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Original article

Effect of broccoli sprouts on thyroid function, haematological, biochemical, and immunological parameters in rats with thyroid imbalance

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ABSTRACT

Broccoli sprouts may exert a negative influence on thyroid function as they are a rich source of glucosinolates, in particular glucoraphanin. Under the study in a long-term experiment broccoli sprouts were tested as an element of rats diet, combined with deficient iodine, or sulfadimethoxine ingestion – two models of hypothyroidism. Evaluations were performed for serum TSH and thyroid hormones completed with analyzes of selected haematological, biochemical and immunological (IL-6, IL-10) parameters, as well as cytosolic glutathione peroxidase (GPX1), thioredoxin reductase (TR) in the thyroid, and plasma glutathione peroxidase (GPX3). A thermographic analysis was conducted to provide auxiliary indicators for determining a potential thyroid dysfunction under the specific experimental conditions. The levels of TSH, fT3 and fT4 remained unchanged following broccoli sprouts ingestion, which was even found to have a protective effect against sulfadimethoxine induced thyroid damage. Moreover, TR activity significantly increased in response to sprouts ingestion. In animals with hypothyroidism, broccoli sprouts were found to exert a beneficial influence on the antioxidant balance of the thyroid gland. In comparison to the rats with iodine deficiency, broccoli sprouts addition to the diet was observed to decrease IL-6 level. No significant differences in IL-10 concentration were determined.

Neither addition of broccoli sprouts to the diet, nor sulfadimethoxine and iodine deficiency, caused negative changes in red blood cell parameters, glucose and uric acid concentrations, or kidney function. However, such a dietary intervention resulted in reduced WBC and PLT levels, and it may adversely interfere with liver function in rats, most likely due to a higher dietary intake of glucosinolates.

1. Introduction

Broccoli sprouts have been long recognized as one of the most significant and well known elements of functional food. The sprouts have been shown to exert chemoprotective effects [1,2] and to reduce cholesterol or lipid levels [3]. They are also a powerful bactericide against *Helicobacter pylori* infections [4]. They may improve insulin resistance in type II diabetes and many other positive effects have been reported for them [5]. No evidence has been found so far for broccoli sprouts to interact with thyroid function in models of potential hypothyroidism. Only Shapiro et al. [6] have published results of their clinical phase I study where safety, tolerance and metabolism of broccoli sprouts were investigated in healthy volunteers. Evaluation of the effect of broccoli on the thyroid function is interesting, as the plant is protective against thyroid carcinoma [7,8]. On the other hand, different brassica vegetables can be responsible for impairment of thyroid gland function in different species, particularly in poultry, pigs and rats [9–11]. Dal Maso et al. [8] indicated that historically reported adverse effects of very high consumption of cabbages and other cruciferous vegetables (e.g. induction of thyroid cancer) are still rooted in public opinion. The influence of such hints is observed in many dietetic recommendations regarding these vegetables presumable involvement in the induction of hypothyroidism. Despite more recent epidemiological studies exploring associations between cruciferous vegetables and thyroid diseases which give only limited support to the previous hypotheses, due to repeated public exposure to the other statements, they have become considered confirmed recommendations. Therefore, the safety of broccoli sprouts as a significant element of functional food should be revisited with respect to their role in thyroid function.

The main aim of this investigation was to study the effects of

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broccoli sprouts on the thyroid gland and on certain haematological, biochemical, and immunological parameters of a rat organism. Three diet models were applied in the experiment, namely, a normal diet, a model based on a diet with iodine deficiency causing thyroid hyperplasia [12,13], and another one based on sulfadimethoxine (SDM) added as an ingredient (0.025%) to the animal drinking water and causing thyroid damage by inhibiting thyroid hormone synthesis [14]. Serum concentration of thyroid-stimulating hormone (TSH), free thyroid hormones (triiodothyronine (fT3), and thyroxine (fT4) served as animal response parameters, while red blood cell count (RBC), haemoglobin (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), thrombocyte count (PLT) represented haematological parameters; glucose (Glu), uric acid (UA), urea (U), aspartate transaminase (ASPAT), alanine transaminase (ALAT), creatinine (Crea), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), alkaline phosphatase (PAL) were used as biochemical parameters; and last but not least interleukin 6 (IL-6), interleukin 10 (IL-10) as immunological parameters. Enzyme activities of cytosolic glutathione peroxidase (GPX1) and thioredoxin reductase (TR) in the thyroid, ferric reducing ability of plasma (FRAP), plasma glutathione peroxidase (GPX3) activity, along with evaluated body temperature complete the list of parameters under investigation.

2. Materials and methods

2.1. Plant material

Voucher specimens of broccoli seeds (*Brassica oleraceae convar. botrytis var. cymosa*) were deposited at the Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College (No#BOCB/PP/PL 1036). Four day old sprouts were harvested by the Uniflora Company, Poland. After sprouting, the materials were lyophilized to obtain dry material suitable for preparation of animal fodder. In lyophilized broccoli sprouts sulforaphane concentration was evaluated by UPLC–MS/MS method, and the mean value was 113.33 ± 12.58 mg/100 g dw. Qualitative HPLC analysis of methanol extracts of broccoli sprouts revealed a number of phenolic acids: chlorogenic, *p*-coumaric, ferulic, gentisic and sinapic acids, and also robinin and traces of myrycetin, luteolin, quercetin and apigenin. The quantitative HPLC analysis was used for predominant polyphenols: chlorogenic acid (37.26 ± 0.6 mg/100 g dw); *p*-coumaric acid (27.75 ± 0.70 mg/100 g dw); ferulic acid (73.85 ± 3.50 mg/100 g dw); gentisic acid (80.80 ± 4.79 mg/100 g dw); sinapic acid (140.53 ± 3.17 mg/100 g dw); robinin (1.04 ± 0.10 mg/100 g dw). The results of fatty acids profile showed that saturated acids in broccoli sprouts consisted 11% of total pool of fatty acids, with palmitic (5.7%) and stearic (2.8%) acids being the dominating compounds. The relative content of unsaturated fatty acids in the analyzed broccoli sprouts was found to be 89%, with predominant oleic acid (45.5%), linoleic (20.8%) and alpha-linolenic acids (17.06%).

2.2. Animals

The 72 male (mean weight 249.5 ± 9.1 g) 4-week-old Wistar rats were maintained in plastic cages in an air-conditioned animal room in the Animal House of the Faculty of Pharmacy, Jagiellonian University Medical College for one week before the experiment at the temperature of 22 ± 2 °C, with a relative humidity of 50 ± 5%, and 12 h periods of light and darkness. After 1 week of acclimatization, the rats were divided into 6 groups, each consisting of 12 animals, and fed one of the following diets: a standard diet (C); an iodine deficiency diet (DI); a diet with 7% of lyophilized broccoli sprouts (B); an iodine deficiency diet with 7% of lyophilized broccoli sprouts (BDI); a standard diet with 0.025% SDM administered to animals with drinking water (S); or a diet containing 7% of lyophilized broccoli sprouts and 0.025% SDM administered in their drinking water (BS). The rats had unlimited access to fodder and water. The diets were prepared by The Morawski Fodder Company (Poland). All the compounds present in all the diet variants (apart from C and B group) were certified as being iodine free. Detailed descriptions of the diets composition, the fodder intake, and the average amount of sulforaphane after hydrolysis are presented in Table 1. The protocols for animal experiments were approved by the Animal Experimentation Committee of Jagiellonian University, Kraków, Poland (No. 76/2014). After 8 weeks blood was collected from the abdominal aorta under thiopental anaesthesia for hormone assays and other parameters determinations, apart from haematological parameters, which were evaluated as described further. Prior to analyzes samples were stored at −80 °C.

2.3. Haematological evaluation

Blood samples of about 600 µL were obtained from rat tail veins and placed in plastic Microvette 100 K3E tubes (Sarstedt). A complete blood count was performed using an ABX COBAS MICROS Haematology automated cell counter, ROCHE. The following parameters were determined: RBC, Hb, Hct, MCV, MCH, MCHC, WBC, and PLT, presented then as 10^{12}/L, g/dL, %, fL, pg, g/dL, 10^9/L, and 10^9/L, respectively.

2.4. TSH, fT3, fT4 analysis

Thyroid hormone analyzes of serum free T4 (fT4), free T3 (fT3) and TSH levels were performed with immunoassay kits (DRG MedTek PL), according to the manufacturer's instructions. The methods have been validated for rat serum. An automatic reader (Synergy-2, BioTek/USA with syringe rapid dispensers) was used in the immunoassays. Hormone analyzes were evaluated for all rats in all groups. The concentrations of fT4, fT3 and TSH were presented as ng/dL, pg/mL and µIU/L, respectively.

2.5. Biochemical analysis

All biochemical analyzes of plasma were performed with kits (Biomerieux, France), and in accordance with the manufacturer's instructions. An ALIZE automatic biochemical analyzer (Lisabio, France) was used in the assays. Biochemical parameters were evaluated for each rat in all the groups. The concentration of Glu, U, TG, TC, HDL was presented as mmol/L, and µmol/L in the case of creatinine. ASPAT, ALAT and PAL activity were expressed as U/L.

2.6. Measurement of cytokine levels

Rat IL-6, and IL-10 ELISA kits were obtained from Diaclone (Besançon, France) and the determination of the levels of IL-6, and IL-10 were performed according to the manufacturer's instructions. The minimum detectable doses equal to 19.0 and 1.5 pg/mL, respectively, were found. Cytokine determinations were performed for 6 rats per group.

2.7. Enzyme activity and antioxidant plasma capacity analysis

The methods for determining parameters such as FRAP, GPX, TR were essentially the same as in our previous paper [15] appropriately adapted to using 48-well or 96-well plates according to Smith et al. [16]. The thyroid tissue samples were homogenized in phosphate buffer pH = 7.4. GPX3 and FRAP were evaluated in plasma. The change of absorbance during FRAP determination was measured after 8 min of incubation and the reducing ability of the sample was expressed in ferrous ion equivalents (µmol Fe^{2+}/L of plasma). TR and GPX1 were investigated in the thyroid tissue. Protein content was determined by the Bradford method (BioRad). In all of the above mentioned methods, an automatic reader (Synergy-2, BioTek/USA) with syringe rapid