

Effect of hydroalcoholic extract of caraway on thyroid gland structure and hormones in female rat

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Summary

Caraway is one of the medicinal plants used for digestion problems, however, it has been reported that the long term use of this plant in large doses might cause hyperthyroidism symptoms such as leanness, hair loss, severe sweating and tremulousness. Therefore, the aim of this study was to evaluate the effect of hydroalcoholic extract of caraway on the level of thyroid hormones and morphologic changes of thyroid gland in rats. For this purpose, 60 rats were divided into 5 experimental and control groups. Experimental groups were orally given 100, 400, 800 and 1600 mg/kg of extract for 45 days by gavage. The same volume of saline was given to the control group. Blood samples were taken before and after the experiments for the measurement of hormones. The rats were dissected under deep anesthesia and their thyroid glands were removed, processed and stained with H&E. Results show that T₃ and T₄ were increased and TSH was decreased significantly at high doses compared to those in the control group, but no change was observed in the structure of the gland and the size of follicles. It seems that caraway can temporally increase thyroid hormones and cause hyperthyroidism, but further studies are required to clarify the issue.

Key words: Caraway, Thyroid, Herbal medicine, Rat

Introduction

Humans have always been exposed to a host of natural hormonally active agents that are present in food and fermented beverages. Phytoestrogens that exist in some plants have various positive effects with respect to human health (Nilsson, 2000). However, there is some evidence that the ingestion of certain herbal medicines can cause hormonal imbalances in the body.

Skakuyaku-kanzo-to is a traditional herbal medicine that decreases total serum testosterone level in the rat (Takeuchi *et al.*, 1989). Kiwi can interfere in the spermatogenesis process and reduce the number of sperm (Panjehshahin *et al.*, 2005). *Foeniculum vulgare* which contain phytoestrogen can reduce the testosterone hormone in rats (Dehghani *et al.*, 2005). S-petasin is a bioactive compound isolated

from the leaves or roots of "*petasites hybridus*" (used Chinese herbal medicine) that inhibits the production of corticosterone from the cortex of the adrenal gland in rats (Chang *et al.*, 2002). Other researches show the weight reducing Japanese herbal medicines, "Dream shape" and "Ever youth", cause thyrotoxicosis (Ohye *et al.*, 2005). In Iranian traditional medicine, caraway is recommended and prescribed for weight loss. Those who ingest caraway over a long time develop symptoms resembling hyperthyroidism such as tachycardia, hair loss, sweating, increasing body temperature and tremors (Jazayeri, 1981).

With an increase in thyroid activity, marked changes in thyroid hormone production, metabolism and action occur. This may result in an increased prevalence of sub-clinical thyroid disease that is associated with thyroid dysfunction (Peeters,

2008). Therefore, the aim of this study was to evaluate the effect of caraway on the morphology and hormone levels of the thyroid gland in rats.

Materials and Methods

Caraway was obtained from a local supplier in Kerman, Iran, during summer and identified by Dr Jafary, botanist. The voucher number specimen (No. 883601) was deposited in the herbarium of Yasuj University, Iran.

Hydroalcoholic extract was prepared from its seed by percolation method, yielding 11.6 g from each 100 g of seeds.

Sixty Sprague-dawley female rats weighing 230-250 g were obtained from the Animal House of Shiraz University of Medical Sciences and were acclimated to the laboratory condition for one week, prior to the experiment. The animals were maintained in a controlled temperature (22-24°C), light and dark period (12:12) and were given free food and tap water *ad libitum*. Female rats were divided into five equal groups. Experimental groups were given a daily gavage administration of 100, 400, 800, 1600 mg/kg for 45 days. The control group received water (total volume was 1 ml). Blood samples were taken from the tail vein before starting and at the end of the experiment and centrifuged at 3000 rpm for 10 min. Then the plasma were collected and kept at -20°C for hormone analysis. The concentration of T₃, T₄ and TSH were determined using Immuntech Immuno-radioassay kit, France. The analytic sensitivity for TSH was 0.025 mIU/L, T₄ was 13 nmol/L and T₃ was 0.3 nmol/L.

Inter assay coefficient of variation for TSH was 5.7%, T₃ was 7.7% and T₄ was 8.6% and Intra assay coefficient of variation for TSH was 3.7%, T₃ was 6.3% and T₄ was 6.2%. At the end of the experiment, the rats were sacrificed under deep anesthesia and their thyroid glands removed, fixed in 10% buffered formalin, prepared and histological slides were stained by H&E method for histopathological evaluation. The results were analyzed using ANOVA. P-value less than 0.05 were considered statistically significant by Tukey HSD test.

Results

Figure 1 shows that the weight of rats in the group which consumed 1600 mg/kg of hydroalcoholic extract of caraway was significantly lower than the control group (P<0.05). The amount of T₃, T₄ and TSH in different groups is shown in Figs. 2, 3 and 4, respectively. According to these results, T₃ and T₄ were increased in the 1600 mg/kg group relative to control group (P<0.05), while TSH was decreased (P<0.05). In lower doses, T₃ and T₄, and TSH have no significant changes. Histological study showed that the microscopic structure of the thyroid gland in different groups was the same and no significant changes in histology. Follicular arrangement, capsule, level of the colloid and follicular cells was observed (Figs. 5 and 6).

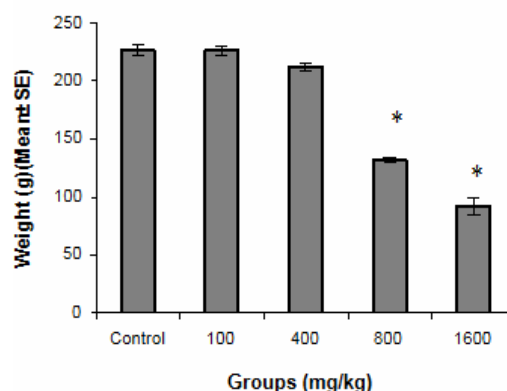


Fig. 1: Weight of diabetic rats after administration of different doses of caraway extract. *: P<0.05 relative to control

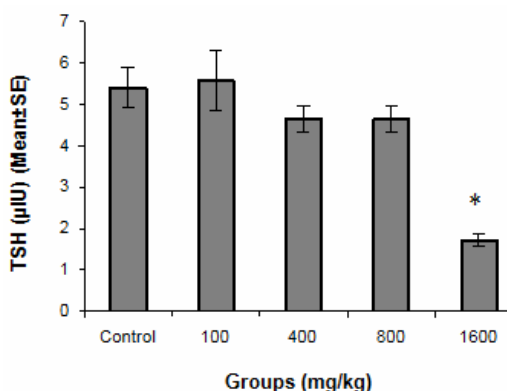


Fig. 2: Levels of TSH in diabetic rat serum after administration of different doses of caraway extract. *: P<0.05 relative to control

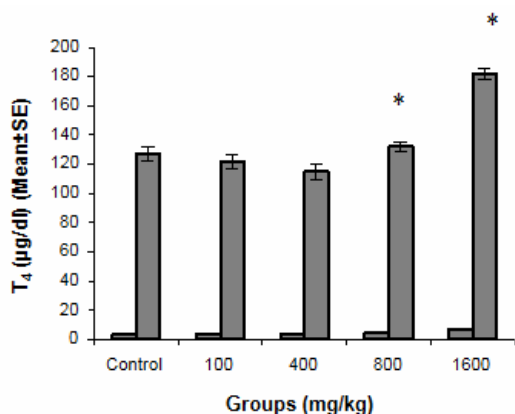


Fig. 3: Levels of T₃ hormone in diabetic rat serum after administration of different doses of caraway extract. *: P<0.05 relative to control

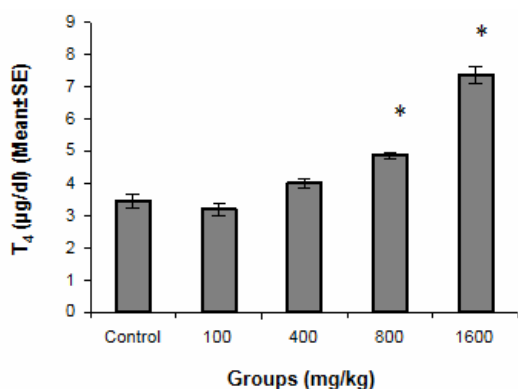


Fig. 4: Levels of T₄ hormone in diabetic rat serum after administration of different doses of caraway extract. *: P<0.05 relative to control

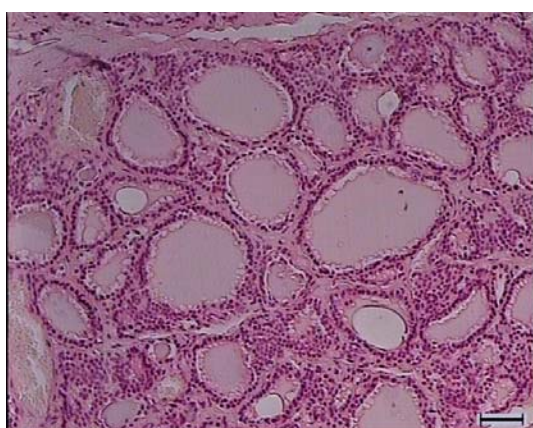


Fig. 5: Follicles of thyroid gland in control group (H&E, ×100; Scale bar = 5 µm)

Discussion

Thyroid hormones have an important role on growth and development of the body

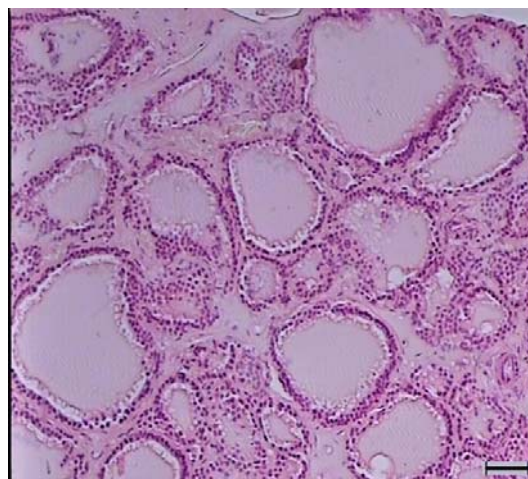


Fig. 6: Follicles of thyroid gland 1600 mg/kg group (H&E, ×100; Scale bar = 5 µm)

and regulate metabolism (Huang *et al.*, 2008). Many factors can influence the concentration of these hormones and therefore disturb the general body metabolism. Thiocyanate from tobacco, smoke, perchlorate and drugs which contain different amounts of iodine can influence the structure and function of thyroid hormones (Steinmaus *et al.*, 2007). Medicinal plants of their extracts can also have the same effect (Ohye *et al.*, 2005). In Iranian traditional medicine, caraway is one of the plants which causes leanness, hair loss, hand tremor and weight loss. If it is overused, these effects resemble hyperthyroidism symptoms (Jazayeri, 1981).

The present study shows that the daily use of high doses of caraway in rats causes significant weight loss, decrease of thyrotropin levels and an increase in T₃ and T₄ hormones. There are reports that two medicinal plants (Ever youth and Dream shape), which are recommended for weight loss in Japan, increase the level of T₃ and T₄ and decrease thyrotropin (Ohye *et al.*, 2005). It seems that there should be some compounds in caraway which cause some change in thyroid function.

Caraway contains flavonoids and antioxidants which help in healing intestinal wounds (Khayyal *et al.*, 2001). The two important compounds are carvone (Bouwmeester *et al.*, 1998) and limonene monoterpene (Bouwmeester *et al.*, 1998; de Sousa *et al.*, 2007). D-limonene is one of the most abundant terpenes in nature. The main

structure of many Citrus oils is D-limonene. This compound is known as a safe material in fruits and vegetables and used as a food flavour (Sun, 2007). Despite its anti-tumor and low toxicity properties (Hohl, 1996; Sun, 2007). D-limonene may increase the chance of hyperplasia and adenocarcinoma in rat kidney (National Toxicology Program, 1990, Ser-347; Whysner and Williams, 1996; Sun, 2007). This effect is due to the aggregation of a special protein in rat called alpha (2u)-globulin (Whysner and Williams, 1996). The alpha (2u)-globulin sulfamethazine can induce tumor in thyroid follicular cells in rat and mouse which result in changes in thyroid hormones (Dybing, 2002). Therefore, it seems that in the first place, this protein increases T₃ and T₄ in the thyroid gland, but in these histological studies no particular structural changes that could be related to an increase in thyroid hormones were shown. Different reports also showed that limonene and carvone have an antiproliferative effect (Chen *et al.*, 2006), therefore, it seems less likely that caraway increased T₃ and T₄ via increasing the number of thyroid cells. It is likely that caraway exerts its effects on physiology and the function of follicular cells rather than on the structure. Other reports revealed that over use of D-limonene in rat and mouse causes weight and hair loss (National Toxicology Program, 1990, Ser-347). Carvone may also have some effects. Some reports showed that carvone can induce exchanges in sister chromatids and chromosomal aberrations in CHO cells (National Toxicology Program, 1990, Ser-381). One of the possibilities is that carvone changes the function of organelles such as Golgi apparatus and endoplasmic reticulum in follicular cells by a change in gene expression. This might result in increasing T₃ and T₄ production. Another possibility is that the enhancement of sodium-iodide transport causes the absorption of iodide, and therefore, higher T₃ and T₄ production of caraway might exert its effect on iodothyrosine deiodinases (Gereben *et al.*, 2008). On the other hand, (R) – (–) – carvone can have an inhibitory effect on CNS (Bouwmeester *et al.*, 1998). It seems that (R) – (–) – carvone can decrease TRH and TSH through the inhibition of

hypothalamic function and then hypophysis. This action in turn can increase thyroid hormones by negative feedback. Meanwhile, it seems that the effect of caraway on increasing thyroid hormones could be temporal. It has been mentioned in Iranian traditional medicine that those who consume caraway over a long period may show hyperthyroidism manifestation, then return to normal situation after withdrawal (Jazayeri, 1981).

Ohye *et al.* (2005) also showed that after discontinuation of the medicinal plants “Ever youth” and “Dream shape” in Japan, which were recommended for weight loss by increasing T₃ and T₄, these hormones return to normal and hyper-thyroidism symptoms were removed. In conclusion, it seems that the components in caraway can increase T₃ and T₄ hormones and decrease TSH through an unknown mechanism. Continuation of this situation can lead to hypertoxicois. Further investigation is needed to clarify the issue.

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References

- Bouwmeester, HJ; Gershenzon, J; Konings, MC and Croteau, R (1998). Biosynthesis of the monoterpene limonene and carvone in the fruit of caraway. *Plant Physiol.*, 117: 901-912.
- Chang, LL; Tseng, YC; Lin, YL; Wun, WS and Wang, PS (2002). Effects of S-petasin on corticosterone release in rats. *Chin. J. Physiol.*, 45: 137-142.
- Chen, J; Lu, M; Jing, Y and Dong, J (2006). The synthesis of L-carvone and limonene derivatives with increased antiproliferative effect and activation of ERK pathway in prostate cancer cells. *Bioorg. Med. Chem.*, 14: 6539-6547.
- Dehghani, F; Panjehshahin, MR; Mirzaee, Z and Mehrabani, D (2005). Effect of foeniculum vulgare organic extract on blood sex hormones and reproductive tissues of male

- rats. *J. Appl. Anim. Res.*, 27: 17-20.
- de Sousa, DP; de Farias Nóbrega, FF and de Almeida, RN (2007). Influence of the chirality of (R) - (-) - and (S) - (+) - carvone in the central nervous system: a comparative study. *Chirality*. 19: 264-268.
- Dybing, E (2002). Development and implementation of the IPCS conceptual framework for evaluating mode of action of chemical carcinogens. *Toxicology*. 181-182: 121-125.
- Gereben, B; Zavacki, AM; Ribich, S; Kim, BW; Huang, SA; Simonides, WS; Zeöld, A and Bianco, AC (2008). Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr. Rev.*, 29: 898-938.
- Hohl, RJ (1996). Monoterpenes as regulators of malignant cell proliferation. *Adv. Exp. Med. Biol.*, 401: 137-146.
- Huang, YH; Tsai, MM and Lin, KH (2008). Thyroid hormone dependent regulation of target genes and their physiological significance. *Chang Gung Med. J.*, 31: 325-334.
- Jazayeri, GH (1981). *Tongue of plants*. 6th Edn., Tehran, Sepehr Press., PP: 279-281.
- Khayyal, MT; el-Ghazaly, MA; Kenawy, SA; Seif-el-Naser, M; Mahran, LG; Kafafi, YA and Okpanyi, SN (2001). Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. *Arzneimittelforschung*. 51: 545-553.
- National Toxicology Program (1990). NTP toxicology and carcinogenesis studies of d-carvone (CAS No. 2244-16-8) in B6C3F1 mice (gavage studies). *Natl. Toxicol. Program Tech. Rep. Ser.*, 381: 1-113.
- National Toxicology Program (1990). NTP toxicology and carcinogenesis studies of d-limonene (CAS No. 5989-27-5) in F344/N rats and B6C3F1 mice (gavage studies). *Natl. Toxicol. Program Tech. Rep. Ser.*, 347: 1-165.
- Nilsson, R (2000). Endocrine modulators in the food chain and environment. *Toxicol. Pathol.*, 28: 420-431.
- Ohye, H; Fukata, S; Kanoh, M; Kubota, S; Kuma, K; Miyauchi, A and Sugawara, M (2005). Thyrotoxicosis caused by weight-reducing herbal medicines. *Arch. Intern. Med.*, 165: 831-834.
- Panjeh-shahin, MR; Dehghani, F; Talaei, T and Panahi, Z (2005). The effects of hydroalcoholic extract of kiwi on sperm count and motility and the level of estradiol and testosterone in male rat. *Arch. Iranian Med.*, 8: 211-216.
- Peeters, RP (2008). Thyroid hormones and aging. *Hormones*. 7: 28-35.
- Steinmaus, C; Miller, MD and Howd, R (2007). Impact of smoking and Thiocyanate on perchlorate and thyroid hormone associations in the 2001-2002 national health and nutrition examination survey. *Environ. Health Perspect.*, 115: 1333-1338.
- Sun, J (2007). D-limonene: safety and clinical applications. *Altern. Med. Rev.*, 12: 259-264.
- Takeuchi, T; Nishii, O; Okamura, T and Yaginuma, T (1989). Effect of traditional herbal medicine, shakuyaku-kanzo-to on total and free serum testosterone levels. *Am. J. Chin. Med.*, 17: 35-44.
- Whysner, J and Williams, GM (1996). D-limonene mechanistic data and risk assessment: absolute species-specific cytotoxicity, enhanced cell proliferation, and tumor promotion. *Pharmacol. Ther.*, 71: 127-136.