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Effect of thyroxine-induced – hyperthyroidism on blood picture and the levels of iron in male rabbits

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ABSTRACT
The disorder known as hyperthyroidism is brought on by the thyroid gland producing too many thyroid hormones. The majority of research examining the connection between serum levels of iron blood picture and hyperthyroidism is conducted on human subjects. Thus, this study's objective was to ascertain how male rabbits' levels of iron were affected by thyroxine-induced hyperthyroidism. Twelve (12) mature male rabbits of similar weight and age were split into three groups: one for low dose (50µg/kg body weight of levothyroxine given orally), one for high dose (100µg/kg body weight of levothyroxine given orally), and a control group (tap water). Thyroid hormone levels were determined by taking blood samples from the rabbits prior to administering levothyroxine. For three weeks, the rabbits received daily doses. Following the conclusion of the treatment period the animals were weighed and each of the three groups had blood samples taken. The study's findings unequivocally demonstrated that administering levothyroxine caused the levels of thyroid hormones in the treated rabbits' sera to rise significantly. Loss of weight is one of the symptoms of hyperthyroidism. The body weight of the treated rabbits decreased slightly, but not in a way that was statistically significant. Levothyroxine treatment led to a substantial decrease in mean corpuscular hemoglobin but a significant rise in red cell distribution width, mean corpuscular volume, hemoglobin, and hematocrit. Blood ferritin and total iron-binding capacity were significantly increased whereas blood iron levels were significantly reduced with levothyroxine treatment.
It is advised that patients with hyperthyroidism or hypothyroidism who are taking levothyroxine regularly monitor their levels of these parameters in order to prevent further complications, as these results unmistakably demonstrate that treatment with the medication significantly alters the levels of these parameters .
Keywords: Thyroxine, Hyperthyroidism, Iron, Blood

ARTICLE HISTORY

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1. INTRODUCTION

The disorder known as hyperthyroidism results from the thyroid gland producing too much thyroid hormone (Bahn *et al*., 2011; Chowdhury *et al*., 2024). Hyperthyroidism is included in the condition known as thyrotoxicosis, which is brought on by an excess of thyroid hormone for whatever reason (Bahn *et al*., 2011). Conversely, some refer to them as the same thing (Devereaux and Tewelde, 2014). Individual differences exist in the signs and symptoms, which can include restlessness, weakened muscles, difficulty sleeping, an irregular heartbeat, heat sensitivity, diarrhea, thyroid enlargement, and weight loss (Devereaux and Tewelde, 2014). Thyroid hormone excess affects iron metabolism apart from the widely recognized consequences of hyperfunction, such as on the cardiovascular system and bone metabolism. For example, iron is a crucial component in the basic metabolic process of oxygen transport. Because high iron concentrations are harmful, the body's metabolism of iron is tightly controlled (Munoz *et al*., 2011). The metabolism of iron depends critically on a number of proteins. Iron that is bound to transferrin is transported by the plasma and taken up via receptor-mediated endocytosis. The main protein involved in buffering and storing excess iron is intracellular ferritin (Munoz *et al*., 2011). Hepcidin is a peptide hormone generated from the liver and is a key player in maintaining iron homeostasis. It reduces iron in plasma. Ferritin levels in hyperthyroid patients have been shown to be temporarily increased in case reports and case series, although they eventually reduced after the patients reached a euthyroid condition (Macaron and Macaron, 1982). While some research (Takamatsu *et al*., 1985) discovered a favorable correlation between thyroid hormone and ferritin levels, other studies (Sakata *et al*., 1991) did not.

All of these studies on the relationship between hyperthyroidism and iron levels are from human subjects. Therefore, this research was carried out to look into the impact of hyperthyroidism on the levels of iron and some vitamins in male rabbits.

2. MATERIALS AND METHODS

Animals

Twelve healthy, six-month-old male rabbits (weighing 1.5–1.7 kg) were purchased from a farmer in the area and kept in separate cages within a space with regular lighting and temperature fluctuations. The rabbits had unlimited availability of food and water. Two weeks before the trial, the bunnies were housed and cared for normally.

Drug

A synthetic version of the hormone thyroxine is called levothyroxine sodium (Berlthyrox 100 µg tablets, Berlin-Chime AG, Germany). A nearby pharmacy provided the levothyroxine, which was utilized in two separate dosages.

Experimental Procedure

Three groups of adult local rabbits were created: the first group received tap water as a control, the second group received an oral dose of 50 µg/kg body weight of levothyroxine, and the third group received an oral dose of 100 µg/kg body weight of levothyroxine (Harrison *et al*., 2010). However, blood samples were taken and thyroid hormone levels were tested before the hormone was given to the rabbits. For three weeks, the rabbits received daily doses. The rabbits were weighed at the conclusion of the three-week period, after which blood samples were collected from each of the three groups. Blood samples were taken from each

rabbit and placed in ethylene diamine tetra acetic acid (EDTA) tubes for hematological measures. For biochemical and hormonal data, blood was collected and placed in tubes without EDTA.

Hematological Analysis

XP-300 Automated Hematology Analyzer, Sysmex American, Inc. was used to evaluate hematological parameters, such as red blood corpuscles (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW).

Biochemical and Hormonal Analysis

Following a 5-minute centrifugation at 4000 rpm, serum was extracted and utilized to analyze the following levels:

Triiodothyronine (T3)

Elecsys T3 Cobas, a ready-to-use kit from Roche Diagnostics GmbH, Sandhofer Strasse (Germany), was used to measure T3 (Klee, 1996).

Thyroxine (T4)

Using a ready-to-use kit from Roche Diagnostics GmbH, Sandhofer Strasse (Germany), called ElecsysT4, T4 was assessed (Nelson and Wilcox, 1996) .

Thyroid stimulating hormone

An ElecsysTSH ready-to-use kit from Roche Diagnostics GmbH, Sandhofer Strasse (Germany) was used to quantify TSH (thyrotropin) (Sakai *et al*., 2009).

Iron

The iron concentration was determined using a ready-to-use kit (Fabricant: Biolabo SAS, France) using the methodology of Hennesy *et al* (1984) .

Ferritin

A ready-to-use kit called Elecsys from Roche Diagnostics International Ltd., Switzerland, was used to quantify the ferritin concentration in the samples .

Total Iron Binding Capacity (TIBC)

TIBC was calculated using a ready-to-use kit (manufacturer: Biolabs Sas, France) in accordance with Tietz (1999).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 4.00 (GraphPad software, San Diego, USA). A one-way Analysis of variance (ANOVA) with Tukey and HSD is used to display the statistical significance between the group means. In order to ascertain the significance between two means, the student's t test was employed. The mean's standard error (SEM) was used to express the results as mean ±. A statistically significant result was defined as a P-value of less than 0.05.

3. RESULTS

The levothyroxine-treated rabbits appeared to have hyperthyroidism symptoms, such as weight changes and lethargies, based on their physical examination. The concentrations of T3 in the sera of the experimental and control groups both before and after levothyroxine therapy are shown in Figure 1. Before treatment, the control group's mean T3 concentration (nmol/l) was 1.53 ± 0.03, and after the trial, it was 1.44 ± 0.09 in the serum. Between the two means, no statistically significant difference was seen. ($p > 0.05$). The concentration in the 50 µg/kg group increased from 1.67 ± 0.01 (pre-treatment) to 1.82 ± 0.04 (post-levothyroxine treatment). There existed a statistically noteworthy shift in the concentrations ($p = 0.0099$). Prior to therapy, the mean concentration of T3 in the 100 µg/kg was 1.66 ± 0.01; following levothyroxine medication, this increased to 1.93 ± 0.03. With a p-value of 0.0002, this shift was very significant. Furthermore, it is evident from these studies that the effect is dose-dependent.

The mean T4 concentrations (nmol/l) in the sera of the two experimental groups and the control group both before and after levothyroxine administration are displayed in Figure 2. The mean concentration in the control group was 47.68 ± 6.14 prior to treatment and 48.95 ± 5.29 at the end of the experiment. Between the two means, there was no discernible change ($p > 0.05$). The average level in the low dose group was 45.95 ± 3.41 prior to levothyroxine medication, and it increased to 62.8 ± 2.91 following levothyroxine administration. There was a statistically significant shift in the concentrations (0.0094). The mean T4 concentration in the rabbits receiving large doses of levothyroxine rose from 46.15 ± 2.99 pre-dosing to 60.28 ± 2.20 post-dosing. $P = 0.0089$ indicates that this increase was statistically significant. These findings make it abundantly evident that the effect is dose dependent.

The mean TSH concentrations (µIU/ml) in the sera of the three groups are shown in Figure 3. Before the experiment began, the control group's serum had a mean concentration of 0.009 ± 0.002, and by the conclusion of the experiment, it had a mean concentration of 0.011 ± 0.002. The means did not substantially vary from one another ($p > 0.05$). Before levothyroxine dosage, the low dose group's mean serum TSH concentration was 0.014 ± 0.002, however following levothyroxine treatment, this concentration dropped to 0.006 ± 0.001. A statistically significant decline was seen in the levels ($p = 0.0076$). Following levothyroxine administration, the mean TSH concentration in the high dose group decreased as well, going from 0.015 ± 0.002 prior to therapy to 0.008 ± 0.001. Additionally, this decrease was statistically significant ($p = 0.0411$).

Loss of weight is one of the indicators of hyperthyroidism. Weights assigned to the control group, the group treated with 50µg levothyroxine, and the group treated with 100µg levothyroxine are shown in Figure 4 both before and after the treatment period. Prior to treatment, the control group's mean weight (kg) was 1.58 ± 0.03. At the conclusion of the experiment, the control group's mean weight climbed marginally to 1.68 ± 0.03. Nevertheless, this rise failed to

obtain statistical significance ($p > 0.05$). After taking levothyroxine, the low dose group's weight decreased from 1.55 ± 0.03 prior to treatment to 1.50 ± 0.02. Yet, no statistically significant difference was seen in weight ($p > 0.05$). The beginning weight of 1.58 ± 0.03 in the high dose-treated group was lowered to 1.5 ± 0.02 after treatment; however, no statistically significant difference was seen in this reduction ($p > 0.05$).

The mean RBC counts (x106/µl) in the blood of rabbits treated with levothyroxine and control groups are displayed in Figure 5. In the control group, the mean count was 4.12 ± 0.26. In the groups treated with low and high doses, this number climbed to 5.64 ± 0.36 and 7.19 ± 0.50, respectively. The control group's mean and the low dose-treated group's mean showed marginally significant differences ($p = 0.0499$). Nonetheless, a statistically significant difference in mean existed between the high dose-treated group and the control group ($p = 0.0008$). Additionally, there was a marginally significant difference ($p = 0.0461$) in the means of the two treated groups, which would suggest that levothyroxine's effects are dose-dependent.

The mean hemoglobin concentrations (g/dl) in the blood of the treated and control rabbits are shown in Figure 6. The control group's blood contained an average concentration of 8.35 ± 0.43. The mean concentrations in the rabbits treated with low and high doses were 11.98 ± 0.60 and 15.78 ± 1.25, respectively. The low dose-treated group's mean was substantially different from the control group's ($p=0.0326$). Additionally, there was a mean difference between the high dose-treated group and the control group that was found to be extremely significant ($p = 0.0004$). Furthermore, there existed a marginally significant distinction ($p = 0.0259$) in the means of the two groups that received treatment.

The mean percent hematocrit (%) of the blood from the treated and control rabbits is shown in Figure 7. In the group receiving low dosage therapy, the mean percent hematocrit was 36.88 ± 1.77; Among those receiving a high dose, it was. 43.43 ± 3.64 The mean percent hematocrit in the control group was 27.53 ± 1.46. The mean of the group receiving low dosage treatment did not differ markedly from the mean of the control group ($p > 0.05$). Nonetheless, a noteworthy distinction was found to exist between the means of the high dose-treated group and the control group ($p = 0.0037$). Additionally, there was no discernible change between the two treated groups' means.

The mean MCH (pg) for the two treatment groups along with the control group are displayed in Figure 8. In the low dose-treated group, the mean MCH was 21.13 ± 0.19; in the high dose-treated group, it was 19.75 ± 0.32 The control group's mean MCH was 22.23 ± 0.25. The low dose-treated group's mean was substantially different from the control group's ($p = 0.0349$). Nonetheless, the means of the groups receiving high doses of treatment differed significantly ($p = 0.0002$) from the control group. Additionally, a notable distinction ($p = 0.0110$) was seen between the means of the two groups that received treatment .

The mean MCHC concentrations (g/dl) in the blood of the treatment and control groups are shown in Figure 9. The mean concentrations of the groups treated with low and high doses were 32.15 ± 0.86 and 30.9 ± 0.49, respectively, whereas the mean concentration of the control group was 32.8 ± 0.13. The means of the three treatment groups showed no discernible differences ($p > 0.05$) .

The mean MCV (fl) for the control group as well as the two treatment groups is shown in Figure 10. The control group's mean MCV was 63.5 ± 0.96, while treatment groups receiving low dose and high dose had increases to 65.3 ± 0.88 and 68.63 ± 1.36, respectively. The mean of the group receiving low dosage treatment did not differ significantly from the mean for the control group ($p > 0.05$). The group that received a high dose showed a statistically significant difference in mean values from the control group ($p = 0.0216$). Between the average of the two treated groups, there was no discernible change ($p > 0.05$) .

The mean RDW-SD (fl) for the groups treated with levothyroxine and the control group is shown in Figure 11. The mean of the control group was 31.7 ± 0.51, whereas the two-treated group's mean RDW climbed to 32.1 ± 1.16 and 48.95 ± 5.45, respectively. The mean of the group receiving low dosage treatment did not differ significantly from the control group's mean ($p > 0.05$). Nonetheless, a noteworthy distinction was found ($p = 0.0110$) between the means of the high dose-treated group and the control group. Furthermore, a noteworthy distinction ($p = 0.0125$) was seen between the means of the two groups that received treatment.

Figure 12 shows the outcomes of the impact of levothyroxine on serum iron concentration. The control group's serum had a mean concentration of iron (µg/dl) of 192.5 ± 4.1, but the sera of groups treated with low and high doses had lower concentrations, at 169.3 ± 6.3 and 130.8 ± 10.6, respectively. The mean of the low dose-treated group did not differ significantly from the control group's mean ($p > 0.05$). The average disparity between the group receiving a high dose and the control group was, nevertheless, statistically significant ($p = 0.0006$). Additionally, a noteworthy distinction ($p = 0.0134$) was seen between the means of the two treatment groups.

The mean ferritin concentrations (µg/l) in the sera of the treated and control groups are shown in Figure 13. The control group's serum had a mean concentration of 11.8 ± 0.50, but the low dose and high dose treatment groups' sera had mean concentrations of 15.03 ± 0.46 and 27.25 ± 4.33, respectively. The mean of the group receiving low dosage treatment did not substantially deviate from the mean of the control group ($p > 0.05$). Nonetheless, a noteworthy distinction was found to exist between the means of the group receiving high dose and the control group ($p = 0.0050$). Additionally, a noteworthy distinction ($p = 0.0190$) was seen between the means of the two treatment groups .

The mean TIBC (µg/dl) in the sera of the levothyroxine-treated and control groups is displayed in Figure 14. The control group's serum had a mean TIBC of 288.5 ± 4.17, but the sera of the groups receiving low and high dose had average TIBC of 312.5 ± 4.48 and 448.8 ± 26.52, respectively. The mean of the group receiving low dose did not differ markedly from the reference group's mean ($p >$

0.05). Nonetheless, the mean of the group receiving high dose of medication differed significantly (p = 0.0001) from the reference group. Additionally, there was a highly statistical difference between the two treated groups' average (p = 0.0005). This suggests that levothyroxine's effects are dose-dependent.

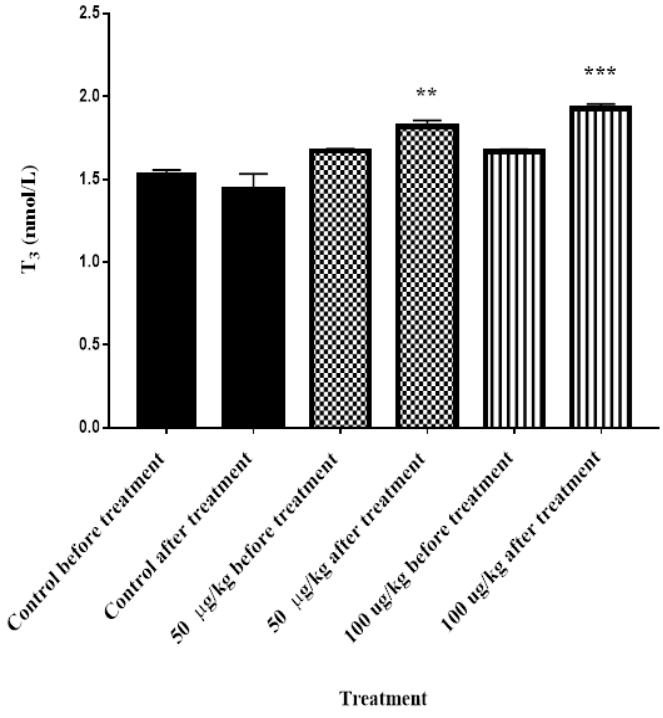

Figure 1: The average T3 concentrations in the sera of the treated and control rabbits both before and after levothyroxine administration. Mean ± SEM is shown for N = 4. Asterisks denote notable variations between the means of the same therapy before and after treatment.

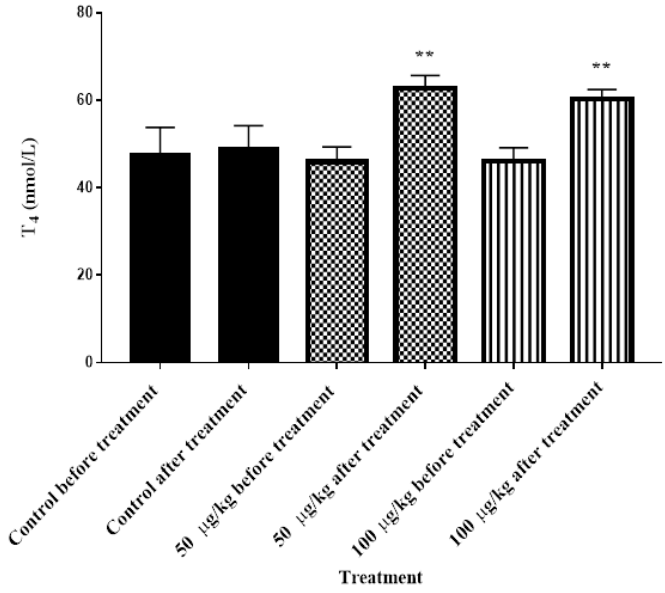

Figure 2: The mean T4 levels in the blood of the animals receiving levothyroxine and the animals receiving no therapy. Mean ± SEM is shown for N = 4. Asterisks denote significant differences between the averages of the same therapy before and after treatment.

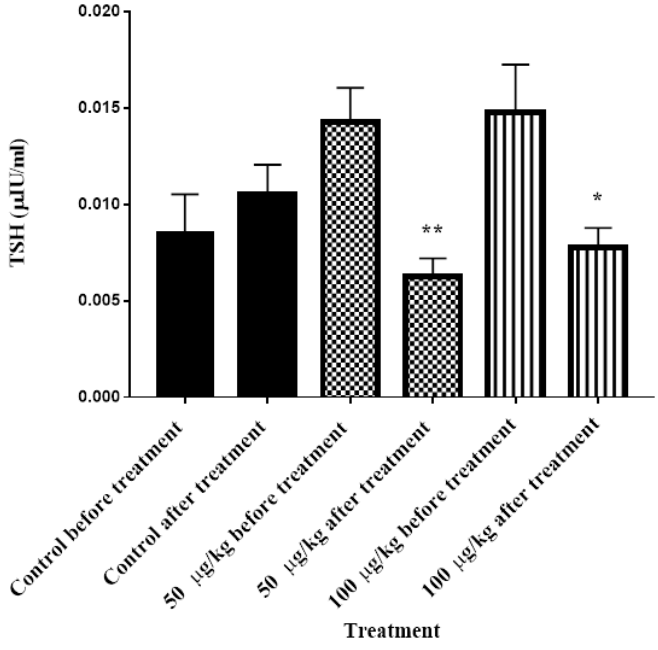

Figure 3: The average TSH levels in the blood of bunnies treated with levothyroxine and bunnies in the control group, both prior to and following therapy. Mean ± SEM is shown for N = 4. Asterisks denote significant differences between the means of the same therapy before and after treatment.

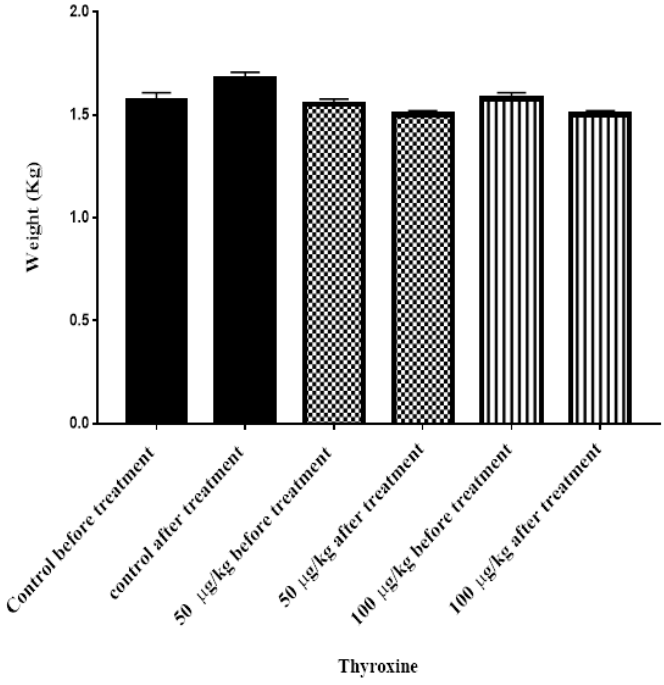

Figure 4: Mean weights of the rabbits treated with low and high dosages of levothyroxine, as well as the control group, before and after therapy. Outcomes are Mean ± SEM. N is 4. The means did not differ significantly from one another.

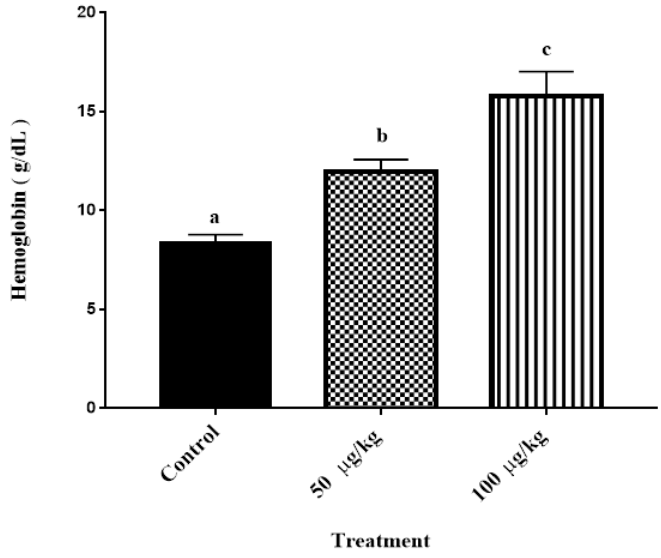

Figure 6: Hemoglobin concentration mean in rabbits treated with levothyroxine and control blood. All results are mean ± SEM. 12 is N. Significant variations between the means are indicated by different letters.

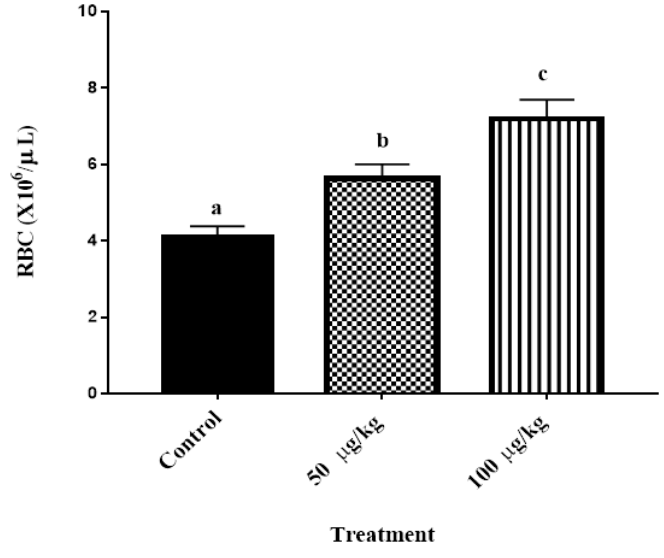

Figure 5: RBC mean in the blood of rabbits treated with levothyroxine and control groups. All results are mean ± SEM. 12 is N. Significant variations between the means are indicated by different letters.

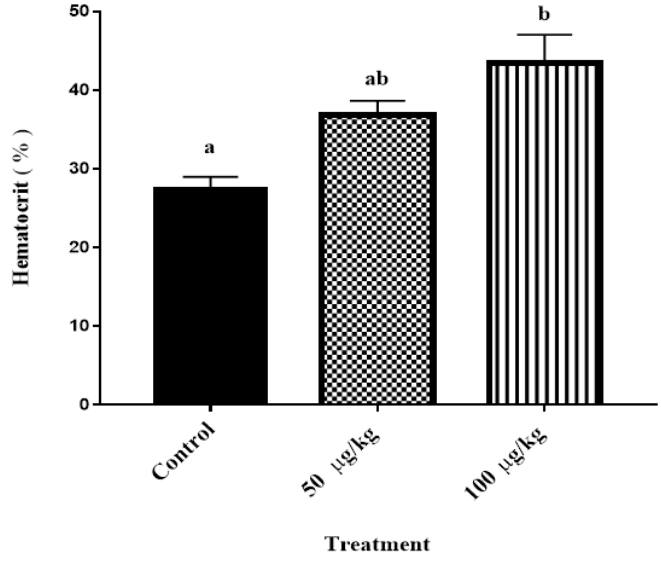

Figure 7: Mean percentage of hematocrit in rabbits treated with levothyroxine and control blood. All results are mean ± SEM. 12 is N. Similar letters signify that there are no appreciable variations in the means. Significant variations between the means are indicated by different letters.

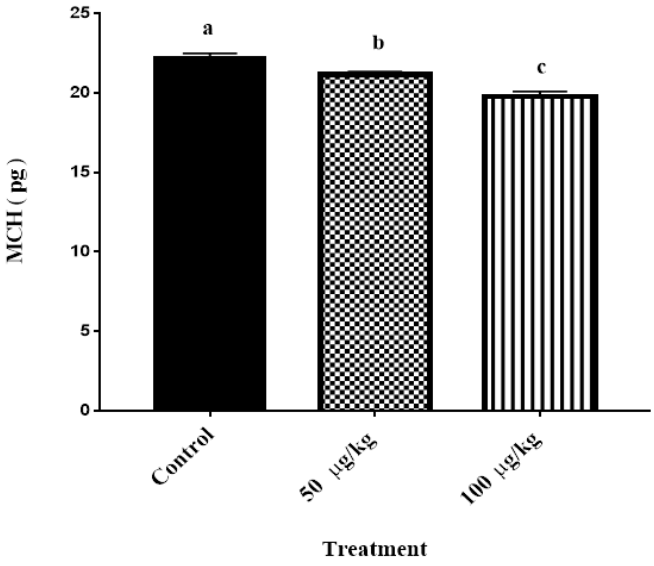

Figure 8: Mean MCH in the blood of rabbits treated with levothyroxine and control. N = 12; results are mean ± SEM. Similar letters signify that there are no appreciable variations in the means. Significant variations between the means are indicated by different letters.

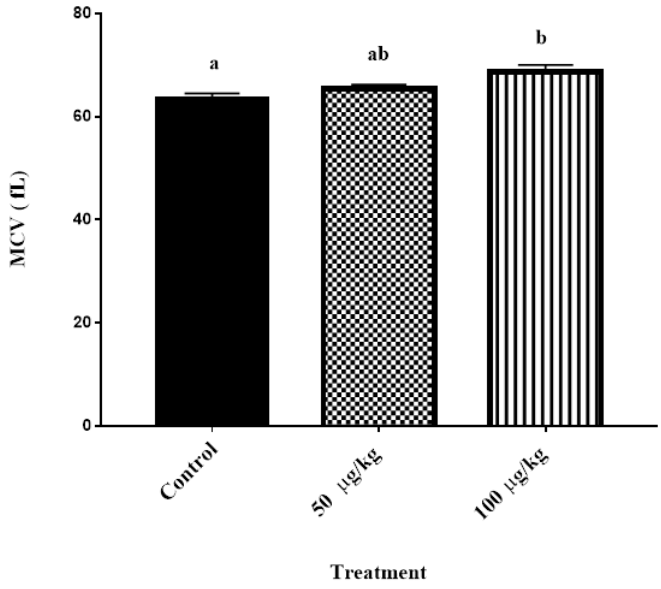

Figure 10: Mean MCV in the blood of rabbits treated with levothyroxine and control. N = 12; results are mean ± SEM. Similar letters signify that there are no appreciable variations in the means. Significant variations between the means are indicated by different letters.

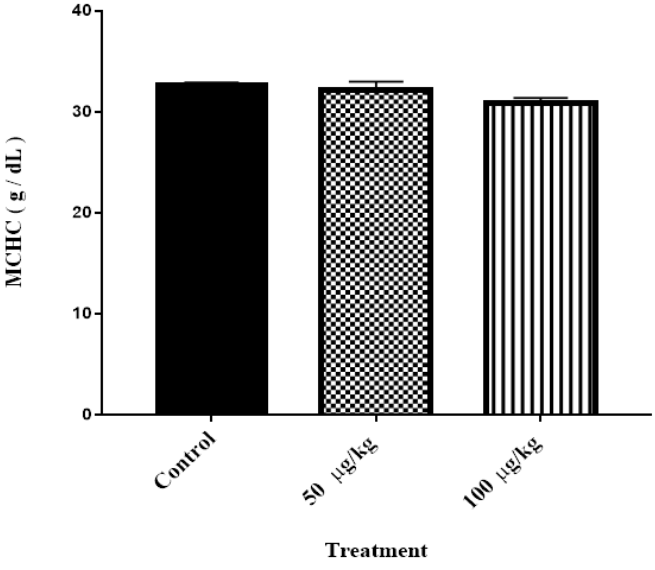

Figure 9: Mean MCHC in the blood of rabbits treated with levothyroxine and control. N = 12; results are mean ± SEM. The means don't differ much from one another.

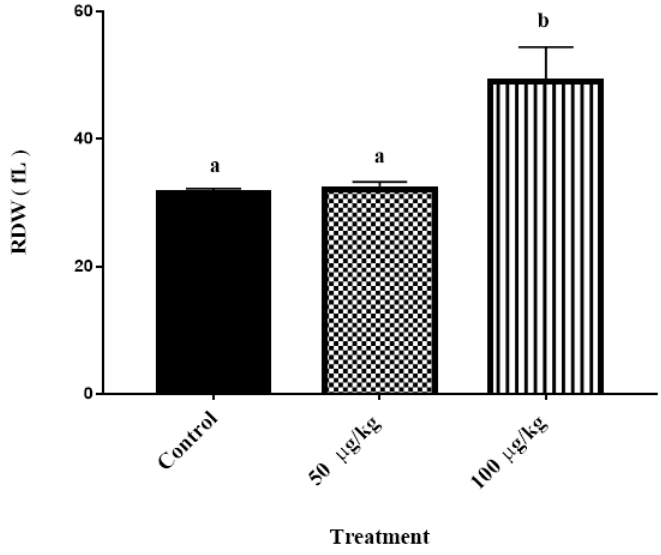

Figure 11: Mean RDW-SD in the blood of rabbits treated with levothyroxine and control. All results are mean ± SEM. 12 is N. Similar letters signify that there are no appreciable variations in the means. Significant variations between the means are indicated by different letters.

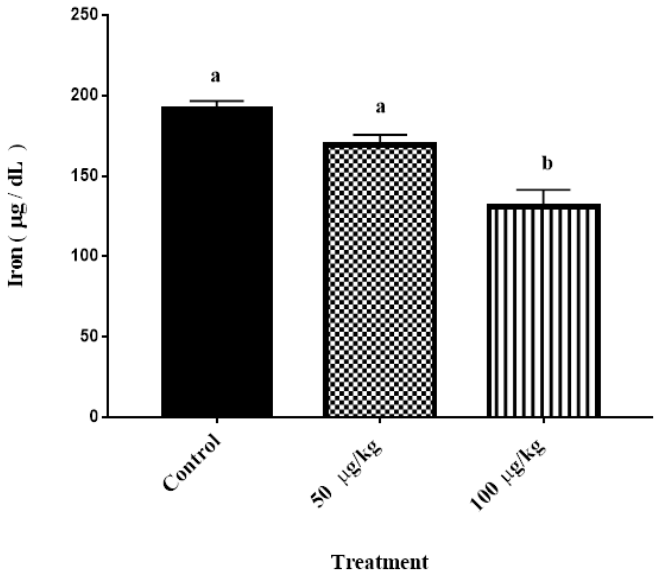

Figure 12: Mean iron concentration in the blood of rabbits given levothyroxine and reference rabbits. The findings are expressed as mean ± SEM. N = 12. Similar letters signify that there are no appreciable variations in the averages. Significant variations between the averages are indicated by different letters.

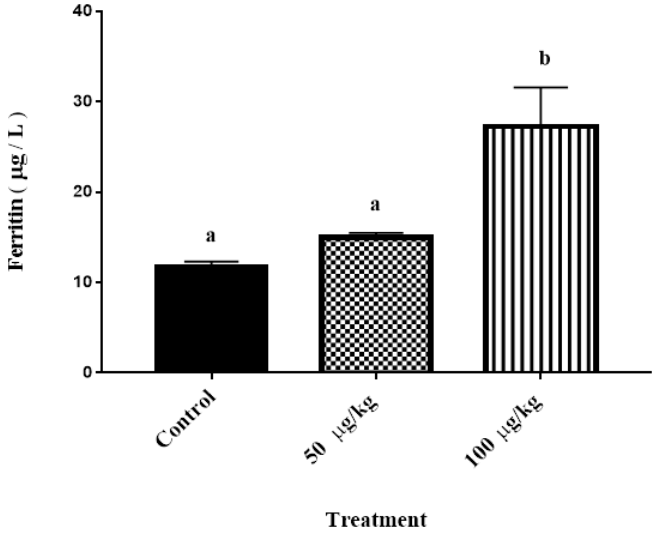

Figure 13: The average ferritin content in the sera of rabbits treated with levothyroxine and those under control. All results are mean ± SEM. 12 is N. Similar letters signify that there are no appreciable variations in the means. Significant variations between the means are indicated by different letters.

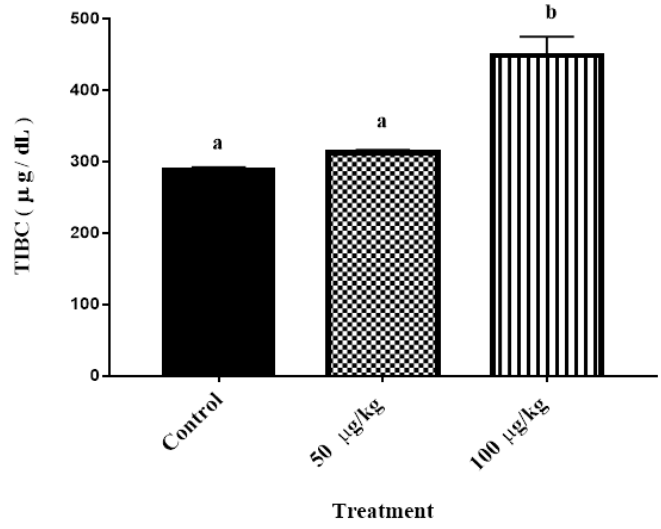

Figure 14: Mean TIBC in the sera of rabbits treated with levothyroxine and those that were not. N = 12; results are mean ± SEM. Similar letters signify that there are no appreciable variations in the means. Significant variations between the means are indicated by different letters.

4. DISCUSSION

Regardless of the source of the hormones, hyperthyroidism, also known as thyroidicosis, is a disorder marked by elevated thyroid hormone levels in the blood. The thyroid hormone (T4), levothyroxine, also referred to as L-thyroxin, was given orally to the rabbits in this study for three weeks at 50 and 100 µg/kg dosages in order to induce hyperthyroidism. Every treated rabbit showed signs of hyperthyroidism, with T3 and T4 levels markedly greater than baseline (before to treatment) and control rabbit levels. Oral administration of 50 µg/kg of levothyroxine sodium caused hyperthyroidism in rabbits, according to a study by Lafta (2016). Moreover, Utkan *et al.* (2007) used intraperitoneal administration of 200 µg/kg of T3 to induce hyperthyroidism in albino rabbits. Jiang *et al*. (2000) administered intramuscular doses of T4 to rabbits daily for seven days at a rate of 200 µg/kg body weight, resulting in hyperthyroidism. Rats were able to develop hyperthyroidism (T3 and T4) when thyroid hormones were given parenterally by Williams and Lefkwitz (1997). They used two distinct injection techniques. Male rats received 500 µg/kg of T3 subcutaneously every day for three days, while female rats received 800 µg of thyroxin intramuscularly 2 times a day for 7 days. The findings of the experiments were similar for both injection procedures.

One of the signs of hyperthyroidism is weight changes. The thyroxin-treated rabbits' weight declined marginally after the course of treatment. One of the impacts of thyroid hormone is how much weight a person weigh. Thyroid hormones have anabolic actions that stimulate protein synthesis when produced in physiological levels and catabolic effects that increase protein oxidation when secreted in excess (Triralle *et al*., 1996). Due to an increase in protein catabolism brought on by thyroid hormone regulation of proteolytic and other lysosomal enzyme activity in muscle tissue, muscle atrophies are seen in hyperthyroidism, a disorder in which the

thyroid releases too much hormones (Demartino and Goldberg, 1978). Increased basal metabolism is another impact of thyroid hormones. Moreover, body weight falls when basal metabolism rises. The thyroid hormone dramatically lowers the buildup of fat. Despite the fact that hyperthyroidism is known to enhance hunger, hyperthyroidism has been linked to weight loss. Thyroid hormone also greatly lowers fat deposition, which helps with weight loss. In 1961, Grossie and Turner discovered that giving female rats a thyroxine injection decreased their weight gain by less than 10% in contrast to the reference group. The quantity of food consumed and the hunger both rises, as do the fluids, secretions, and movements of the digestive tract. Diarrhea is often associated with increased thyroid hormone secretion (Ebert, 2010).

Thyroid hormone was given to the rabbits, which resulted in an increase in RBC in this investigation. It seems that the elevation was dose-dependent. Zahediasl *et al*. (2010) discovered that rats given oral levothyroxine for 30 or 60 days did not exhibit a statistically significant change in RBC count. After three and six weeks, Kandir and Keskin (2016) observed no discernible change in the RBC count between hyperthyroid and control rats. Messarh *et al*. (2011), on the other hand, found that after five weeks, rats treated with L-thyroxine had lower RBC counts. On the other hand, research on humans has shown that hyperthyroidism results in erythrocytosis, or an increase in red blood cell count (Kawa *et al*., 2010). Conversely, Dorgalaleh *et al*. (2013) failed to discover a statistically noteworthy variation in the numbers of red blood cells between hyperthyroid patients and healthy control subjects. Erythropoiesis is induced by thyroid hormone, which causes marrow erythroid hyperplasia and a rise in red cell mass (Ford and Carter, 1988; Singh and Catlett, 1998). Although evidence for direct (Golde *et al*., 1977) and indirect (Dainiak *et al*., 1986; Fandrey *et al*., 1994) effects on erythroid precursors has been described, the mechanisms underlying these activities are still unknown. The hormones can either directly (Fandrey *et al*., 1994) or indirectly (Ford and Carter, 1988) raise erythropoietin levels. The kidney is driven to enhance erythropoietin production and release when tissues are deprived of oxygen due to the thyroid hormone impact. Then, erythropoiesis in the bone marrow is stimulated and accelerated by erythropoietin. Five of the twenty-one hyperthyroid patients that Das *et al.* (1975) examined were anemic. The patients had raised serum erythropoietin levels and enhanced erythropoesis with erythrocytosis, according to the scientists' findings. They deduced that these effects were induced by erythropoietin. But only T3 enhanced oxygen consumption in mouse, but both T3 and its calorically dormant form, enhanced RBC iron utilization (Donati *et al*., 1965). As a result, thyroid hormone's erythropoietic and calorigenic effects might not be connected.

Treatment with thyroid hormones resulted in an increase in hemoglobin concentration. This is to be expected considering the rise in hemoglobin-containing red blood cells. Patients with hyperthyroidism had greater hemoglobin levels, according to Kawa *et al*. (2010). In addition, Dorgalaleh *et al*. (2013) found that although RBC counts did not rise, hemoglobin concentration did. These individuals had hyperthyroidism. In hyperthyroid rats, Zahediasl *et al*. (2010) found that after 60 days, there was no significant rise in the RBC count, but there was a substantial increase in hemoglobin concentration. Since there was also an increase in MCH and MCV levels, this could be the result of aberrant hemoglobin and red blood cell production. After six weeks, Kandir and Keskin (2016) observed no discernible shift in the hemoglobin content in hyperthyroid rats. Considering that no other hematological parameters changed noticeably, this is to be expected.

In these rabbits, levothyroxine therapy raised the hematocrit percentage. The RBC count increased in tandem with this increase. Hyperthyroidism was found by Kossler *et al*. (1987) to raise hematocrit and RBC in rats. However, hematocrit did not significantly change in rats treated with thyroxine, according to Kandir and Keskin (2016) and Messarah *et al*. (2011) who saw a decrease in hematocrit in thyroxine-treated rats. In contrast to the standard reference group, Zahediasl *et al*. (2010) found that after 60 days, rats with hyperthyroidism reported higher hematocrit levels but no appreciable shift in RBC count. Kawa *et al*. (2010) found that an increase in hematocrit has been linked to hyperthyroidism in humans. Conversely, Dorgalaleh *et al*. (2013) discovered that hyperthyroid individuals had a lower hematocrit.

In this investigation, levothyroxine therapy raised the values of MCV and MCH. This suggests that levothyroxine medication has a major impact on the size of RBC generated and the distribution and concentration of hemoglobin in each red blood cell. In rats that were hyperthyroid for 30 days, MCH and MCV values did not significantly alter, but after 60 days of hyperthyroidism, they dramatically rose, according to Zahediasl *et al*. (2010). In contrast, Kandir and Keskin (2016) observed that after 3 and 6 weeks of hyperthyroidism in rats, there were no appreciable alterations in MCV, MCH levels, or MCHC. Kawa *et al*. (2010) found that hyperthyroidism elevated the MCV value in people, a sign of macrocytosis. Additionally, they found that MCV levels, a sign of microcytosis, were below the normal range in 42% of hyperthyroid patients. Additionally, they discovered that patients with hyperthyroidism had considerably decreased MCH and MCHC per red blood cell, indicating hypochromic RBCs. Geetha and Srikrishna (2012) and How *et al*. (1979) both discovered low MCV values in hyperthyroid individuals. When comparing hyperthyroid patients to controls, Singh *et al*. (2016) reported their results, which indicated that only MCV and MCH were statistically different; other hematological variables did not alter statistically. However, recently Yang *et al*. (2025) reported that patients with hyperthyroidism showed significant reductions in red blood cell parameters, including HGB, HCT, MCV, HCH, and MCHC. Their research showed that increased thyroid hormone concentrations may cause changes in peripheral blood cell counts by inducing cell cycle arrest in bone marrow cells, leading to proliferation and differentiation impairment.

In this investigation, levothyroxine medication caused a considerable rise in the RDW value. An indication of RBC size variation within a blood sample is the RDW. According

to Szczepanek-Parulska *et al*. (2017), RDW, a measurement of the amount of RBC anisocytosis, is higher in individuals with deficiencies in folate, vitamin B12, and iron, and thyroid function disturbance. Other clinical conditions that may also affect RDW include inflammatory processes, cardiac diseases, and rheumatoid arthritis. High RDW values were found in hyperthyroid individuals by Geetha and Srikrishna (2012). RDW can indicate a folate or vitamin B12 shortage in addition to increasing iron-deficiency anemia (Szczepanek-Parulska *et al*., 2017). In comparison to euthyroid controls, hyperthyroidism was linked to noticeably higher RDW values in an investigation carried out in 2013 by Dorgalaleh *et al*.

In this research, levothyroxine medication resulted in a significant reduction in iron concentrations and a rise in ferritin and TIBC concentrations. Iron levels in the liver of rats given L-thyroxine-induced hyperthyroidism were noticeably higher (Deshpande and Nadkarni, 1992). On the other hand, hyperthyroid rats had a 38% increase in liver ferritin production rate. They came to the conclusion that increased ferritin synthesis in the liver and possible circulatory leakage could be partially to blame for hyperthyroidism's high serum ferritin levels. Ferritin concentrations in the serum of hyperthyroid patients increased, according to Onat *et al*. (2003), although serum iron levels matched those in the reference group in every way. High levels of plasma erythropoietin (EPO), elevated erythropoiesis, and hypercellularity in the bone marrow are characteristics of hyperthyroid conditions (Rivlin and Wagner, 1969; Popovic *et al*., 1977). Thus, there is no iron overload but an increase in iron turnover as a result of higher iron usage (Donati *et al*., 1965). Faster iron incorporation into heme can account for the observed drop in plasma iron levels. Ferritin levels in hyperthyroid patients were briefly high in case reports and case series, but they subsequently reduced after the patients reached a euthyroid condition (Macaron and Macaron, 1982; Van de Vyver *et al*., 1982). While some research (Takamatsu *et al*., 1985) found that the levels of thyroid hormone and ferritin were positively correlated, other studies (Sakata *et al*., 1991) did not.

5. CONCLUSION

Characterized by a generalized acceleration of metabolic processes, hyperthyroidism exerts profound systemic effects on multiple organ systems, significantly altering cellular oxygen consumption, nutrient utilization, and mineral homeostasis. The aim of this study was to evaluate the dose-dependent effects of daily thyroxine-induced hyperthyroidism over a three-week period on the hematological profile and serum iron levels in male rabbits, while observing its subsequent impact on overall body weight. A 3-week sub-chronic daily exposure to thyroxine in male rabbits induces distinct, dose-dependent alterations in the blood picture and iron homeostasis, without causing statistically significant systemic wasting. While daily administration of both 50 µg/kg and 100 µg/kg body weight doses for 21 days resulted in a slight, non-significant reduction in body weight, they triggered profound internal metabolic shifts. At the lower daily dose (50 µg/kg), thyroxine initiated moderate elevations in erythrocytic parameters (RBC, Hb, and Hct) alongside a reduction in serum iron due to accelerated utilization. These effects were severely exacerbated at the higher daily dose (100 µg/kg), leading to profound iron depletion indicating severe physiological stress and altered hematopoiesis. Ultimately, these findings demonstrate that a 3-week daily course of thyroxine-induced hyperthyroidism alters hematological profiles and depletes iron stores in direct proportion to the dose concentration, serving as a critical indicator of metabolic stress prior to the onset of significant physical weight loss and necessitating careful monitoring in clinical and experimental settings.

6. DECLARATIONS

Funding

This work was self-funded by the authors.

Data availability

The data is available when requested via the corresponding author.

Conflict of interest

The authors declare no competing financial or nonfinancial interests.

Ethical approval

Ethical approval was granted by Al-Mukhtar committee for Biosafety and Bioethics (MCBB), reference number: NBC: 007. A. 26. 77.

Informed consent

All participants provided written informed consent for the use and publication of their data.

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