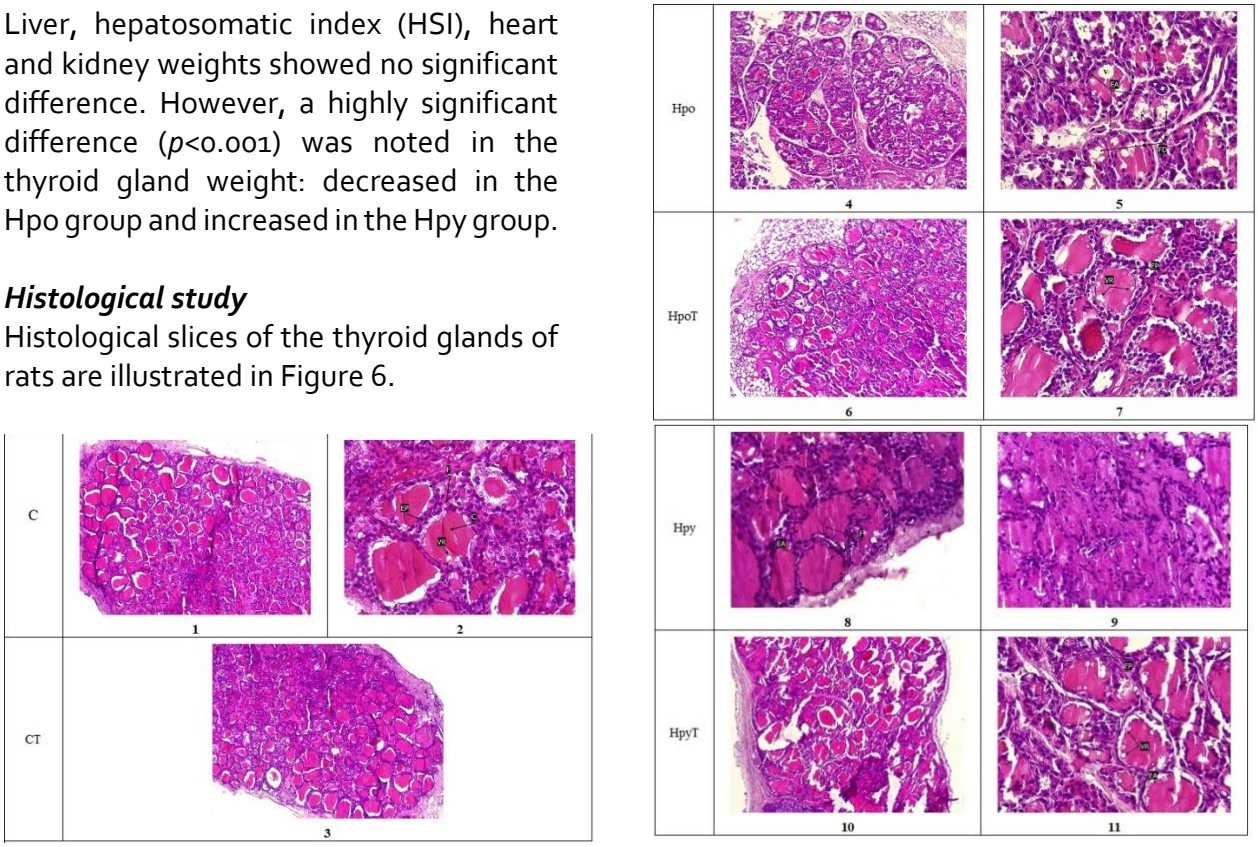

Figure 6. Histological section of thyroid stained with *H&E*

Figure 6.1 (C: 10 ×10) illustrates normal thyroid with follicles (F) lined with a single layer of cubical follicular cells

Figure 6.2 (C: 40 ×10) Presence of resorption vesicles (VR) filled with colloid (C) and covered with prismatic epithelium tissue (EP)

Figure 6.3 (CT: 40 ×10) Normal thyroid

Figure 6.4 (Hpo: 10 ×10) was characterized by follicular degeneration and massive eosinophilic colloid (*)

Figure 6.5 (Hpo: 40 ×10) demonstrates disrupted and fused thyroid follicles (FD) with desquamated epithelial cells in their lumen. Dark nuclei and vacuolated cytoplasm (V) are seen in most follicular cells

Figure 6.6 (HpoT: 10 ×10) represents an active thyroid, with variable size of thyroid follicles (F)

Figure 6.7 (HpoT: 40 ×10) Presents a resorption vesicle (VR), covered by a prismatic epithelium tissue (EP)

Figure 6.8 (Hpy: 10 ×10) increases resorption of colloid (little or no colloid), large follicles of irregular shape and thinner epithelial layer suggesting disruption of follicular walls and fusion of neighboring follicles with desquamated thyrocytes inside the lumen of some follicles noticed

Figure 6.9 (Hpy: 40 ×10) demonstrates total destruction of the follicles, absence of the cubic epithelium, with the presence of a cluster of cells and nuclei

Figure 6.10 (HpyT: 10 ×10) shows an active thyroid with the presence of lesions

Figure 6.11 (HpyT: 40 ×10) shows a primary degenerative process characterized by the reappearance of thyroid follicles of variable size with resorption vesicles (VR) and covered by a prismatic epithelium tissue (EP), but some flattened (EA) cells with flat dark nuclei and lesions are also seen.

The present study was conducted to evaluate the effect of dried *B. incrassatum* tubers powder in experimental hypo and hyperthyroidism. The alterations in thyroid function after Carbimazole and LT_4 exposure were confirmed by histological examination of the thyroid follicles by *H&E* and *MT* according to Hadi & Hamza,[20] and Hammood & Hamza[21] for hypothyroidism. For hyperthyroidism, it was referred by Chi & Guilmette[22] and Park et al.[23]

G1 (C) and G2 (CT) revealed a normal thyroid gland structure with uniform thyroid follicles lined by cuboidal cells containing a colloid in the lumen. No evidence of fibrosis or inflammation was noted (Figures 6.1, 6.2 and 6.3).

The groups affected by thyroid dysfunction and not treated with Talghouda powder (Hpo, Hpy) had an altered thyroid structure, with inactivated follicles (Figures 6.4 and 6.8). The thyroid follicles of Carbimazole administered rats (G3) were closely packed and displayed abundant granular cytoplasm with

reduced lumen size and colloid. Vacuolization of the cytoplasm and widening of the interfollicular space appeared in sections (Figure 6.5).

G5 treated with LT_4 showed complete loss of the normal structure of the thyroid gland. The researchers observed disruption of the basal laminae of some follicles (Figure 6.9). Some follicles appeared distended with colloid lined with squamous epithelium, while others were degenerated (Figure 6.8). Disruption of the apical membranes of some thyrocytes with desquamated cytoplasm and nuclei inside the follicles was also noted.

However, the diseased rats treated with Talghouda (HpoT, HpyT) found a follicular reactivation with the improvement of the tissue damage of the thyroid gland. The densities of colloid staining varied, and some follicles were still disrupted with desquamated cells in the lumens (Figures 6.6 and 6.10). Meanwhile, the thyrocytes appeared less vacuolated.

Our hyperthyroidism model showed a histological change caused by TSH suppression. It is well known that low TSH levels affect the function and structure of the thyroid gland. In hypothyroidism, Carbimazole®, after ingestion, is converted to Methimazole (active form) which acts as a false substrate for thyroid peroxidase, blocking the iodination of tyrosine residues in thyroglobulin and the coupling of iodotyrosines to iodothyronines.[23] The slides revealed a restoration of altered tissues caused by Carbimazole® and levothyroxine® in rats treated with Talghouda. This vegetable seemed to have restored the thyroid cell's structure (Figures 6.7 and 6.11). The thyroid gland regained its normal cellular structure and appeared similar to the control group. Treated animals showed regenerated follicles of variable size with functionality, confirmed by resorption vesicles and prismatic epithelium tissue.

However, the structure of thyroid parenchyma is also similar to those of euthyroid groups (Figure 7).

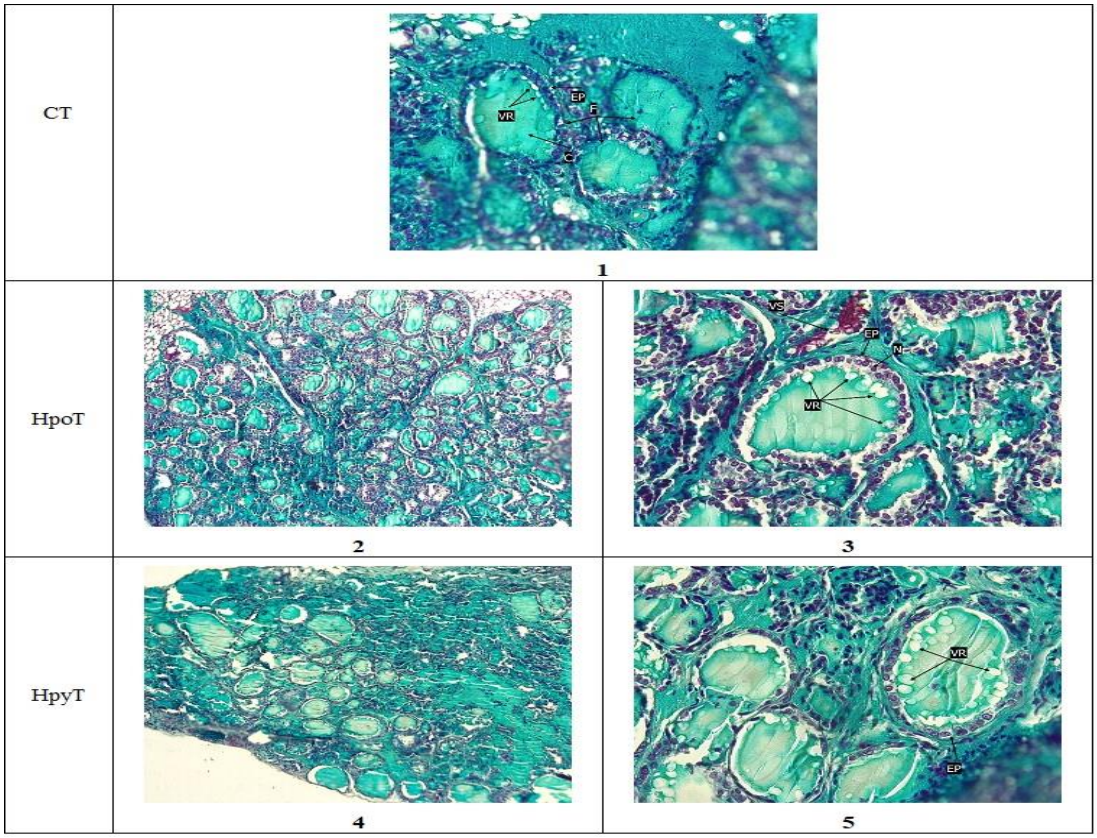

Figure 7. Histological section of thyroid stained with *MT*

Figure 7.1 (CT: 40 ×10) thyroid follicles (F) filled with colloid (C), presence of secretion vesicles (VR) covered by a prismatic epithelium tissue (EP)

Figure 7.2 (HpoT: 10 ×10) represents an active thyroid, with variable size of thyroid follicle

Figure 7.3 (HpoT: 40 ×10) presents a resorption vesicle (VR), follicles (F) filled with colloids (C), covered by a prismatic epithelium tissue (EP), with visible round nuclei (N), surrounded by blood capillaries (VS)

Figure 7.4 (HpyT: 10 ×10) shows an active thyroid with the presence of lesions

Figure 7.5 (HpyT: 40 ×10) shows a primary degenerative process characterized by the reappearance of thyroid follicles of variable size with remnants of colloid (C), resorption vesicles (VR) and covered by a prismatic epithelium tissue (EP). Some dark nuclei and lesions are also seen

Follicles are well adhered to enclosing an active colloid. An experiment made by Bentayeb et al.[25] on the effect of *B. incrassatum* on the immunological expression of estrogen receptors in the adrenal gland suggested that the organic extract, and its composition, mainly β-Sitosterol and β-caryophyllene, had a modulating activity of estrogen receptor. They had a role in preventing and treating breast cancer and the prevention of osteoporosis.

The identification of the bioactive compound(s), which act on hypo and hyperthyroidism, was not studied in this experiment, but nutritional and phytochemical analyses were carried out. According to the literature, several phytosterols, isoflavones such as genistein, and polyphenols stimulated thyroid function by increasing thyroid hormone levels and signaling. However, the underlying mechanisms were not studied[26]. Relevant studies had reported an antithyroid effect of quercetin (flavonoid), which reduced the expression of mRNA-NIS in thyroid cells and inhibited TPO activity, the iodide uptake of the thyroid and the expression of thyroid-

specific genes TG and TSHR[27]. Coumarins, which were the characteristic compounds of the Umbelliferae family, had a value of 17.94 ± 5.25 mg EC/g extract tuber. This molecule can act as an anti-hyperthyroid substance. It seems that this role is mediated by the regulation of extra thyroid conversion from T_4 to T_3 (by changes in the activity of Thyroxine 5-desiodase) and the inhibition of T_4 production in the thyroid gland.[27] In addition, alkaloids content was 0.214 g, suggested to have a double action, in particular arecoline.[28]

Moreover, alkaloids increased noticeably serum levels of T_3 and T_4, associated with a decrease in serum concentrations of TSH during acute exposure. Meanwhile, the long-term treatment (10 mg/kg BW/ d. for 15d.) induced ultra-structural degeneration of thyroid follicular cells with a reduction in T3 and T4 serum levels followed by an increase in TSH. The induction of hypo- and hyperthyroidism affected rats' body weight and hematological profile. In the Hpo group, a decrease in weight was noted. The same event was observed by Hadi & Hamza[20] due to Methimazole (MMI) effect. The hematological profile was altered by hypothyroidism, which might have induced some forms of anemia in humans and experimental animals. Antithyroid agents based on Thioamide such as MMI could reduce RBC count, HT and Hb by suppressing bone marrow.[29]

According to our data, hematological parameters decreased significantly in the Hpo group and might continue to decrease by the progression of hypothyroidism.[30] The decrease observed in the Hpy group reflected hypochromic anemia of inflammatory origin, probably caused by the inflammation of the thyroid gland.[31] Messarah et al revealed that the decrease in erythrocyte parameters, in hyperthyroidism, anemia was probably due to severe hemolysis in response to oxidant/antioxidant system misbalance induced by long-term treatment with thyroxin.[32] A decrease in WBC and lymphocyte levels reflects the fragility of the immune system. Recent studies have shown that innate immune cells could be as important as thyroid hormone target cells. Tight regulation of cellular thyroid hormone availability and action was performed by thyroid hormone transporters, receptors, and the deiodinase enzymes.[33] Furthermore, ingestion of dried Talghouda tuber powder in HpoT and HpyT groups revealed an improvement in body weight and hematological profile. On the one hand, a study on B. incrassatum organic extract showed a non-significant increase in the number of RBCs, GMV, MCHT and MCHC, in rabbits treated with Talghouda extract.

On the other hand, Hb, HT and PLT at a dose of 50 mg/Kg bw/d were shown to be significantly increased.[34] Moreover, Talghouda's antioxidant activity (Table 3) had a role in improving hematological parameters by its capacity to improve the functions of the bone marrow, a major site of erythropoiesis.[35] Evaluation of organ weights (Table 6) noted a decreasing thyroid weight in the Hpo group and an increase in the Hpy group. Thyroid dysfunction was shown to induce a change in absolute and relative thyroid weight.[36]

CONCLUSION

In conclusion, *Bunium incrassatum* or Talghouda improved nutritional status and an immune booster by restoring the thyroid architecture and its functions due to its phytochemical composition and antioxidant activity. However, these results are preliminary and need to be supported by complementary research.

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