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STUDY OF THE SYNERGISTIC EFFECT OF METHIMAZOLE AND AQUEOUS EXTRACT OF LICORICE ON THE LEVEL OF THYROID HORMONES IN RATS

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Abstract
This study was conducted at the University of Tikrit, College of Education for Pure Sciences, Department of Biology, in 2024, on the licorice plant, to study the synergistic effect of the drug methimazole and the aqueous extract of the licorice plant, to reveal the effective biological active compounds contained in this plant and the effect of these compounds on the level of T3 thyroid hormones. And T4 and TSH, are the active compounds detected in the aqueous extract of the licorice plant, which are phenols, flavonoids, alkaloids, glycosides, and tannins. In this study, 10 rats were divided into two groups. The first group was the control group, and the second group was the group treated with the drug methimazole and the aqueous extract of the licorice plant. The results showed that the treatmentwith the drug Methimazole and the aqueous extract of the licorice plant affected the level of thyroid hormones, as it was observed that there was a significant decrease ($P \leq 0.05$) in the level of the thyroid hormones T3 and T4 and the thyroid stimulating hormone (TSH) in the blood serum of the group treated with methimazole and the aqueous extract of the licorice plant compared to the control.
Keyword: methimazole, licorice, thyroid hormones

Introduction

In recent years, hyperthyroidism has become a common disorder that requires medical treatment with antithyroid drugs from the thionamide class, which work to prevent the synthesis of thyroid hormone and thus reduce high thyroid hormone concentrations in the blood circulation. They can be of great value in controlling hyperthyroidism. Long - term hyperthyroidism. Antithyroid medications also play an important role in the preoperative stage. Methimazole is an antihyperthyroidism drug of the thionamide type that prevents the synthesi s of thyroid hormones. It was first introduced to science in 1949 AD. It is used in the treatment of hyperthyroidism, especially in people who They have complex treatment options such as surgery or radioactive iodine therapy (Davaran and Shafiei,2020). Methimazole was approved for medical use in the United States in 1950 under the trade name Tapazole by the US Food and Drug Administration (FDA). Since then, methimazole has emerged as one of the most widely prescribed drugs for the treatment of hyperthyroidism, especially in cases where the treatment... Surgical or radioactive iodine is not suitable. Methimazole has a chemical formula of C4H6N2S and a molecular weight of 114.17 g/mol in the form of a white crystalline powder. Its melting point is 417.15 K and its boiling point is 553.15 K. After reaching the boiling point, it decomposes (Sun et al., 2021). Methimazole inhibits the enzymatic process mediated by thyroid peroxidase. Central to the mechanism of action of methimazole is its strong inhibition of thyroperoxidase, an enzyme important for the synthesis of thyroid hormones. Peroxidase catalyzes the addition of iodine to the tyrosine residue on thyroglobulin, leading to their subsequent conjugation and the formation of T4 and T3. The structure of thionamide allows Methimazole binds irreversibly to the peroxidase enzyme in its active site, effectively disrupting iodine and conjugation reactions. It is rapidly absorbed from the gastrointestinal tract with an absorption rate of about 70-80%. It is widely distributed throughout the body and is concentrated in the thyroid gland. It is not bound to protein in the blood. The half-life of the drug in serum is approximately 6-8 hours and the biological effect of the drug can persist for more than 12 hours after oral administration. Methimazole is metabolized mainly rapidly and extensively in the liver (Xue et al., 2021). Nature has always been a wonderful source of therapeutic materials, as it provides us with many medicinal plants that produce valuable phytochemicals, as each plant is essentially an integrated pharmacy, its components varied in proportions established by God Almighty. Among these plants is the Licorice plant, which is used in the treatment of many diseases. Diseases, as its roots are the main

source of an important source of many medicines because it contains the active ingredient known as Glycyrrhiza , which is derived from the Greek word glykos, meaning sweet, and rhiza, meaning root, meaning sweet root(Batiha et al;2020). Licorice is a perennial plant belonging to the leguminous family Leguminaseae, which has more than 30 species widely distributed globally (Cerulli et al., 2022). It is a wild plant known by several common names, including licorice, licorice stem, licorice stick, and the delicious root and wood fragrance plant (Pastorino et al., 2018).The roots contain many biologically active chemical compounds and thus have important therapeutic properties in treating bacterial, viral and fungal infections. Some of the active compounds derived from them are of great importance in treating some cancerous diseases, as the compound Glabridin, which is a chemical compound found in licorice, is a type of isoflavonoid. It works This compound is an anti-cancer cell division, in addition to its use in treating gastrointestinal ulcers, as it works to increase the mucous glands of the digestive system. Many studies have proven that the licorice plant has antioxidant activity (Minnetti et al., 2022 Koyuncu et al., 2021).

Materials and methods

This study was conducted for the period from 6/22/2023 until 7/22/2023 in the Animal House Unit / College of Veterinary Medicine / Tikrit University, where the rats were subjected to appropriate environmental conditions in terms of ventilation and temperature of 25 °C and a lighting period of 12 hours. The animals were fed with the standard and mixed ration. From yellow corn 35% wheat 35% soybeans 20% concentrated protein 10%. (Ward,1970).

Distribution of study animals

The laboratory rats were distributed into two groups, each group containing 5 rats. Each group was placed in a separate cage and provided with water and food in sufficient quantities during the experiment period of 30 days. Each animal was weighed separately before the experiment began , taking into account the similarity in weights between the groups.

The weight was then recorded . Weighing them weekly after starting the experiment during the remaining period , the study animals were divided into two groups.The first group (control): This group was given water and food for 30 days.The second group (the methimazole and licorice group). The animals of this group were given the drug methimazole at a concentration of 10 mg/kg of body weight and the aqueous extract of licorice at a concentration of 500 mg/kg of body weight. It was dissolved in distilled water and the animals were dosed orally daily for 30 days.

Estimation of T3 concentration

The concentration of the T3 hormone was estimated by following the steps included with the ready-made analysis kit of the American company Monobind and according to the manufacturer's instructions for the ELISA technique (Braverman, 1996).

Estimation of the concentration of thyroxine tetraiodine

The T4 hormone concentration was estimated by following the steps included with the ready-made analysis kit (Kit) of the American company Monobind and according to the manufacturer's instructions for the ELISA technique (Mazzafferi and Gharib, 1998).

Estimating the concentration of thyroid-stimulating hormone

The TSH concentration was measured by following the steps included with the ready-made analysis kit (Kit) of the American company Monobind , and according to the manufacturer's instructions for the ELISA technique (Fisher, 1996).

Estimating cholesterol level

The concentration of cholesterol in blood serum was estimated by using the enzymatic colorimetric method, as this method relies on enzymatic oxidation of free cholesterol and cholesterol ester, as shown in the following equations (Bowers and Bostick, 1982).

Estimation of triglyceride concentration

The concentration of triglycerides in the blood serum of the study samples was estimated using the enzymetic method (Bowers and Bostick, 1982).

Estimating the level of antibodies to the enzyme thyroid peroxidase

The level of TPO-Ab in the blood was estimated using the diagnostic kit prepared by the Chinese company Shanghai Yehua Biological Technology. The sandwich-type ELISA method was used using a small plate coated with antibodies specific for TPO-Ab and added by the producing company, and samples and standard solutions were added to the existing small holes. After that, the specific avidin peroxidase-HRP complex antibody is added to each hole in the plate, then incubated and washed according to the method , then the TMP solution is added to each hole. After adding the reaction termination solution, the optical density is measured spectrophotometrically at a wavelength of 450 nm (Ruf et al .,1988).

Statistical analysis

Statistical analysis of the results was conducted using the ANOVA test, and significant differences were determined according to Duncan's multiple ranges test with a significance level of (0.05). (Steel & Torries, 1980).

Results

The results of the current study, as shown in Table (1), showed a significant decrease (P≤0.05) in the level of the thyroid hormones tetraiodothyroxine (T4) and triiodothyronine (T3) and a significant increase in the level of thyroid-stimulating hormone (TSH).

Table No. (1) Effect of methimazole and aqueous extract of licorice plant on the levels of TSH, T3, and T4

NO	Parameters / Groups	Mean ± SD		
		T3 ng/ml	T4 µg/dl	TSH µUI/ml
1	Control	0.5950±0.1118 ab	3.9420 ± 0.687 a	0.0050±0.0000 c
2	Methi + Lico	0.4444 ±0.0665 cd	0.7004± 0.1469 f	0.0388± 0.0424 b

Table No. (2) effect of methimazole and the aqueous extract of the licorice plant on the level of TG, COL, and AbTPO.

NO	Parameters / Groups	Mean ± SD		
		CHOL mg/dl	TG mg/dl	TPOAb Pg/ml
1	Control	59.10±18.28 cd	62.29±11.62 a	53.81±4.79 a
2	Methi + Lico	62.65±3.91 c	59.93±4.87 a	46.59±7.18 c

The results of the current study in Table (2) showed that there was a significant increase at the level of cholesterol (P ≥ 0.05) and the absence of any significant difference in the level of triglycerides. The results of the current study also showed that there was a significant decrease at the level of (P ≥ 0.05) in the levels of the body. Anti-thyroperoxidase enzyme.

Discussion

The results of this study, as shown in Ta ble (1), showed a significant decrease in the level of thyroxine T4 and thyronine T3 in the second group with combined treatment. The reason for this decrease is due to the synergistic effect of the drug methimazole and the aqueous extract of the licorice plant . The reason for the decrease in thyroxine T4 and thyronine is due to the synergistic effect of the drug methimazole and the aqueous extract of the licorice plant. T3 in the third group treated with methimazole and the fourth group treated with propylthiouracil indicated that methimazole and propylthiouracil inhibited the action of the thyroperoxidase enzyme, which is an essential enzyme in the process of manufacturing thyroid hormone s, by inhibiting the process of iodine oxidation and the process of conjugating iodine absorbed in the thyroid gland to tyrosine (Kahaly *et al.,* 2018). Studies have also indicated that the reason for the decrease in the level of thyroxine T4 and thyronine T3 is due to the bioinhibition of the synthesis of thyroxine T4 and thyronine T3 within the thyroid gland through the influence of methimazole, which inhibit the action of the enzyme thyroperoxidase, the enzyme responsible for the synthesis of thyroid hormones within the gland. Methimazole also prevents is the process of converting thyroxine T4 to thyronine T3 by inhibiting the action of the deiodinase type-1 enzyme, which plays a major role in metabolizing thyroid hormones and converting them from thyroxine T4 to the active form thyronine T3 by removing one iodine atom. This process takes place in the surrounding tissues outside the thyroid gland (Allaithi and Kadhem,2023).The licorice plant contains effective biologically active compounds represented by phenols and flavonoids. These compounds affect the expression and activity of many enzymes and proteins that are involved in thyroid function (Pistollato *et al.,* 2019). Phenolic compounds and flavonoids affect the process of metabolism of thyroid hormones in peripheral tissues by affecting many reactions, including deiodination reactions by deiodinase enzymes. Changes in these metabolic pathways may greatly affect the process of metabolism of thyroid hormones. In addition, phenolic compounds inhibit the activity of the enzyme. Thyroperoxidase , which plays an essential role in the manufacture of thyroid hormones through iodine oxidation and binding to tyrosine (Dos Santos *et al.,* 2011).Treatment with methimazole led to a decrease in the level of thyroid hormones and an increase in the level of thyroid-stimulating hormone (TSH). The decrease

in the level of thyroid hormones affects the hypothalamic-pituitary-thyroid axis, or what is called positive feedback, to produce more thyroid-stimulating hormone (TSH). A low level of thyroid hormones stimulates the hypothalamus to release TRH, which in turn stimulates the anterior lobe of the pituitary gland to secrete amounts of TSH to stimulate the thyroid gland to produce more of its secretions (Lam *et al.,*2021).The active compounds in the aqueous extract of the licorice plant participate directly or indirectly in the feedback mechanism. These compounds can also stimulate the hypothalamic cells to secrete the thyrotropin hormone (TRH), which stimulates the anterior lobe of the pituitary gland to secrete the thyroid-stimulating hormone (TSH). (Zehnder and Trevor, 2009).The high level of cholesterol is attributed to the synergistic effect of the drug methimazole and the aqueous extract of the licorice plant, as the use of the drug methimazole leads to a decrease in the activity of low-density lipoprotein (LDL) receptors, which are receptors found on the surfaces of cells. These receptors play an important role in transporting cholesterol through lipoprotein endocytosis. Low density LDL is loaded with cholesterol (Duntas and Brenta 2018). Hypothyroidism is often associated with an increase in total cholesterol and low-density lipoprotein levels. This increase is due to a decrease in the process of converting cholesterol into bile acids (Waheeb *et al.,* 2020). The use of aqueous extract of the licorice plant results in hypothyroidism. Hypothyroidism is accompanied by a decrease in the ability of the liver to get rid of cholesterol by retaining it in the liver and converting it into bile acids or into steroid horm (Sheymaa,2012) .he results of the current study showed that there was a decrease in the level of antibodies to the enzyme thyroperoxidase. This decrease is due to the fact that methimazole reduces the levels of cytokines in the blood and also affects the process of transforming the immune response from the cellular immune response (T helper) to the extracellular immune response (T). helper 2 The response that stimulates immune cells to produce antibodies (Giusti,2019). Regarding treatment with the aqueous extract of the licorice plant, the results of the current study showed a significant decrease in the level of antibodies to the thyroperoxidase enzyme. The reason for this decrease is that licorice extracts have the ability to reduce the regulation of the expression of pro-inflammatory cytokines, interleukin IL-1 and interleukin IL-6 (Yu J *et al.,*2015).

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