Original Article

Effect of Excessive Green Tea versus Fluoride and Caffeine on Body Weight and Serum Thyroid Hormones in Male Mice

***[1]Abulfadle K. A., [2]Bakhaat G. A., [3]Shaik R. and [4]Tantry B. A.**

[1] Department of Physiology, Aljouf University College of Medicine, Sakaka, KSA, and Zagazig University Faculty of Medicine, Zagazig, Egypt.
[2] Department of Histology, Aljouf University College of Medicine, Sakaka, KSA, and Al-Azhar University Faculty of Medicine, Assiut, Egypt.
[3] Department of Biochemistry, Aljouf University College of Medicine, Sakaka, KSA.
[4] Department of Microbiology, Aljouf University College of Medicine, Sakaka, KSA.

Abstract

Green tea is a worldwide used beverage rich in fluoride and caffeine. To study the effect of excessive green tea intake versus fluoride and caffeine on body weight and serum thyroid hormones in male mice. Also, to confirm the effect of excessive green tea intake on thyroid gland was due to its fluoride and caffeine content or not. Thirty Male albino mice 25-30 gm weight were divided into five groups. Control (C) group (6 mice) receiving distilled water. Green tea (GT) treated group; 6 mice were given a 5 gm% tea hot water extract as their sole water supply. Fluoride (FL) treated group, 6 mice were given fluorine excess solution as their only water supply. Caffeine (Caf) treated group, 6 mice were treated with caffeine (6.25 mg/kg ip). Propylthiouracil (PTU) treated group, 6 mice were treated with PTU (10 mg/kg ip). Each of the five groups is treated with the corresponding substance for 30 days, then sacrificed under ether anesthesia, blood was collected to study serum levels of T3 and T4. There was a significant decrease in serum T3 in PTU treated group (0.72±0.03), GT treated group (0.96±0.04), FL treated group (0.93±0.04) and Caf treated group (1.46±0.08) in comparison to the C group (1.71±0.05).Also, there was a significant decrease in serum T4 in PTU treated group (15.71±1.26), GT treated group (30.07±2.29) and FL treated group (22.03±1.95)in comparison to the C group (51.1±3.15). But, there was an insignificant change in serum T4 in Caf treated group (45.55±2.73) in comparison to the C group (51.1±3.15). Green tea unfractionated aqueous extract decreased serum T3 and T4 and at the same time, increased final total body weight without a significant effect on the relative thyroid weight in comparison to the control group and this may be at least partly due to its content of fluoride and caffeine. Further work should be done on green tea free fluoride and caffeine to confirm this result.

Keywords: Thyroid gland, thyroid hormones, green tea, fluoride, caffeine, propylthiouracil.

* Corresponding author: Department of Physiology, Aljouf University College of Medicine, Sakaka, KSA, and Zagazig University Faculty of Medicine, Zagazig, Egypt.
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Introduction

Tea is a widely consumed beverage all over the world. Nowadays, green tea becomes involved in many consumer products such as vitamins, drinks, creams, soaps, shampoos and cosmetics (Mukhtar & Ahmad, 2000). The leaves of Camellia sinensis give origin to the green tea. Some studies found that green tea consumption in certain quantities per day provide protection against some health troubles such as liver disease, osteoporosis, stroke, viral infection, and cancer (Fujita, 1994, Weisburger, 1996, Hodgson, 2008). But, Schmidt *et al.,* (2005) studies showed that excessive consumption of green tea caused some hazards like liver cells acute cytotoxicity. Thyroid is an endocrine gland that shares in the control of body metabolism by secreting the thyroid hormones, triiodothyronine (T3) and thyroxine (T4). These hormones alsocontrol the growth and function of many other body systems (Zhao *et al.,* 1998). Some studies found that green tea extract induced an enlargement in the thyroid of normal rats (Sakamoto *et al.,* 2001; Satoh *et al.,* 2002). In addition to the enlargement of thyroid gland, Chandra, De, and Choudhury (2011) declared that green tea extract decreased the serum level of both T3 and T4.But, the green tea contains fluoride absorbed from the soil and accumulated in its leaves. (Koblar, Tavčar, & Ponikvar-Svet, 2012) and the green tea beverages contain. Thus, green tea is a major source of fluoride intake. Although fluoride intake in small amount (0.05 mg/day/kg body weight for all ages over 6 months) is of value in preventing dental cavities, yet, its excessive long time intake causes some hazards as osteoarthritis (Koblar *et al.,* 2012) (Shyu & Chen, 2013). There is an area of controversies in studies done on the effects of excessive fluoride intake and thyroid gland functions. Some studies confirmed that excessive fluoride intake affected thyroid gland weight and decreased serum level of both T3 and T4 (Hu, *et al.,* 2007; Liu, *et al.,* 2008). On the other hand, Xiaoli, *et al.,* (1999) stated that fluoride excessive intake decreased serum level of T4, but, increased the serum level of T3. On the contrary other studies found no effect of excessive fluoride intake on thyroid gland function (Baum, *et al.,* 1981, Eichner, *et al.,* 1981; F, G, & Z, 1994). Caffeine (1, 3, 7-trimethylxanthine) represents also one of the components in green tea (E Spindel *et al.,* 1980). Although some benefits are gained from caffeine consumption, as prevention of Parkinson's disease, yet, it was found that overuse of caffeine is addictive and has other hazards as thyroid carcinogenesis (Peterson *et al.,* 2005; Sinija & Mishra, 2008). On the other hand, the effect of caffeine on thyroid hormone secretion has some controversies. Some studies stated that it changed T4 and T3 blood levels (E Spindel *et al.,* 1980) (ER Spindel & Wurtman, 1984), while others found that it had no effect on thyroid hormone secretion (Bartsch *et al.,* 1996). Few studies on the effect of the un-fractionated green tea extract on thyroid gland weight and hormones are available, although, green tea is used as whole beverage rather than taking any single component of it. So, it is essential to study the effect of un-fractionated green tea extract on mice with euthyroid function. But, as previously mentioned green tea contains fluoride and caffeine which may affect thyroid gland function. Thus, in this present study, the effects of high doses of green tea, fluoride and caffeine on the thyroid gland weight and hormones was evaluated to confirm if green tea had an effect on thyroid gland and if so, it was due to its content of fluoride and caffeine or not.

Materials and Methods

In this study thirty male albino mice about 25-30 gm weight were obtained from Aljouf College of Medicine animal house, Sakaka, Saudi Arabia. They were provided feed and water ad libitum and maintained under standard pathogen free laboratory conditions for one week before the experiments for adaptation. Experiments were performed according to national and institutional regulations for animal use. Mice were divided into five groups. Control (C) group (6 mice) receiving distilled water. Green tea (GT) treated group; 6 mice were given a 5 gm% tea hot water extract as their sole water supply. The 5 gm% aqueous tea extract was prepared every other day by adding 100 ml of boiling water to 5 gm of green tea followed by filtration after letting the mixture stand at room temperature for30 min (Jiang,

Glickman, & de Boer, 2001). Fluoride (FL) treated group, 6 mice were given fluorine excess solution as their only water supply. Fluorine excess solution was prepared by the addition sodium fluoride into distilled water (30mg/L) (Zhao *et al.*, 1998). Caffeine (Caf) treated group, 6 mice were treated with caffeine (6.25 mg/kg ip) (El Yacoubi *et al.*, 2000). The caffeine solution was prepared fresh daily and injected ip in a volume of 10 ml/kg. Propylthiouracil (PTU) treated group, 6 mice were treated with PTU (10 mg/kg ip) (Kar *et al.*, 2002). Each of the five groups is treated with the corresponding substance for 30 days.

Chemicals

Green tea was commercially obtained from the market while sodium fluoride, caffeine and propylthiouracil were obtained from Sigma-Aldrich.

Estimation of Serum T3 and T4

Just after sacrifice, blood samples were collected for each mouse under etheranesthesia. These blood samples were allowed to clot at room temperature, and were centrifuged at 3000 rpm for 10 min and serum was recovered and stored frozen at -20oC till used for quantitative immunoassaying of T3 andT4 using commercially available kits as recommended by the manufacturer (cat#SE120091, SE120090, Mouse ELISA Kits, Sigma-Aldrich).

Body Weight and Relative Thyroid Weight

The body weight (gm) of each mouse was recorded on the first day before treatments start (initial body weight) and the day of sacrifice (final body weight). Just after sacrifice, the thyroid glands were taken out, trimmed off the attached tissues and weighed. The relative weight of thyroid gland (mg) was expressed per100 gm body weight (Chandra & De, 2010).

Statistical Analysis

The data was expressed as mean ± standard error of the mean (SEM) and it was analyzed by using (IBM SPSS Statistics Version 21 Software for Windows) for statistical significance using one-way ANOVA and Tukey HSD for Post Hoc Multiple Comparisons. $P<0.05$ was considered as statistically significant.

Results

The effect of propylthiouracil (PTU), green tea (GT), fluoride (FL) and caffeine (Caf) on both serum T3 and T4 (ng/ml) in male albino mice was shown in Table I-III. There was a significant decrease in serum T3 in PTU treated group (0.72±0.03), GT treated group (0.96±0.04), FL treated group (0.93±0.04) and Caf treated group (1.46±0.08) in comparison to the control (C) group (1.71±0.05).Also, there was a significant decrease in serum T4 in PTU treated group (15.71±1.26), GT treated group (30.07±2.29) and FL treated group (22.03±1.95) in comparison to the control (C) group (51.1±3.15).

Table 1: Effect of green tea, fluoride and caffeine on serum T3 (ng/ml) and serum T4 (ng/ml).

Group	T3	T4
Control (C)	1.71±0.05	51.1±3.15
Propylthiouracil (PTU)	$0.72\pm0.03^{***}$	$15.71\pm1.26^{***}$
Green tea (GT)	$0.96\pm0.04^{***}$	$30.07\pm2.29^{***}$
Fluoride (FL)	$0.93\pm0.04^{***}$	$22.03\pm1.95^{***}$
Caffeine (Caf)	$1.46\pm0.08^{*}$	$45.55\pm2.73^{\#}$

Data is expressed as Mean±SEM. $^{*}P<0.05$ in comparison to the control. $^{***}P<0.001$ in comparison to the control. $^{\#}P>0.05$ in comparison to the control.

On the other hand, there was an insignificant change in serum T4 in Caf treated group (45.55±2.73) in comparison to the control (C) group (51.1±3.15).

Table -II and Figure- III were showed the effect of green tea, fluoride and caffeine on relative weight of the thyroid gland (in mg/100 gm body weight) in male albino mice.

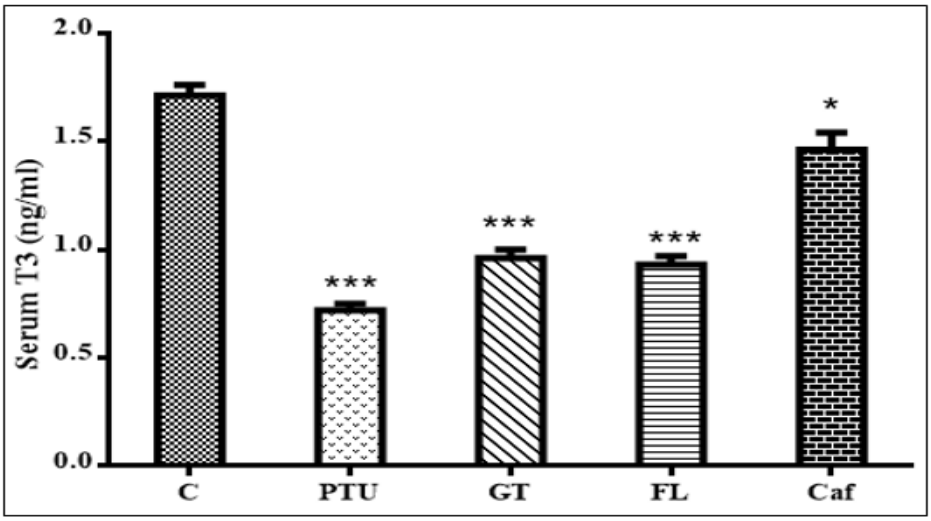

Fig. 1: Effect of green tea, fluoride and caffeine on serum T3 (ng/ml).

Data is expressed as Mean±SEM. $^{*}P<0.05$ in comparison to the control. $^{***}P<0.001$ in comparison to the control. C, control group; PTU, Propylthiouracil treated group; GT, green tea treated group; FL, fluoride treated group; Caf, caffeine treated group.

There was a significant increase in the relative weight of the thyroid gland in PTU treated group (10.86±0.45) in comparison to that of the control group (9.56±0.22), but, there was an insignificant increase in the relative weight of the thyroid gland in GT treated group (10.19±0.15), FL treated group (10.48±0.26) and Caf treated group (9.58±0.16) in comparison to that of the control group (9.56±0.22). Table -III and Figure –IV were revealed the effects of green tea, fluoride and caffeine on total body weight (in gm) in male albino mice.

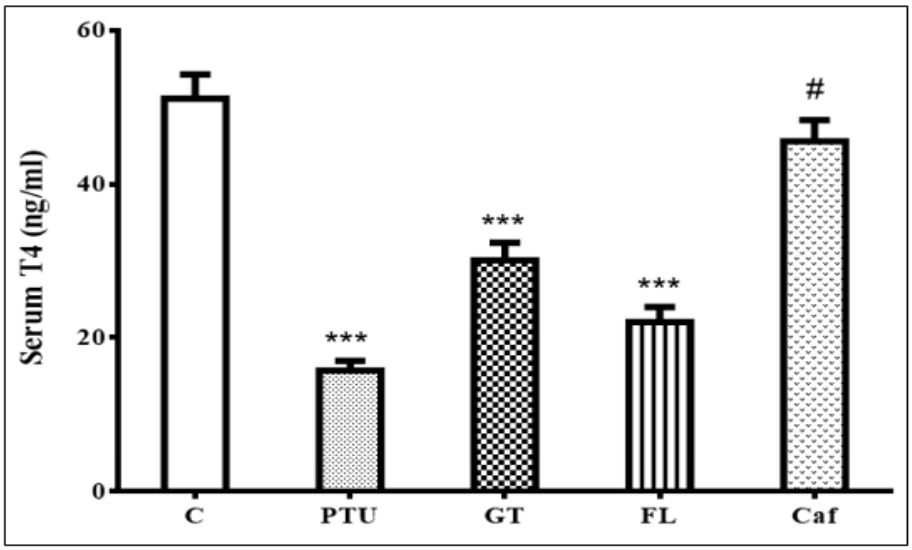

Fig. 2: Effect of green tea, fluoride and caffeine on serum T4 (ng/ml).

Data is expressed as Mean±SEM. $^{***}P<0.001$ in comparison to the control. $^{\#}P>0.05$ in comparison to the control. C, control group; PTU, Propylthiouracil treated group; GT, green tea treated group; FL, fluoride treated group; Caf, caffeine treated group.

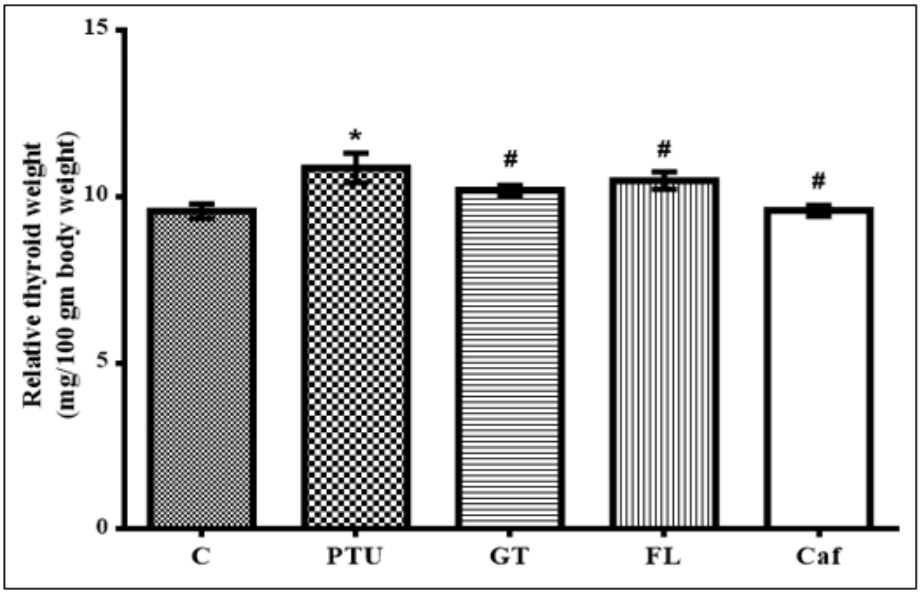

Fig. 3: Effect of green tea, fluoride and caffeine on relative weight of the thyroid gland (mg/100 gm body weight). Data is expressed as Mean±SEM. *P<0.05 in comparison to the control. #P>0.05 in comparison to the control. C, control group; PTU, Propylthiouracil treated group; GT, green tea treated group; FL, fluoride treated group; Caf, caffeine treated group.

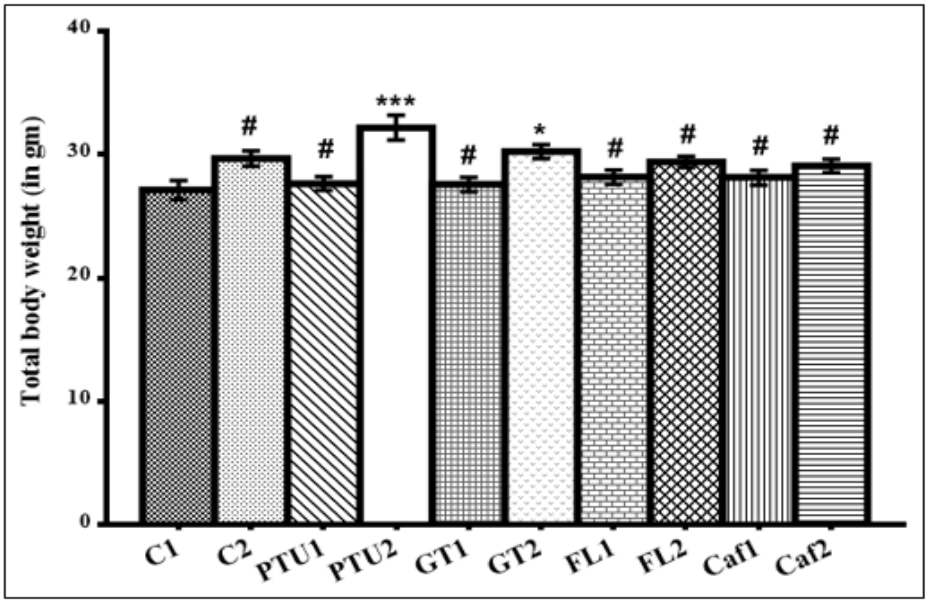

Fig. 4: Effect of green tea, fluoride and caffeine on total body weight of the thyroid gland (mg/100 gm body weight). Data is expressed as Mean±SEM. *P<0.05 in comparison to the control initial weight (C1). ***P<0.001 in comparison to the control initial weight. #P>0.05 in comparison to the control initial weight.C1, control initial weight; C2, control final weight, PTU1, propylthiouracil treated group initial weight; PTU2, propylthiouracil treated group final weight; GT1green tea treated group initial weight; GT2, green tea treated group final weight; FL1, fluoride treated group initial weight; FL2, fluoride treated group final weight; Caf1, Caffeine treated group initial weight; Caf2, Caffeine treated group final weight.

There was a significant increase in total body weight in PTU2 group (32.16±1) and GT2 group (30.23±0.56) in comparison to that of the control initial weight (C1) group (27.12 ± 0.77), but, there were no significant changes in total body weight in FL2 group (29.39±0.45) and Caf2 group (29.08±0.54) in comparison to that of the control initial weight (C1) group (27.12 ± 0.77).

Table 2: Effect of green tea, fluoride and caffeine on relative weight of the thyroid gland (mg/100 gm body weight).

Group	Relative weight of the thyroid gland (mg/100 gm body weight)
Control (C)	9.56±0.22
Propylthiouracil (PTU)	10.86±0.45*
Green tea (GT)	10.19±0.15#
Fluoride (FL)	10.48±0.26#
Caffeine (Caf)	9.58±0.16#

Data is expressed as Mean±SEM. *P<0.05 in comparison to the control. #P>0.05 in comparison to the control group.

Discussion

Green tea is widely used as a beverage and traditionally as an experience based medication (Henderson *et al.,* 2002). So, this study was conducted to confirm if excessive use of green tea affected thyroid gland function or not. Our study showed a significant decrease in serum levels of both T4 and T3 in the GT treated group in comparison to the control one which is supported by Divi and Doerge (1996) who explained this effect on the decreased deiodinase (5´DI) enzymeactivity by the green tea which inhibit synthesis and release of thyroid hormones and the flavonoids content of green tea. Also, there was a significant increase in the final total body weight in the GT treated group (GT2) in comparison to the initial total body weight of the control group (C1) although there was an insignificant increase in the relative weight of the thyroid gland in the GT treated group in comparison to the control group. On the contrary, Allain, Thomas, McGregor, and Salisbury (1995) reported a significant decrease in total body weight with hypothyroidism and they explained this by the increase in protein catabolism and the decrease of the muscle mass. As fluoride is a component of green tea, its effect was also studied on the thyroid gland hormones and weight. Our results showed a significant decrease in serum levels of both T4 and T3 in the FL treated group in comparison to the control one which was supported by (Hu *et al.,* 2007, Liu *et al.,* 2008, Quanyong *et al.,* 2009, Susheela, 2003, H. Wang *et al.,* 2009, Zhan *et al.,* 2006). Also, Wan *et al.,* (2001) recorded a decrease in serum T3 and T4 in the endemic fluorosis patients although Xiaoli *et al.,* (1999) reported that, in endemic fluorosis areas in China, serum level T4 was reduced while T3 was increased in children aged 8 - 12 years. On the contrary, Zhao *et al.,* (1998) found that with iodine deficiency, excessive fluorine intake increased serum level of both serum T3 and T4 in iodine treated mice. Also, X.-h *et al.,* (2001) recorded a normal serum level of both T3 and T4 with high iodine and fluoride intake. Thus, fluoride may decrease thyroid hormones by affecting iodine absorption and trapping in thyroid, and by affecting thyroid enzyme system (Xiaowei & Xiaohong, 1994). This was confirmed by some studies which showeda significant inhibition of radioiodine thyroid uptake by the high fluorine intake (Bachinskiĭ *et al.,* 1984, Sidora *et al.,* 1982, Yu, 1985; Zhang, 1982). Moreover, our results showed insignificant changes in both final total body weight and relative thyroid weight in the FL treated group in comparison to the control one. This result was supported by (Baum *et al.,* 1981; Eichner *et al.,* 1981; F *et al.,* 1994).On the other hand, some animal studies indicated that there was a significant change in the relative thyroid gland weight in rats (Hu *et al.,* 2007; LIU *et al.,* 2008; Quanyong *et al.,* 2009; Wang H *et al.,* 2009; Zhan *et al.,* 2006). Also, Wilson (1941) found thyromegally among children

lived in endemic fluorosis areas. The discrepancy between the results of our study and these studies may be due to species difference or the duration of excess fluoride exposure. Also, as caffeine is present in green tea, thus we studied its effect on thyroid gland hormones and weight. Our results cleared that there was a significant decrease in serum level of T3 without a significant change in serum T4 in Caf treated group in comparison to that of the control group and this effect may be due to inhibition of the peripheral conversion of T4 to T3. These results were supported by (Cloze *et al.,* 1983). On the other hand, E Spindel *et al.,* (1980) found a significant decrease in both serum T4 and T3following caffeine administration and they explained this by the decrease in serum TSH in response to caffeine intake. Also, Bartsch *et al.,* (1996) found no changes in thyroid hormones in response to caffeine intake. Our results also showed insignificant changes in both final total body weight and relative thyroid weight in the Caf treated group in comparison to the control group. These results were supported by Bartsch *et al.,* (1996) and Mohr *et al.,* (1984). On the contrary, Wolff and Varrone (1969) stated that there was a marked increase in the thyroid gland size in response to a caffeine similar methylxanthine, theophylline. Also, Son *et al.,* (2003) found thyroid changes with high doses of caffeine in rats with iodine deficiency. From all the previously discussed results of our study, it is clear that green tea decreased thyroid hormones and increased total final body weight without a significant change in the relative thyroid weight in comparison with the control group. Also, high doses of fluoride decreased thyroid hormones without significant changes in total final body weight or in the relative thyroid weight in comparison with the control group. Moreover, high doses of caffeine decreased T3 without significant changes in T4, total final body weight or the relative thyroid weight in comparison with the control group. Thus, fluoride and caffeine present in green tea may be considered as a mechanism by which green tea affected thyroid gland function. Further work should be done on green tea free fluoride and caffeine to confirm these results.

Conclusion

It is emphasized that green tea unfractionated aqueous extract decreases serum T3 and T4 and at the same time, increases final total body weight without a significant effect on the relative thyroid weight in comparison to the control group and this may be at least partly due to its content of fluoride and caffeine. However, further work should be done on green tea free fluoride and caffeine to confirm this result.

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