The Egyptian Journal of Hospital Medicine (October 2017) Vol.69 (3), Page 2137-2142

Effect of Egyptian Red Cabbage on Some Physiological Parameters in Hyperthyroidimic Rats

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ABSTRACT
Background: this study aimed to illustrate the protective effect of red cabbage extract against hyperthyroidism induced by L-thyroxin in male albino rats. **Materials and methods:** Eighteen animals were divided randomly into three groups. **Group A**: healthy rats, **Group B:** normal rats were subcutaneous injected with 500 µg/kg body weight/day L-thyroxin once daily for two weeks to induce hyperthyroidism and **group C**: hyperthyroid rats received red cabbage extract. At the end of the experiment, blood samples were collected for biochemical analysis. **Results**: hyperthyroid rats induced by L-thyroxin had significant decrease ($P<0.05$) in serum levels of triodothyronine (T3), thyroxin (T4), protein profile, lipid profile (TC, HDL) as well as a significant increase in kidney function test (BUN, uric acid), in liver enzymes (ALP, ALT), and a significant decrease in TSH, TG, VLDL compared to the normal control group. Oral administration of red cabbage extract ameliorated most of the tested parameters.
Keywords: hyperthyroidism- red cabbage- rat- kidney function.

INTRODUCTION
Thyroid hormones (TH) are required for the normal function of most tissues of the body, playing essential roles in growth, development, differentiation, and metabolism, with major effects on O_2 consumption and metabolic rate [(1)]. Hyperthyroidism is associated with an increased metabolic rate due to increased rate of oxygen consumption in target tissues. Acceleration of aerobic metabolism by thyroid hormones enhances the generation of oxidative stress[(2)].

Despite the fact that day-by-day herbal drugs are gaining much importance for their affordable and safe nature, scientific investigations towards the mitigation of thyroid disorders by the plant extracts are meager [(3)]. Large number of these plants and their isolated constituents have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and immunomodulatory effects. Cruciferous vegetables are the only source of sulfur-containing compounds called glucosinolates that are responsible for their bitter flavor[(4)]. Glucosinolates are digested into isothiocyanates that reduce inflammation and fight bacteria. The red pigment comes from a flavonoid, cyanidin, that functions as an antioxidant. In addition to these important phytochemicals, cabbage contributes to overall health with fiber and a range of vitamins and minerals[(4)] .

Among natural plants, red cabbage (RC) (*Brassica oleracea*) has been found to have antioxidant, ant hyperglycemic,anticancer, and hypocholesterolemic[(5)] properties. Cabbage is an excellent source of vitamin C and also contains significant amounts of glutamine and amino acid that has anti-inflammatory properties.The principle constituents of RC are isothiocyanates (glucosinolate), vitamins A, B, C and anthocyanins which is a group of phenolic natural pigments that found to have the strongest antioxidizing power of 150 flavonoids . RC extract has also preventive oxidative stress induced in livers[(6)]

MATERIALS AND METHODS
Preparation of RED CABBAGE extract
Red cabbage leaves were sliced into small pieces and oven-dried at 50℃. Dried plants (8g) were extracted in 1000 ml of 70% aqueous ethanol using ultrasonic treatment at an intensity of 70 W/ cm^2 and oscillation frequency at 20 kHz for 5 min. Ultrasonic irradiation was applied by means of a Branson 450 digital sonifier (20 kHz, 450 W) equipped with a cylindrical titanium alloy probe (12.7 mm in diameter). After overnight maceration, the extract was filtered through gauze and ethanol was evaporated under reduced pressure at 50ºC by using a rotary evaporator. The remaining water extract was dried using a freeze dry system under reduced

Received: 3 /8 /2017
Accepted: 13/ 8 /2017
DOI : 10.12816/0041072

pressure. The dried extract was dissolved in distilled water before administration to hyperthyroidimic rats.

Experimental Animals

This experiment was carried out on 18 male Swiss albino rats weighing 100–120 g obtained from the Animal Farm of the El-Nile Company for Pharmaceutical Products (El-Nile, Cairo, Egypt). Animals were housed in metallic cages and maintained under standard conditions of temperature, humidity and 12 hr light/dark cycle along the experimental period. Food and water were available throughout the experiment *ad libitum*. Rats were left to acclimatize for one week before starting the experiment.

Experimental Design

In the current study, 18 male Swiss albino rats (100-120) were divided randomly and assigned into three equal experimental groups (6 rats in each group) as the following: **Group I: (**Control group) comprised of normal rats and maintained standard pellet diet and tap water *ad libitum* for two weeks. **Group II:** (L-Thyroxin group) rats were injected subcutaneously with 500 µg/kg body weight of L-Thyroxin dissolved in physiological saline for 15 successive days. **Group III: (**Red cabbage + L-Thyroxin) comprised of normal rats injected with L-Thyroxin as in the group II in concomitant with oral administration of RCE extract (100 mg/kg/day) ten minutes after L-Thyroxin injection for two weeks. All the treatments were given between 9.00 and 11.00 h of the day to avoid circadian variation.

Body weight measurement

Body weight was recorded weekly beginning on zero time (the time prior to treatment) and continued until the end of the treatment.

Blood sample collection

At the end of the experimental period, the blood samples were collected from the retero-orbital sinus after overnight fasting and anesthetized by ether. Serum was separated by centrifugation at 2500 g for 15 minutes at room temperature to estimate biochemical parameters.

Biochemical analysis

Assessment of biochemical parameters:

In the present study total protein (TP) and albumin concentrations were estimated, then serum globulin concentrations were calculated according to the formula:

Globulin (g/dl) = total protein (g/dl) –albumin (g/dl)

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, creatinine, BUN concentrations as well as lipid profile that including total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were also determined. Concentrations of TSH and thyroid hormones (T3 and T4) and testosterone were measured. All parameters were estimated using Bio Merieux SA kits, France.

The ratio of serum albumin/ globulin was determined. However, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were also calculated after calculation of serum LDL-C (low-density lipoprotein cholesterol) and VLDL (very low-density lipoprotein cholesterol) using the Friedwald's[7] and Norbert[8] formulas, respectively as following:

Friedewald's equation: LDL (mg/dl) = TC- {HDL + [TG/5]}.

Norbert equation: VLDL = TG/5

Statistical analysis

The results were expressed as Mean ± SEM of the mean. Data were analyzed by one way analysis of variance (ANOVA) and were performed using the Statistical Package (SPSS) program, version 20. The Bonferroni test was used as a method to compare significance between groups.

The study was approved by the Ethics Board of Al-Azhar University

RESULTS

Body weight: animals that received L- thyroxin has a highly significant decrease in body weight ($p<0.01$), while those administrated with red cabbage showed insignificant changes as compared to control rats (Table 1).

Protein profile

The present study showed that administration of thyroxin to normal rats significantly($p<0.05$) decreased the total protein and showed insignificant change in albumin, globulin, albumin\ globulin ratio levels as compared to control . Meanwhile, the administration of the plant extract to hyperthyroidimic rats recorded insignificant change in the concentration of these parameters as compared to the control group (Table 2).

Liver functions

The data in table 3 revealed insignificant change in serum ALT activity, and significant increase ($p<0.05$) in AST and ALP activities in hyperthyroid

group as compared with the control rats. Meanwhile, the administration of plant extract recorded insignificant change in the concentration of these parameters as compared to control group (Table 3).

Kidney functions

The data in table 4 showed that there was insignificant change in serum creatinine in all groups, and highly significant increase ($p<0.01$) and a significant increase ($p<0.05$) in serum BUN for hyperthyroidism and red cabbage -treated group respectively. Also There was a significant increase ($p<0.05$) in uric acid in both treated groups.

Percentage of change in L-thyroxin-treated rats of BUN, uric acid was 20.8% and 9.9 respectively. After using red cabbage, this ratio changed to 70% and 35%.as compared to control group.

Lipid profile

T4-induced a significant decrease ($p<0.05$) in HDL and a significant increase ($p<0.05$) in TG while there was insignificant change in TC,HDL,LDL,VLDL,TC/HDL,LDL/HDL as compared to control animals .The present results showed that Treatment of hyperthyroidimc rats with red cabbage extract recorded a significant increase ($p<0.05$) in triglyceride level(TG). Further more,red cabbage recorded insignificant change in TC,LDL,HDL,VLDL,LDL/HDL ,TC/HDL as compared to control group (Table 5) .

Hormones

The data in table (6) show that T_3,T_4 significantly increased ($p<0.05$) ,while serum TSH was significantly decrease ($p<0.05$) in hyperthyroid rats, accompanied with insignificant change in testosterone level when compared to control rats .Red cabbage treatment recorded insignificant change in the concentration of all these hormones as compared to the control group (Table 6).

Table 1: Changes in the Body weight in the control and treated groups

Groups	Control	hyperthyroid rats	red cabbage- treated hyperthyroid rats
Body weight	2.5±0.6	-3±o.8**	2 ±0.42

Values represent mean ±SE(standard error).(p*<0.05, p**<0.001as compared to control group).

Table 2: Changes in the total protein, albumin, globulin, albumin/globulin and albumin/creatinine levels in the control and treated groups

GroupS / Parameters	Control	hyperthyroid rat	red cabbage
Total protein (g/dl)	6.3±0.18	5.2±0.3* 17.5%	5.9±0.1 6.3%
Albumin (g/dl)	3.4±0.2	2.5±0.4 26.5%	3.1±0.1 8.8%
Globulin (g/dl)	2.9±0.2	2.7±0.01 6.9%	2.8±0.2 3.4%
Albumin/Globulin(g/dl)	1.17±0.1	0.9±0.13 23%	1.1±0.19 5.9%

Values represent mean ±SE(standard error).(p*<0.05, p**<0.001as compared to control group).

Table 3: Changes in the ALT, AST and ALP levels in the control and treated groups

GroupS / Parameters	Control	hyperthyroid rat	red cabbage
ALT(U/L)	22.5±1.6	24.5±1.7	24.5±1.6 8.9%
AST(U/L)	35.5±1.1	40±1.1* 12.7%	38±1.2 7%
ALP(U/L)	61±1.5	66±1.1* 8.2%	64±1.2 4.9%

Values represent mean ±SE(standard error).(p*<0.05, p**<0.001as compared to control group).

Table 4: Changes in the BUN ,creatinine and uric acid levels in the control and treated groups

Groups / Parameters	Control	hyperthyroid rat	red cabbage
BUN (mg/dl)	18.2 ±0.5	22±0.2** 20.8%	20.±0.1* 9.9%
Creatinine (mg/dl)	0.4±0.1	0.35±0.01 50%	0.3±0.1 25%
Uric acid (mg/dl)	4.0±0.5	6.8 ±1.1* 35%	5.4±0.1* 35%

Values represent mean ±SE(standard error).(p*<0.05, p**<0.001as compared to control group).

Table 5: Changes in the lipid profile in the control and treated groups

Groups / Parameters	Control	hyperthyroid rat	red cabbage
T C(mg/dl)	74±0.9	72±0.5	73.5±0.6
T G(mg/dl)	65±0.8	69±0.9*	68±1.1*
HDL(mg/dl)	40±0.9	36±1.43*	40.1±0.5
LDL(mg/dl)	21±0.5	22.2 ±0.2	21.4±0.5
VLDL(mg/dl)	13±0.5	13.8±0.2	14±0.2
LDL/HDL(mg/dl)	0.52**±0.1**	0.61±0.3	0.53±0.1
TC/HDL(mg/dl)	1.85**±0.4**	**2±0.3**	1.69±0.1

Values represent mean ±SE(standard error).(p*<0.05, p**<0.001as compared to control group).

Table 6: Changes in the TSH, T3, T4 and Testosterone levels in the control and treated groups

Groups / Parameters	Control	hyperthyroid rat	red cabbage
TSH(µu/dl)	3±0.1	1.9 ±0.4*	2.3±0.54 23.3%
T3(µu /dl)	1.9±0.1	6.5±1.5*	2±0.1 5.3%
T4(µg/dl)	5.5±0.3	11±1.6*	6.5±0.5 18.2%
Testosteron(µu /dl)	3.6±0.3	4±0.4	3.7±0.1 2.8%

Values represent mean ±SE(standard error).(p*<0.05, p**<0.001as compared to control group).

DISCUSSION

Hyperthyroidism is the result of excess synthesis and release of thyroid hormones. Thyrotoxicosis is the hyper metabolic state associated with elevated levels of free thyroxine

(fT4), free triiodothyronine (fT3), or both and decrease in TSH level[9]. Several studies have shown that vegetables play a protective role against the development of human diseases. Vegetables like cabbage belonging to the cruciferous family exert a protective effect against many chronic degenerative diseases[9].

Glucosinolates are particularly abundant in cabbage and are believed to be the bioactive compounds responsible for many of the biological effects attributed to them .However, cabbage is also an important source of other essential compounds such as polyphenols, carotenoids and phytosterols that exert an anti-inflammatory and antioxidant effect[10]. as well as flavonoids and alkaloids that were reported to have antioxidant effect. Treatment with L-Thyroxin lead to significant reduction of body weight. Red cabbage has a thyroid function suppressing action. In the present study, increased plasma T_3 and T_4 levels and decreased in TSH levels were observed in the hyperthyroid animals induced by thyroxin. Red cabbadge has a thyroid function suppressing action so lead to increase body weight. . Red cabbage group recorded insignificant change in all measured hormones in comparison with control rats. This data agree well with the results of **Jin *et al.*[11]**.Goitrogens interfere with production of thyroxin in the thyrocytes[12]. Lipid profile results showed insignificant change of all lipids except TG increaseand HDL decrease in the hyperthyrodimic group compared to the control group . Red cabbage intake increased activity of the HMG - CoA reductase, levels of total cholesterol, LDL-C, tend to decrease in experimentally rats induced hyperthyroidism. This may be due to increased LDL receptor or gene expression resulting in enhanced LDL receptor activity, which results in increased catabolism of LDL and IDL. Moreover, triiodothyronine (T3) upregulates LDL receptors by controlling the LDL receptor gene activation. This T3-mediated gene activation is done by the direct binding of T3 to specific thyroid hormone responsive element**[13].** Red cabbage extract effect on the lipid profile pattern in hyperthyroidism animals induced by thyroxin, was decrease in triglyceride and increase the levels of cholesterol to normal pattern as in healthy group due to anthocyanin content. The extract contains procyanidins which has been shown to decrease triglycerides and increase HDL-cholesterol levels in rats In another short-term animal study, anthocyanin glycosides (leucopelargonin derivatives) from the bark of F. Bengalensis have shown hypocholesterolemic and antioxidant activities. The liver is regarded as the major organ of metabolism. The liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are considered as biomarkers of liver toxicity and are used in the evaluation of hepatic disorders. The result showed that increasing plasma ALP, AST level were observed in the hyperthyroid animal induced by thyroxin .Cabbage extract supplemented group recorded significant change in comparison with control rats[9] . Considerably elevated AST in response to increased plasma levels of T3 and T4. This study concluded that red cabbage have a protective effect against hepatocellular damage in hyperthyroid animals induced by L-thyroxine and these results agrees well with that reported by ***Menak et al.* [14].** Kidney is vulnerable to damage because of larger perfusion and the increased concentration of excreted compounds that occur in renal tubular cells[15] BUN is the major nitrogen-containing metabolic product of protein catabolism and creatinine is another product of protein metabolism. The increase in serum BUN, uric acid levels in L-thyroxin treated group indicates impairment in the normal kidney function of the animal . The mechanism of removing them from the blood might have been affected. It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney, it is well known that, many biochemical and histopathological findings confirmed renal damage in hyperthyrodimic animals. In the current investigation increased serum BUN and uric acid and decreased levels of creatinine reflect the diagnosis of renal dis function**[15].** Administration of C.E. nearly protects the kidney function were it recorded less percentage of change in this parameter in comparison with normal rats. The amelioration in liver and kidney function tests after using red cabbage may be due to its content of cabbage contains Anthocyanin, which is a phenolic natural pigment present in red cabbage extract, it was demonstrated to have the autoxidizing power of 150 flavonoids. Hence RC polar extract has significant anti hyperthyroid activity that may, at least in part, modulate the oxidative stress caused by hyperthyroid induced generation free radicals[12].The significant reduction in total protein content in hyperthyrodimic rats is in concomitant with our results , and this may be due to

reduction in the three major phases in protein secretion, intracellular transport and discharge. The reduction in total protein may be due to significant increase in protein excretion by impaired kidney. Non-enzymatic glycation of albumin was the potential to alter its biological structure and function . Hypoalbuminemia is one of the factors responsible for the onset of ascites related to liver fibrosis . This study concluded that red cabbage have a protective effect against increase in protein excretion in hyperthyroid animals induced by L-thyroxine[16] . Red cabbage is goitergen so it reduced T3,T4which lead to amelioration of protein profile . In the present study, increased plasma testosterone levels were observed in the hyperthyroid animals induced by thyroxine. Red cabbage recorded insignificant change in comparison with control rat. Cabbage is loaded with vitamins and minerals. The body of men produce female hormone oestrogen when they put on weight. Oestrogen reduces the effectiveness of testosterone. Cabbage contains indole-3-carbinol, which reduces levels of oestrogen, allowing testosterone to do its function[17] .

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