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The Effects of Garlic (*Allium sativum*) Extracts on the L-Thyroxine-Induced Hyperthyroidism in Male Rats

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Abstract. Thyroid hormone level variations relate to physiological and clinical anomalies. Many studies showed that garlic has protective effects on various body systems. This study aimed to determine the beneficial effects of garlic extracts against l-thyroxine-induced male rat hyperthyroidism. 25 male rats were randomly grouped into five groups: healthy rats (Control), rats subcutaneously injected with l-thyroxine 0.3 mg/kg BW, and rats given orally doses (100, 150, and 200 mg/kg BW/d) of garlic extracts. Blood samples were taken from the heart at the end of the experiment to identify T3 and T4 using Elisa. The results showed that hyperthyroid rats had a significant increase ($p < 0.05$) in serum concentrations of T3 and T4 relative to the negative control group, and no significant increase in serum concentrations of T3 and T4 relative to the negative control group at the doses of 100 and 200 mg/kg. However, at the dose of 150 mg/kg BW, the serum concentrations of T3 and T4 decreased. Hyperthyroid rats receiving 100 g/kg garlic extracts were more beneficial in enhancing the behavior of hyperthyroid rats than those in the control group. In conclusion, garlic extracts could affect the level of T3 and T4 in a dose-dependent manner.

INTRODUCTION

The thyroid is an essential endocrine organ in the human body. Its shape resembles a butterfly on both sides of the trachea [1]. The thyroid secretes two significant regulatory hormones: triiodothyronine (T3), tetraiodothyronine (T4). Both hormones control the crucial biological processes in the body [2]. Thyroid hormone (TH) controls natural growth and growth metabolic processes, and it is well known that hormone activity associates with body mass and energy consumption [3]. Differences in these hormone levels contribute not just to the difference in the metabolic rate, but also a variety of health conditions. Hyperthyroidism, in particular, often ends up with common health conditions, such as DM and heart diseases if they are not treated properly [4].

Hyperthyroidism is an endocrine disorder characterized by an excessing activity of the thyroid gland [5], mean produces high levels of thyroxine (T4) and triiodothyronine (T3) into the blood-stream [6]. The high level of thyroid hormones (TH) inhibits the production of thyrotropin (TSH) in the pituitary gland. Consequently, the concentrations of TSH hormone are lower than the normal level [7]. High concentrations of thyroxine (T4) and triiodothyronine (T3) lead to variation in mental state, anxiety, and nervousness [8]. Hyperthyroidism may cause oxidative damage to the liver [9], osteoporosis, heart failure, and heart attack [10]. The liver is the main primary target of the thyroid hormone and has major medical and biological repercussions [11]. Many medications to treat hyperthyroid have effects on the biochemical such as cholesterol, lipid profile [12], oxidation, antioxidant, malondialdehyde (MDA), catalyze enzyme (CAT), liver enzymes, and cardiac function [13]. Besides, the medications to treat hyperthyroid may lead to damages in the tissue of the thyroid gland and other organs [14].

Natural productions are crucial in pharmaceutical medications [15]. In addition, herbs, medicinal plants, and crude drugs are sources to combat various diseases including hyperthyroidism [16]. Herbs and medicinal plants have been

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used from the earliest times for their medicinal properties [17]. Herbs have been used to treat diseases since many years ago and over time grown over as supportive medicine because they are readily and cheaply available healthcare alternatives [18].

Garlic (*Allium sativum* L) is among the most widely grown vegetables worldwide [19]. Garlic is a source of many biologically active molecules, including phenolic acids, organic sulfur compounds, flavonoids, alleles, thiosulfate, and vitamins [20]. The beneficial properties of garlic to health are related to the bioactive compounds. In particular, its phenolic compounds, present in relatively high amounts, showed interesting pharmacological properties [21]. This study investigated the impact of garlic (*Allium sativum* L) extracts on hyperthyroidism caused by L-thyroxine in rats. Observing the changes in the blood level of thyroid hormones is to examine the various pharmacological effects of garlic (*Allium sativum* L) extracts.

EXPERIMENTAL DETAILS

Preparation of the Garlic Extracts

Fresh garlic (*Allium sativum* L.) was collected from Malang city in Indonesia and prepared in Pharmacology Laboratory in Universitas Brawijaya. Dry and ground bulbs of garlic (about 100 g) were submitted to extraction with 900 mL ethanol (98%) by the maceration method. The supernatant was evaporated by rotavapor. The alcoholic garlic extracts were stored at -20 °C until the day use. The garlic extracts were then diluted for oral administration in double-distilled water.

Experimental Animals

In this research, 25 healthy male rats (190-200 g weight) were used. Five rats per cage were housed in five cages. The rats were fed and housed at a steady temperature of 22 ± 2 °C in a room with a constant temperature of 22 ± 2 °C with % 50 relative humidity and 12 hours light/dark cycle period. All the experimental procedures were approved by the Health Research Ethics Committee, Faculty of Medicine at Brawijaya University (Approval number: 101/ EC/KEPK/05 /2020).

Experimental Design

25 male Wistar rats were divided into five main identical experimental groups (five rats) for every group as follows: healthy rats in the control group (Group I), the hyperthyroid group containing rats subcutaneously injected with L-thyroxine daily (0.3 mg/kg BW) for 14 days (Group II), treatment 1 group containing hyperthyroid rats + 100 mg/kg BW of garlic extracts (Group III), treatment 2 group containing hyperthyroid rats + 150 mg/kg BW of garlic extracts (Group IV), and treatment 3 group containing hyperthyroid rats + 200 mg/kg BW of garlic extracts). Garlic extracts were administered orally and all the treatments were given between 10.00 and 11.00 am of the day and were continued for 14 days.

Blood Sample Collection

After 14 days experimental period, the rats were anesthetized with ketamine. Then, the blood samples were collected from the heart, by using centrifugation (at 2500 rpm for 15 mins at room temperature) to separate the serum to determine the level of T3 and T4 using the Elisa method.

Data Analysis

The data were analyzed by using ANOVA static one-way from SPSS v.21.

RESULTS AND DISCUSSION

The Blood of Level Triiodothyronine

The results of measurements of the blood levels of T3 in the negative control, the positive control, and treatments groups are presented in Table 1. The results showed that T3 significantly increased ($p < 0.05$) in the positive control when compared to negative control rats. Garlic extract-treated dose of 100, 150, and 200 mg/kg BW showed that T3 recorded was not significantly different from the negative control group. The dose of 150 mg/kg could slightly decrease the level of T3, but a higher dose increased the level of T3. The blood levels of T3 in the negative control, positive control, and treatment groups are displayed in Figure 1. The overall differences in the blood level of T3 in each group above can also be depicted in a graphic form in Figure 1 showing no significant differences in the blood level of T3 between treatment and the negative control groups, but T2 is nearest to C-. Research conducted by Mahmoodi *et al.* proved that there was no significant changes in measured thyroid hormones [22].

TABLE 1. The level of T3 in each group

Groups	1	2	3	4	Mean ± SD
Control (-)	383.5	498.5	558.5	641	520.3 ± 108.3
Control (+)	523.5	658.5	1021	1733.5	984.2 ± 541.9
T1	383.5	706	1013.5	1281	846 ± 386.6
T2	356	486	503.5	843.5	499.7 ± 244.7
T3	693.5	778.5	1138.5	1123.5	933.5 ± 230.7

Abbreviation: C-: negative control, C+: positive control, T1: treatment group1, T2: treatment group 2, T3: treatment group3 (*p-value: 0.168)

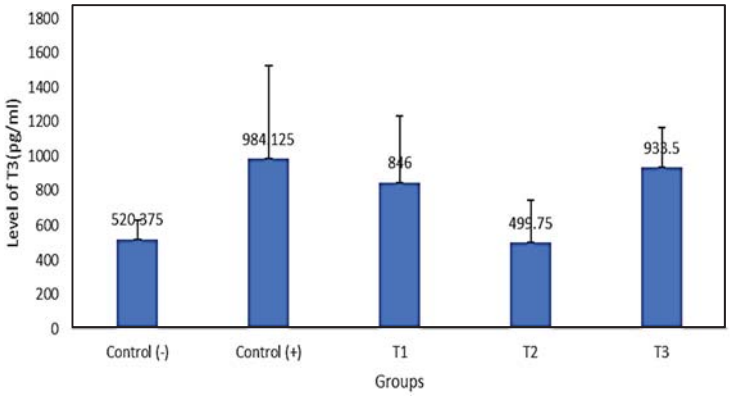

FIGURE 1. The blood level of T3 in each group. The blood levels of T3 in C+ increase compared to that in the C- group. In treatment groups, the blood levels of T3 are different, but the blood levels of T2 are near to C-: negative control, C+: positive control, T1: treatment group1, T2: treatment group 2, T3: treatment group 3.

The Blood Level of T4

The results of measurements of the blood level of T4 between the control, the hyperthyroid, and treatment groups are presented in Table 2. The results showed that T4 significantly increased ($p < 0.05$) in the positive control when compared to the negative control. Garlic extract-treated dose of 100, 150, and 200 mg/kg BW showed that T4 recorded was not significantly different from the positive control group, only T2 showed nearest to C- and it had similar results to the T3 level. Hence, it can be concluded that garlic extracts could affect the level of T3 and T4 in a dosage-dependent way. The blood levels of T4 in the negative control, positive control, and treatment groups are displayed in Figure 2.

TABLE 2. The blood level of T4 in each group

Groups	1	2	3	4	Mean ± SD
Control (-)	403.5	463.5	498.5	856	336.6 ± 96
Control (+)	373.5	546	636	726	542.2 ± 166.1
T1	228.5	243.5	738.5	1038.5	562.2 ± 3961.1
T2	83.5	123.5	248.5	333.5	197.2 ± 114.8
T3	436	481	593.5	1043.5	638.5 ± 278

Abbreviation: C-: negative control, C+: positive control, T1: treatment group1, T2: treatment group 2, T3: treatment group 3 (*p-value: 0.101)

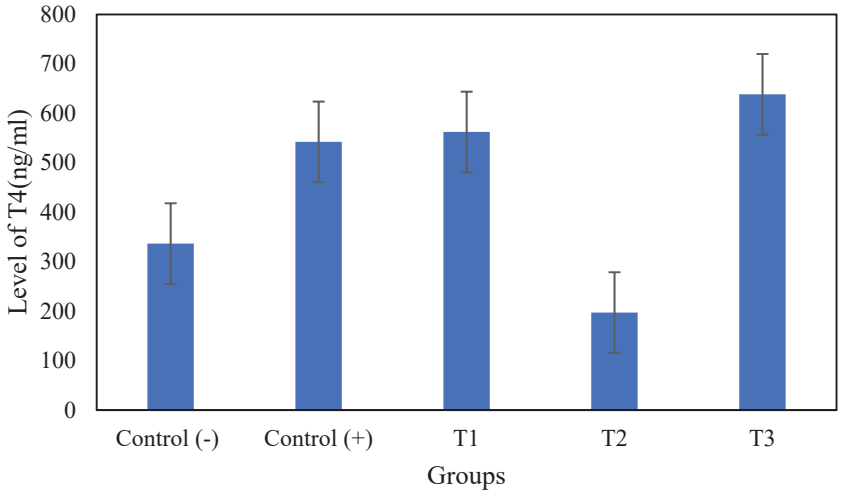

FIGURE 2. The blood level of T4 in each Group. The blood level of T4 in the C+ increased than that in C- group. In treatment groups, the blood levels of T3 are different, but the blood levels of T2 are near to C-: a negative control, C+: positive control, T1: treatment group1, T2: treatment group 2, T3: treatment group 3.

The differences in the blood level of T4 in each group above described in graphic form as in Figure 2. The results of the analysis of variance for the blood levels of T4 data showed no significant differences between all treatments of garlic extracts compared to the control group. It means that garlic extracts could prevent the increase in T4 levels in hyperthyroid rats. In this study, the increasing plasma T3 and T4 levels were shown in the rats induced by thyroxin to get hyperthyroid. The three different dose groups of garlic extracts decreased the blood levels of triiodothyronine (T3) and thyroxine (T4) in the hyperthyroid rats. Garlic contains chemical groups, sulfur, disulfides, and thio that influences the pathway by which the iodide ions present in the thyroid follicle are displaced, thus stalling iodination and leading to depression in T3 and T4 serum levels [23].

The Behavioral Measurement

The results of behavioral measurements by Open Field test in the control, the hyperthyroid, and treatment groups are displayed in Table 3. Table 3 shows that the difference in dose of garlic (*Allium sativum* L) extracts influence or provide different effects on the behavior of hyperthyroid rats (factor scores). The effects of the garlic (*Allium sativum* L) extracts start to see where the behavior of hyperthyroid rats begins (factor scores). In the hyperthyroid rats, induces by L-thyroxine were higher, after the garlic (*Allium sativum* L) extracts were given at a dose of 100 mg/kg BW, compared to the behavior of hyperthyroid rats (factor scores) in the positive control group. Then, the behavior of hyperthyroid rats (factor scores) more increased when they were given higher doses. Thus, based on the descriptive assessment according to the mean, the behavior of hyperthyroid rats (factor scores) can be said that the treatment in the form of garlic (*Allium sativum* L) extracts at a dose of 100, 150, and 200 mg/kg showed different influences, where the higher dose of garlic (*Allium sativum* L) extracts provided would further increase the behavior of hyperthyroid rats (factor scores).

TABLE 3. The average of the behavior of hyperthyroid rats (factor scores)

Groups	N	Mean	Std. Deviation	Minimum	Maximum
Control (-)	2	0.6471276	1.77059392	-0.60487	1.89913
Control (+)	1	1.26480	.	-1.26480	-1.26480
D1	2	0.3275606	0.65817443	-0.79296	0.13784
D2	2	0.2053058	0.80585152	-0.36452	0.77513
D3	1	0.2150537	.	0.21505	0.21505
Total	8	0.0000000	1.00000000	-1.26480	1.89913

Abbreviation: C-: negative control, C+: positive control, D1: treatment group1, D2: treatment group 2, D3: treatment group 3

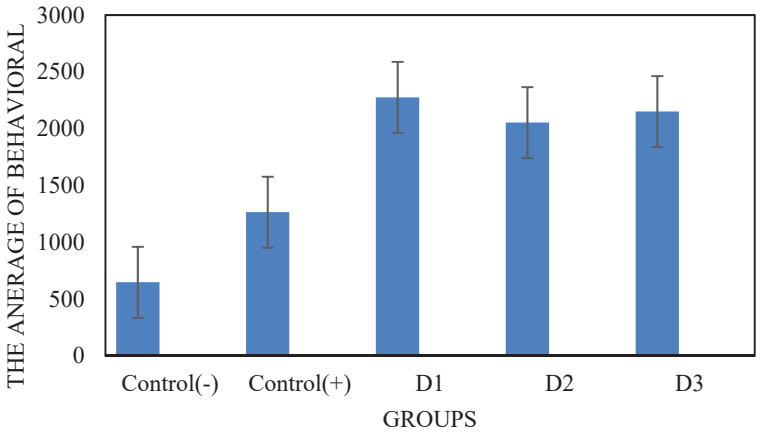

FIGURE 3. The average of hyperthyroid behavior in each group. The blood level of T4 in the C+ is higher than that in C- group. In treatment groups, the behavioral range is different, but in Treatment groups with 100, 150, and 200 mg/kg BW has no difference compared to the normal group.

The overall differences in the behavior of hyperthyroid rats (factor scores) in each treatment above can also be depicted in graphic form as presented in Figure 3. The results of the analysis of variance for the behavior of hyperthyroid rats (factor scores) data showed a significance value of 0.747 ($p > 0.05$). Hence, it can be concluded that there were no differences in the behavior of hyperthyroid rat (factor scores) data in each treatment group of garlic (*Allium sativum* L) extracts. Many studies showed animals have development in brain functions after eating garlic, and this because the antioxidants found in garlic destroy and neutralize the free radicals in the body [24]. In addition, garlic extracts have antioxidants indicating beneficial activities against behavior disorders [25].

SUMMARY

This study concluded that the concentration of 0.3 mg/kg BW of L-thyroxine could induce hyperthyroidism in male rats and garlic extracts tended to protect against hyperthyroidism although they were not significant results. This case may be caused by the too short duration of treatment.. Therefore, we suggest further research to conduct the longer exposure for the treatment.

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