

Potential Effect of Garden Cress (*Lepidium Sativum*) Seeds on Hyperthyroidism in Rats Induced by Lthyroxin

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Abstract: The present work was conducted to estimate the potential influence of garden cress seeds powder (GCSP) at concentrations 5, 10, 15 and 20% on the hyperthyroidism in rats. Thirty six female albino rats, weighing 200 ± 10 g. were classified into two main groups. The first main group (6 rats), kept as a negative control group and the second main group (30 rats) were subcutaneous injected by Lthyroxin to induce hyperthyroidism. The last group were classified into 5 sub groups (6 rats per each) as follow: group 2 was left as a positive control group and groups 3, 4, 5 and 6 were fed on basal diet containing 5, 10, 15 and 20% GCSP during the experimental period (60 days). The results indicated that the rats fed on basal diet containing 5, 10, 15 and 20% GCSP showed significant higher in feed intake, body weight gain, FER, TSH, and high density lipoprotein. Whereas showed significantly lower of T3, T4, TG, TC, LDL-c, VLDL-c, AST, ALT, uric acid, creatinine, and urea nitrogen as compared to positive control group. The diet containing 20% GCSP recorded the highest values of all parameters between all tested diets followed by the diet containing 15% of the same powder. Therefore, GCSP at different levels is beneficial to minimize the risk of hyperthyroidism disease.

Keywords: Garden cress seeds powder, T3, T4, TSH, liver and kidney functions, serum lipid profile, nutritional status.

Introduction

Garden Cress (*Lepidium Sativum*) is an annual herb, belonging to *Brassicaceae* family. Garden Cress is known as garden pepper cress, pepper grass and also known as 'Hab el Rashaad' or "Thufa" (Bafeel and Ali 2009 ; Manohar *et al.*, 2012 and Behrouzian *et al.*, 2014). Seed is known to have health elevate properties it served as raw material for functional foods (Rehman *et al.*, 2012 and Gaafar *et al.*, 2013).

The seeds of garden cress are used in different medicinal applications (Maier *et al.*, 1998). They were used as antidiabetic (Eddouks and Maghrani., 2008), anti-asthmatic, diuretic (Eddouks *et al.*, 2002), hypotensive (Maghrani *et al.*, 2005), anti tumors (Zhang and Talalay 1994; Kassie *et al.*, 2003a), treatment of blood and skin disorders (Aburjai *et al.*, 2001) and had hepato-protective role (Kassie *et al.*, 2002 and 2003b).

Hyperthyroidism means elevation of thyroid function. Hyperthyroidism refers that hyper metabolic situation due to excessive level of thyroid hormone secretion (Truter., 2011 and Palacios *et al.* , 2012).

Thyroid hormones consists of tri-iodothyronine, (T3) and tetra-iodothyronine, (T4) which have a strong impact on oxidative stress and regulating the growth

(Karbownik and Lewinski, 2003 and Sigrun and Heike., 2010). Alterations in thyroid hormone causes hypothyroidism (Hypo) or hyperthyroidism (Hyper) (Wiens and Trudeau., 2006). Lthyroxin promoted hyperthyroidism status and increased susceptibility to lipid peroxidation (Serakides *et al*; 2005). In a trial to treat such disorders, the present study aimed to investigate the effect of GCSP on hyperthyroidism in rats induced by Lthyroxin

Materials and Methods

Materials

Garden cress (*Lepidium sativum* L.) seeds were purchased from Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt. L-thyroxin was purchased from MP Biomedical Company, USA. Casein, cellulose, all vitamins and minerals from El-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, El-Mansoura, Egypt.

Methods

Preparation of raw material

Garden cress seeds were cleaned and rendered free of dust, were dried in an air ventilated oven at 40-60 °C, grinded to powder and sieved in 80 mesh per square inch then stored in polyethylene bags in the refrigerator until used.

Chemical constituents of cress seeds

Moisture, protein, crude fibers, fat content and ash contents were determined such as described in AOAC (2000). Total carbohydrates were calculated by difference. Minerals content including Ca, P, K, Mg ,Fe and Zn were determined according to Chapman and Pratt (1978). After complete the digestion process the minerals were determined using Shimatzu atomic absorption Spectrophotometer (Tokyo, Japan)

Biological Experiment

Animals

Thirty six female albino rats (Sprague Dawley Strain), weighing 200±10g, were obtained from National Research Center, Dokki, Egypt.

Basal Diet

The basal diet was prepared according to Reeves *et al.*, (1993).

Experimental Design

All animals were fed on basal diet and water for one week for adaptation period, after this, rats classified into two main groups. The first main group (6 rats) kept as a negative control group and the second main group (30 rats) were subcutaneous injected by Lthyroxin (0.5 mg/kg.bw) for induction hyperthyroidism according to the previous established method (Saxena, *et al.*, 2012). The last group were classified into 5 sub

group (6 rats per each) as follow: group 2 was left as a positive control group and groups 3, 4, 5 and 6 were fed on basal diet containing 5, 10, 15 and 20% GCSP during the experimental period (60 days). During the experimental period (60 days), the feed intake (FI) was calculated daily and the body weight gain (BWG) was recorded weekly and the feed efficiency ratio (FER) at the end of the experiment such as mentioned by Chapman *et al.*, (1950).

Blood Sampling

Rats were fasted overnight then anaesthetized and sacrificed to collect blood samples, centrifuged to separate serum. and kept frozen at -20°C till analysis.

Biochemical Analysis

Serum levels of T3, T4 and thyroid stimulating hormon TSH were analyzed according to Larsen, (1972), schuurs and van weeman, (1977) and Bhowmich *et al.*, (2007), respectively. Serum total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-c), Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were determined according to Richmod (1973); Fossati and Principe (1982); Friede wald *et al.*, (1972), and Lee, and Nieman, (1996), respectively. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were assayed by Reitman and Frankel (1957) and Kind and King, (1954), respectively. Serum uric acid, urea nitrogen and creatinine were assayed such as mentioned by Fossati *et al.*, (1980), Patton and Crouch (1977) and Bohmer (1971), respectively.

Statistical analysis

Data were statistically analyzed of variance "ANOVA" test at $P \leq 0.05$ according to Snedecor and Cochran (1967).

Results and Discussion

Chemical composition of GCSP

The chemical composition of GCSP is presented in Table (1). The moisture, protein, fat, ash, carbohydrate and crude fiber were 6.15, 26.33, 29.21, 5.12, 33.19 and 6.46 g/100g, respectively. These results are in harmony with Mathews *et al.*, (1993), Andersson *et al.*, (1999), Gokavi *et al.*, (2004), Sumangala *et al.*, (2004), Yogesh *et al.*, (2010), Kadam *et al.*, (2012) and Agarwal and Sharma, (2013) and Doke and Guha, (2014) which reported that the chemical composition of GCSP indicates the presence of high amounts of protein (22.47-25.00%) and lipids (14.00-28.03%) which indicated that seeds have high food energy, with low moisture content (3.92-4.14%), also, crude fiber (6.75-16.50%), Ash (4.25-4.65%), and carbohydrates (32.87-54.00%), respectively.

Table (1): Chemical composition of GCSP (g/100g dry weight basis)

Nutrients	Moisture (%)	Crude protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)	Crude fiber (%)
GCSP	6.15±0.9	26.33±2.8	29.21±4.2	5.12±0.8	33.19±4.6	6.46±0.7

The minerals composition of GCSP is illustrated in Table (2). The minerals content of GCSP were Ca, P, K, Mg, Fe and Zn which were (469, 620, 1240, 416, 11.9 and 7.02 mg/100g) respectively. These results are agree with those obtained by (Gopalan *et al.*, 2000; Gokavi *et al.*, 2004; Yadav *et al.*, 2010 ; Yogesh *et al.*, 2010 and Agarwal & Sharma 2013). The last authors reported that garden cress seeds contained the values of mineral Ca, P, Mg, Na, K, Cu, Fe, Mn and Zn, with an average of 480.72; 637.25; 631.06; 36.25; 1635.62; 12.51; 28.82; 46.07 and 62.69 mg/100g , respectively (Mona *et al.*,2015).

Table (2): Minerals content of GCSP (mg/100g)

Minerals	Ca	P	K	Mg	Fe	Zn
GCSP	469±31	620±65	1240±43	416±12	11.9±0.89	7.02±1.22

Effect of feeding on GCSP on BWG %, FI and FER of experimental rat groups

As shown in Table (3) the positive control (untreated group) had significant decreases in BWG , FI and FER when compared to the negative control group, while the groups which fed on diet containing 5%, 10%, 15 and 20 % garden cress seeds powder showed significantly increase in BWG, FI, and FER in comparing with positive control group. These data are similar to the study of **Panda and Kar, (2007)** who found that hyperthyroidism leads to oxidative stress also affected to body adipocyte and body weight decreases, On the other hand, **Mona *et al.*, (2015)** reported that protective groups which fed on basal diet containing garden cress seeds overproduce weight gain because of its containing 18-24% of fat. It contained (alpha linolenic acid 34%) of total fatty acids which gave it nutritional values. These results confirmed also by **Doke and Guha., (2014)**.

Effect of GCSP on serum level of T3, T4 and TSH of hyperthyroidism rats induced by Lthyroxin

The obtained results in Table (4) indicated that the positive control group recorded significantly higher in T3 and concentrations of T4 while recorded significantly lower in TSH when compared to the negative control group, All the groups treated with garden cress seeds powder recorded significantly higher in T3 and T4 while recorded significantly lower in TSH when compared to the negative control group except the

group treated with garden cress seeds powder at concentration 20% showed no significant. On the other hand all the groups treated with garden cress seeds powder recorded significantly lower in T3 and T4 while recorded significantly higher in TSH in comparing with positive

Table (3): Effect of GCSP on BWG , FI and FER of hyperthyroidism rats induced by Lthyroxin

Groups	Weight gain (g)	Feed intake (g/day)	FER
Normal control group (-ve)	93.76±8.11 ^a	17.94± 2.20 ^a	0.187±0.03 ^a
Positive control group (+ve)	20.64±3.11 ^d	13.94± 2.20 ^b	0.67±0.06 ^d
GCSP 5%	85.13±9.13 ^c	16.66±2.32 ^a	0.185±0.04 ^{ab}
GCSP 10%	88.77±9.17 ^c	16.65±2.21 ^a	0.183±0.03 ^b
GCSP 15%	89.44±9.28 ^b	16.68±2.92 ^a	0.185±0.04 ^{ab}
GCSP 20%	92.44±9.35 ^b	16.66±2.32 ^a	0.180±0.04 ^c

Mean values in each column having different superscript letters denote significant difference at P<0.05

Table (4): Effect of GCSP on serum level of T3, T4 and TSH of hyperthyroidism rats induced by Lthyroxin

Groups	T3 (µg/dl)	T4 (µg/dl)	TSH (µIU/ml)
Normal control group (-ve)	2.12±0.17 ^d	9.87±0.75 ^d	5.13±0.32 ^a
Positive control group (+ve)	6.82±1.7 ^a	18.33±2.55 ^a	1.84±0.16 ^d
GCSP 5 %	4.96±0.11 ^b	15.15±0.80 ^b	2.654±0.13 ^c
GCSP 10 %	3.81±0.10 ^c	10.89±0.09 ^c	3.47±0.37 ^b
GCSP 15 %	3.51± 0.15 ^c	10.59±0.72 ^c	3.98±0.21 ^b
GCSP 20 %	3.04± 0.12 ^c	10.43±0.70 ^d	4.02±0.23 ^{ab}

Mean values in each column having different superscript letters denote significant difference at P<0.05

control group. These data are confirmed with **Al-Jenoobi et al., (2014)** who revealed that garden cress contains goitrogens, which interference iodine absorption in thyroid gland (**Duke, 1992**). Also **Nayak et al., (2009)** and **Ghante et al., (2011)** reported that GCSP contains flavonoids, cardiotoxic glycosides, glucosinolates, sterols, tannins, and triterpene used as pharmacological characteristics to garden cress seed. Hyperthyroidism is due to increase in serum T3 and T4 with lowering of serum TSH, a pituitary hormone that organize thyroid functions (**Elnagar et al. 1998**).

Effect of GCSP on serum lipid profile of hyperthyroidism rats induced by L-thyroxin

As recorded in Table (5) control (+ve) group showed elevated significantly in TG, TC, LDL-c, and VLDL-c, while showed lowered significantly in HDL-c in comparing with normal control group. Animals treated with garden cress seeds powder at concentrations 5%, 10%, 15 and 20 % recorded highest significantly in TC, TG , LDL-c and VLDL-c and showed lowest significantly in HDL-c in comparing with normal control group while showed lowest significantly in these previous parameters and highest significantly in HDL-c compare with positive control group. These results in parallel with **Behrouzian et al., (2014)**, **Doke and Guha (2014)** and **Mona et al., (2015)** who reported that rats which fed on diet fortified with garden cress seeds powder at 5% and 10% had lower mean values of LDL-C and VLDL-C and higher mean values of HDL-C compared with the positive control group. Aqueous extract of *Lepidium sativum L.* improve lipid profile in comparison to control groups (**Amawi and Aljamal., 2012**). GCSP supplementation in the human diet is efficient in depressing cholesterol and triglycerides (**Al Hamedan., 2010 ; Diwakara, et al., 2010 and Chauhan et al., 2012**).

Effect of GCSP on liver and kidney functions of hyperthyroidism rats induced by Lthyroxin

As shown in Table (6), the control (+ve) showed a significant increase in AST, ALT, uric acid, creatinine and urea nitrogen in compared with normal control group, All the groups treated with GCSP recorded significantly higher in AST, ALT, uric acid, creatinine and urea nitrogen in compared with normal control group except the group treated with garden cress seeds powder at concentration 20% showed no significant in creatinine and urea nitrogen in compared with normal control group. On the other hand all the groups treated with garden cress seeds powder recorded significantly lower in AST, ALT, uric acid, creatinine and urea nitrogen in compared with positive control group. Direct effect of hyperthyroidisms on organ damages induced by Lthyroxin through antioxidant effects (**Seong, et al., 2012**). Excessive virility of ALT, AST enzymes in serum caused by oxidative stress induced by hyperthyroidism (**Subudhi, et al., 2008**). Thyroid hormone plays an important role in hepatocyte diffusion of rat liver (**Videla, 2000**).

Table (5): Effect of GCSP on serum lipid profile of hyperthyroidism rats induced by Lthyroxin

Groups	TG mg/dl	TC mg/dl	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl
Normal control group (-ve)	45.16± 2.28 ^c	75.49 ± 3.86 ^d	28.35± 0.71 ^a	38.30± 1.25 ^d	9.03± 0.45 ^d
Positive control group (+ve)	75.43± 4.15 ^a	121.46 ± 4.84 ^a	19.47± 1.18 ^d	86.21± 2.83 ^a	15.85± 0.83 ^a
GCSP 5 %	51.19± 3.46 ^b	81.27± 3.54 ^b	24.03± 1.37 ^c	46.97± 2.75 ^b	10.29± 0.62 ^b
GCSP 10 %	49.18± 2.79 ^b	80.14± 3.74 ^b	27.71 ± 2.06 ^b	42.60± 1.74 ^c	9.83± 0.59 ^c
GCSP 15 %	47.76± 2.75 ^b	77.46± 3.65 ^c	26.92 ± 2.11 ^b	40.97± 1.13 ^c	9.57± 0.48 ^c
GCSP 20 %	47.19± 2.65 ^b	77.11± 3.12 ^c	26.96 ± 2.17 ^b	40.70± 1.56 ^c	9.45± 0.33 ^c

Mean values in each column having different superscript letters denote significant difference at P<0.05

The results are in agreement with **Mona *et al.*, (2015)** who cleared that oral administration of 5 and 10% garden cress seeds powder reduced uric acid, creatinine and urea nitrogen for rats suffering from acute kidney disease, and administration of Curative & protective rats fed on basal diet containing garden cress seeds powder at 5 and 10% caused a significant reduction in the mean values of serum AST and ALT as compared to the positive control group. High concentration of garden cress seeds safe and improves liver functions, and can protect the liver from chemically induced damage confirmed by **Vishwanath *et al.*, (2012)** and **Behrouzian *et al.*, (2014)**. The addition of GCSP in diet lead to lowering in the levels of urea and creatinine, and activity of AST and ALT as compared to cholesterol fed rats (**Al Hamedan, 2010**).

Table (6): Effect of GCSP on liver and kidney functions of hyperthyroidism rats induced by Lthyroxin

Groups	AST (U/L)	ALT (U/L)	Uric acid mg/dl	Creatinine mg/dl	Urea Nitrogen mg/dl
Normal control group (-ve)	43.82± 2.11 ^d	22.51± 1.78 ^d	1.41± 0.42 ^d	1.34± 0.54 ^d	35.99± 2.91 ^d
Positive control group (+ve)	84.07± 5.58 ^a	46.36± 3.27 ^a	2.99± 0.62 ^a	3.91± 0.47 ^a	62.96± 3.57 ^a
GCSP 5 %	60.08± 4.28 ^b	35.99± 3.43 ^b	2.58± 0.16 ^b	2.47± 0.17 ^b	56.39± 4.48 ^b
GCSP 10 %	46.82± 3.02 ^c	26.73± 2.14 ^c	1.81± 0.80 ^{bc}	2.00± 0.27 ^b	40.25± 3.41 ^b
GCSP 15 %	47.08± 4.13 ^c	25.13± 3.43 ^c	1.79± 0.70 ^c	1.85± 0.25 ^c	37.77± 2.35 ^c
GCSP 20 %	46.61± 3.12 ^c	24.53± 2.11 ^c	1.75± 0.60 ^c	1.62± 0.45 ^{cd}	36.24± 2.31 ^{cd}

Mean values in each column having different superscript letters denote significant difference at P<0.05

In conclusion, the results suggest that GCSP is a good source of protein, fat, and minerals (calcium, phosphorus, potassium, magnesium, iron and zinc) which are vital for human nutrition and maintains a good health, beside, improve the BWG, FI and FER, with declining the serum lipid profile, AST , ALT, uric acid, urea and creatinine and reduces hyperthyroidism in rats.

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التأثير المحتمل لمسحوق بذور حب الرشاد فى علاج النشاط الزائد للغدة الدرقية فى

فئران التجارب المستحث بالثيرواكسين

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ملخص

نبات حب الرشاد من الاعشاب التى تنمو فى مصر وغرب آسيا - بذوره غنية بالبروتين، الالياف، الاحماض الدهنية اوميغا 3، الحديد والمغذيات الاساسية والمركبات الفينولية. فى هذا البحث يتم دراسة امكانية تأثير مسحوق بذور حب الرشاد بنسب 5، 10، 15، 20% على الفئران المصابة بارتفاع نشاط الغدة الدرقية، حيث أجريت الدراسة على 36 من فئران التجارب قسمت الى مجموعتين رئيسيتين، المجموعة الرئيسية الاولى (6 فئران) تغذت على الوجبة الاساسية وهى المجموعة الكنترول السالبة و المجموعة الرئيسية الثانية (30 فأر) حقنت بالثيروكسين (0.5ملجم / كجم وزن الجسم) مرتين اسبوعيا لزيادة نشاط الغدة الدرقية ثم قسمت الى المجموعة الثانية كنترول مريض، و المجموعات 3 ، 4 ، 5 ، 6 تناولت بذور حب الرشاد بنسب 5، 10، 15، 20% لمدة 60 يوما مدة الدراسة ، وقد اظهرت النتائج تحسن فى المجموعات التى تناولت حب الرشاد بنسبه المختلفة فى اوزان الفئران و هرمون الثيروكسين و الليبوبروتينات مرتفعة الكثافة كما أوضحت النتائج انخفاض معنوى فى هرمونات T3, T4 والدهون الثلاثية و الكوليستيرول الكلى، الليبوبروتينات منخفضة الكثافة وانزيمات ألانين و إسبارتات أمينو ترانسفيريز وحمض اليوريك و الكيرياتين والنيتروجين مقارنة بالكنترول المريض وحققت مجموعة الفئران التى تناولت 20% حب الرشاد افضل النتائج يليها 15%. لذلك توصى الدراسة بضرورة تناول حب الرشاد لخفض النشاط الزائد للغدة الدرقية.

الكلمات المفتاحية: مسحوق بذور حب الرشاد، ت3، ت4، تى أس أنش، وظائف الكبد والكلى، صورة دهون الدم، الحالة الغذائية.