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# The effects of safflower (*Carthamus tinctorius*) extract on thyroid gland activity in rats

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## Abstract

**Background and Objective:** Safflower (*Carthamus tinctorius*) has a long history in traditional medicine. It contains a wide range of useful compounds such as flavonoids. In this study, the effect of safflower extract on thyroid gland activity was investigated.

**Materials and Methods:** 60 male Wistar rats were studied in four groups. The negative control was left untreated while three experimental groups intra-peritoneally received 100, 200 and 300 mg/kg B.W. of safflower hydroalcoholic extract for 28 days. The serum levels of T3, T4, FT3, FT4 and TSH were measured by radioimmunoassay method on 14th and 28th days of study.

**Results:** The findings showed that short-term use of the extract directly affects thyroid function as the levels of thyroid hormones are decreased significantly, whereas it has no significant effect on TSH since its mean serum concentration remained unchanged. The long-term use of this extract did not show any significant effect, confirming that during the long run, the negative feedback on the thyroid is removed and this gland becomes adapted to some effective compound (s) present in Safflower.

**Conclusion:** The continuous short-term use of the safflower can decrease the level of thyroid hormones temporarily, while in the long term, it does not show the same effect.

**Keywords:** *Carthamus tinctorius*, Thyroid hormones, Thyroid-stimulating hormone, Rats

## 1. Introduction

In traditional medicine, different plant products are used in treatment of various diseases (1). Indeed, until half a century ago, Herbs were considered as the main source of medicine. One of these herbs is Safflower plant (*Carthamus tinctorius*), which belongs to the aster family (2). Safflower is cultivated mainly for its seeds, which is expensed as edible oil and birdseed, or for its flowers, used as dye sources and medicinal purposes (2-5). Safflower dyes can also be used as food additives /natural food colorants; it has Yellow or yellow-orange flowers with ovate and lance leaves (6). Originally from India and Saudi Arabia, Safflower is cultivated in most parts of Europe and the United States as well as north-western and southern areas of Iran, near Tehran, Khorasan and Tabriz (6).

A variety of medicinal benefits are attributed to Safflower plant, including its usage in the treatment of inflammation and tumors, and as laxative and anodyne drugs. Khalid et al. (2017) reported that treatment of skin infections, bone related disorders, menopause and atherosclerosis are some functional properties of safflower oil (7). Modarresi et al. (2005) stated that safflower flowers hydroalcoholic extract has some effects on sperm parameters different from those

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involved in the sperm count increase (8). They stated that the extract can change the sperm form, motility and fertility. Similarly, Bahmanpoor et al. (2012) examined the effect of safflower extract (dried safflower petal aqueous extract) on infertile rats, and noted a positive effect on spermatogenesis and sperm count, and they concluded that safflower can improve fertility (9). In contrast, Louei Monfared and Salati (2013) reported that treatment with C. tinctorius extract has detrimental effects on the ovarian histomorphology and female reproductive hormones, and they suggested that popular consumption of this plant should be reconsidered (10).

The medicinal effects of Safflower are brought about by some naturally occurring compounds such as glycosyl quinochalcones and flavonoid glycosides, which are polyphenolic compounds with antioxidant activities (11).

Kanehira et al in 2013 in a study, an antioxidant isolated from cultured safflower cells, was compared to two natural antioxidants, lignin and quercetin, and were found to exhibit stronger anti-oxidative effects; thus concluded, it may be valuable as a cryoprotective agent (12). Similarly, nicotiflorin, an antioxidant isolated from safflower, has been observed to have neuro-protective effects on memory/dementia in rats (13).

As a very important gland, thyroid plays a crucial role in metabolism through secretion of two essential hormones i.e. thyroxine and tri-iodothyronine. Since medicinal values of safflower consumption have been determined by previous researches, and its possible beneficial effects on thyroid gland has not been studied, we attempted to investigate the possible positive or negative effects of short and long term administration of hydroalcoholic extract of this herb on thyroid hormonal functions.

## 2. Materials and Methods

The approval ID of this manuscript is IR.IAU.KAU.REC.1398.162. 60 male adult (two month old) Wistar rats with a mean body weight of 180 20 g were purchased from animal house of the Islamic Azad University, Kazerun Branch. They were kept there at 25°C and fed a routine diet. They were randomly divided into 4 groups to investigate the possible effects of different doses of *Carthamus tinctorius* extract on their thyroid functions. As the negative control, group 1 was left untreated, whereas Groups 2, 3 and 4, the experimental groups, intra-peritoneally received 100, 200 and 300 mg/kg b.w. safflower hydroalcoholic extract, respectively. The extract was administrated daily for duration of 28 days (14). To compare the short- and long-term effects of the extract, blood serum sampling was carried out on the fasting rats on 14th and 28th days of the study. Blood samples were transferred to the laboratory on ice, and their sera were separated by routine method. Measurement of T3, T4, FT3 and FT4 and TSH were carried out by radioimmunoassay method with Pars Azmoon kits (15, 16).

### 2.1. Extract preparation

Dried flowers were powdered in a blender, and sucked in 70% alcohol for 24 hours at room temperature. The mixture was passed through Whatman no. 1 filter paper, then steamed and dried in a vacuum at 40 ° C. Using freeze-drying at -50°C, the extracted material was milled into powder (14).

### 2.2. Statistical analysis

Data were expressed in SI units, and analyzed using one-way ANOVA and Duncan tests as well as Spearman and T-test using SPSS25 software (17). Duncan's multiple range tests were used to detect significant differences between the means. All values were expressed as mean and standard error (SE) and $P<0.05$ was considered statistically significant.

## 3. Results

The results are presented in Tables 1 to 3. As seen, a significant difference is observed between the mean levels of T3, T4 and FT3 of experimental groups compared to the control group on 14th day ($P<0.05$). However, on the 28th day, there were no such differences between these parameters in experimental groups relative to the control group ($P> 0.05$). In addition, there were no statistically significant differences between the mean levels of TSH in three experimental groups compared to the control on days 14 and 28 ($p>0.05$).

**Table 1.** The mean and standard deviation of parameters studied on day 14

| Parameter | $14^{th}$ day (mean ± SD) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | Number | Sex | Age (Month) | Weight (g) | T3 (ng/mL) | T4 (µg/dL) | FT3 (Pg/mL) | FT4 (µg/dL) | TSH (IU/ml) |
| Negative Control | 60 | Male | 2 | 180±20 | 1.78±0.13 | 4.34±0.19 | 1.48±0.01 | 3.00±0.15 | 3.66±0.17 |
| Group 2 | 60 | Male | 2 | 180±20 | 1.35±0.09 | 2.91±0.16 | 1.28±0.04 | 2.70±0.15 | 4.14±0.14 |
| Group 3 | 60 | Male | 2 | 180±20 | 1.32±0.08 | 3.04±0.15 | 1.25±0.05 | 2.70±0.15 | 3.68±0.19 |
| Group 4 | 60 | Male | 2 | 180±20 | 1.29±0.10 | 2.90±0.15 | 1.23±0.05 | 2.88±0.11 | 4.01±0.21 |

**Table 2.** The mean and standard deviation of parameters studied on day 28

| Parameter | $28^{th}$ day (mean ± SD) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | Number | Sex | Age (Month) | Weight (g) | T3 (ng/mL) | T4 (µg/dL) | FT3 (Pg/mL) | FT4 (µg/dL) | TSH (IU/ml) |
| Negative Control | 60 | Male | 2 | 180±20 | 1.78±0.13 | 4.34±0.19 | 1.48±0.42 | 3.00±0.15 | 3.66±0.17 |
| Group 2 | 60 | Male | 2 | 180±20 | 1.87±0.07 | 4.35±0.07 | 1.3±0.78 | 2.86±0.13 | 3.10±0.12 |
| Group 3 | 60 | Male | 2 | 180±20 | 2.02±0.11 | 4.15±0.11 | 1.32±0.33 | 2.68±0.11 | 3.43±0.22 |
| Group 4 | 60 | Male | 2 | 180±20 | 1.80±0.16 | 4.33±0.17 | 13±0.51 | 2.93±0.15 | 3.64±0.22 |

**Table 3.** Presence or absence of significant differences between the mean values of various parameters in different groups compared to the control group on days 14 and 28

| Parameter | $14^{th}$ day (p_value) | | | | | $28^{th}$ day (p_value) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | T3 | T4 | FT3 | FT4 | TSH | T3 | T4 | FT3 | FT4 | TSH |
| Group 2 | 0.032* | 0.000* | 0.073 | 0.460 | 0.316 | 0.974 | 0.891 | 0.098 | 0.891 | 0.306 |
| Group 3 | 0.013* | 0.000* | 0.022* | 0.445 | 1.000 | 0.519 | 0.310 | 0.102 | 0.310 | 0.845 |
| Group 4 | 0.011* | 0.000* | 0.014* | 0.934 | 0.588 | 1.000 | 0.977 | 0.075 | 0.977 | 1.000 |

* There is a statistically significant difference between the mean concentration of parameter in the experimental group as compared to the control group (Significant at $p<0.05$)

## 4. Discussion

The herbal approach to thyroid dysfunction is invariably necessary to avoid the various side effects of hormonal therapy. A large number of herbs have anti-thyroid activity in both hypothyroidism and hyperthyroidism. For example, several plants are known to be useful in hyperthyroidism treatment, like Bugleweed (Lycopus virginicus), Gypsywort (Lycopus europaeus), Gromwell (Lithospermum ruderale), Water horehound (Lycopus lucidus), Lemonbalsam (Melissa Officinalis), Rose marry (Rosmarinus officinalis) and Sage (Salvia officinalis) (18). To certify the efficacy of specific herbs in normalizing thyroid dysfunction, they must be investigated in-vitro, in-vivo and in clinical states (19). On the other hand, the signs and symptoms of hypothyroidism and hyperthyroidism are often nonspecific and ambiguous. Thus, measurement of

TSH, T3 and T4 serum levels is important for diagnosis of overt and subclinical thyroid dysfunction (20).

In our study on the effects of safflower extract on thyroid gland activity, we found that mean serum levels of tri-iodothyronine (T3) and thyroxine (T4) hormones show a significant decreased on day 14th in all three experimental groups compared to the control group ($P < 0.05$). On the same day, a significant difference is observed between the mean concentration of free three-iodothyronine hormone (FT3) in experimental groups 1 and 2 compared to the control group ($P = 0.022$ and $P = 0.014$) resulting from FT3 decrease in these groups (Table 3). Conversely, there is no significant difference in the level of thyroid-stimulating hormone (TSH) in the experimental groups compared to the control group ($P > 0.05$; Table 3).

Concentrations of T4 and T3 are regulated by circadian (21) and, additionally for TSH, ultradian rhythms (22). Plasticity of the hypothalamic–pituitary–thyroid axis in form of adaptive responses may promote misdiagnosis (23, 24). Diagnosis of subclinical dysfunction is also dependent on the mode of statistical analysis (25-29).

The influence of plant extract on thyroid function has been documented before. For instance, Zareii et al. (2011) reported that barberry root extract increases the levels of T3 and T4 (in contrast to our findings), while the plasma TSH level remains unchanged (30).

Based on our results, it can be concluded that low, medium and high doses of safflower hydro-alcoholic extract have no significant effects on thyroid-stimulating hormone in both short- and long-term consumptions. In this respect, reduced T3 and T4 levels during the first 14 days of the experiment could be the result of inhibitory effects of this extract on TSH receptor site, immunoglobulin effects on thyroid stimulating hormone receptor, decrease in the peripheral conversion of T3 and T4, or direct negative feedback effect on the thyroid itself. Consumption of some plant flavonoid compounds and isoflavonoids, which are also abundant in safflower extract, inhibit thyroid hormone production by inhibiting thyroid peroxidase (31).Some plant flavonoids disrupt thyroid hormone production through inhibition of thyroid peroxidase (32). Flavonoid compounds decrease the production of central and environmental prostaglandins by cyclooxygenase inhibition (33). Therefore, due to the stimulatory effect of prostaglandins on the production and secretion of pituitary-thyroid hormones (31) and due to the abundance of flavonoid components in safflower extract (Yubin et al.2018), it is possible that T3 and T4 decrease and TSH unchanged may be due to the inhibitory effect of flavonoids on prostaglandin production(34).Flavonoids by stimulation of opioid receptors and inhibition of 3-Methyl-D-aspartate receptors also via calcium channel blockage and intracellular calcium reduction (35), have a negative impact on TSH and TRH secretion followed by thyroid hormones.Flavonoids, through inhibition of the monoamine oxidase enzyme, cause increased dopamine levels and due to the inhibitory effect of dopamine on TSH and TRH secretion the reduction of pituitary-thyroid hormones is justified(36). Another cause of the decrease in T3 and T4 levels may be due to the structural similarity of these hormones to flavonoids (37).

Apium graveolens leaf has flavonoid compounds similar to safflower; Kooi et al. (2014) reported that this plant extract reduces thyroid hormones and TSH concentrations (38). Moreover, after 28-day administration of safflower hydroalcoholic extract, no significant difference was observed between mean levels of thyroxine, triiodothyronine and free form of these hormones as well as thyroid-stimulating hormone in three experimental groups relative to the control group (Table 3). It is interesting that even the long-term consumption of high dose of the extract has no apparent effect on the activity of this gland suggesting the development of some type of adaptation.

The relatively stable TSH concentration in experimental groups receiving different doses of safflower hydro-alcoholic extracts showing decreased levels of thyroid hormones (Tables 1-3). Based on the induction of this decreased by short-term use of different doses of safflower extract and the lack of this disorder following long-term administration of this extract, it can be concluded that safflower flower may contain compounds similar to T3 and T4 with suppressive effects on the thyroid gland in short-term. Furthermore, thyroid gland gradually adapts to these compounds and continues its normal functions in the long-term administration of the extract.

## Conclusion

The short-term use of hydro-alcoholic extract of safflower flower induces temporary decrease of thyroid hormones, but TSH level do not change, so it is not called hypothyroidism. Its continuous long-term use, even at a dose of 300 mg/kg body weight, has no significant effect on thyroid function, confirming thyroid gland adaptation to compounds present in safflower flowers.

## Conflict of Interest

All the authors declare that this study was conducted in the absence of any commercial or financial supports that could be considered as a potential conflict of interest.

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